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ДО
ДИРЕКТОРА
И СЛУЖИТЕЛИТЕ
НА ИНСТИТУТА ПО
ЕКСПЕРИМЕНТАЛНА
МОРФОЛОГИЯ, ПАТОЛОГИЯ И
АНТРОПОЛОГИЯ С МУЗЕЙ ПРИ БАН

Поздравителен адрес

УВАЖАЕМИ КОЛЕГИ,

За мен удоволствие и приятно задължение да Ви поздравя от името на Ръководството на Българска академия на науките и лично от свое име, по случай 60-годишния юбилей на Вашия институт.

Институтът по експериментална морфология, патология и антропология с музей има богата история и традиции, чийто корени се крият в средата на миналия век. Неговите основи са положени със създаването на Институтите по експериментална медицина и по експериментална ветеринарна медицина, чийто правоприемник днес е Вашия институт. Тук е мястото да изразя нашето голямото уважение и почит към делото на основателите на института – Акад. Асен Асенов Хаджиолов и Акад. Ксенофонт Иванов, изтъкнати и признати учени в областта на експерименталната морфология и патологията.

Шестдесет години са едновременно много и малко време за един институт. Много са от ъгъла на ретроспективния анализ на постигнатите резултати, малко са от гледна точка на предстоящите програми, амбиции и перспективи. Въпреки многото предизвикателства на времето, ИЕМПАМ винаги е бил авторитетна научна институция, която извършва съвременна научно-изследователска и експертна дейност в областта на клетъчната морфология и патология, антропологията и музейното дело. Националният антропологичен музей е достоен пазител на

националната памет и идентичност. Основание за гордост и удовлетворение са не само Вашите високи научни постижения, но и приложението им в медицинската и ветеринарно-медицинската практика, в биотехнологиите и бизнеса.

Ръководството на БАН високо оценява постигнатото от Вас и Ви пожелава здраве, успехи, ползотворна работа, нови перспективи и завоевания.

С УВАЖЕНИЕ,

ПРЕДСЕДАТЕЛ:

/акад. Стефан Воденичаров/

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Editorial

ДОКЛАД
60 години ИЕМПАМ

Институтът по експериментална морфология, патология и антропология с музей (ИЕМПАМ) е създаден на 1 юли 2010 г., като приемник на Института по експериментална морфология и антропология с музей и Института по експериментална патология и паразитология. Зад тази близка дата обаче се крие богата история, започнала още в средата на миналия век, когато в две последователни години са основани Институтът по експериментална медицина (ИЕМ, 1947 г.) и Институтът по експериментална ветеринарна медицина (ИЕВМ, 1948 г.). Задачите на двата първи биомедицински института е да разработват актуални въпроси и да подпомагат клиничната и ветеринарно-медицинска практика. Не след дълго през 1953 г. на основата на Секция по хистология и ембриология към Института по експериментална медицина е учреден Институтът по морфология, чиято задача е да работи върху проблеми в областта на морфологията и антропологията. През 1986 г. той е преименуван в Институт по клетъчна биология и морфология. През 1995 г. след вливане на Институтата за изучаване на мозъка и на Централната лаборатория по регенерация се създава Институтът по експериментална морфология и антропология, по-късно преименуван в Институт по експериментална морфология и антропология с музей през 2006 г. Успоредно с това по своя път върви и ИЕВМ, който през 1964 г. се превръща в Институт по сравнителна патология на животните, а през 1973 г. – в Институт по обща и сравнителна патология (ИОСП). През 1994 г. обединението на ИОСП и Института по паразитология води до появата на Института по експериментална патология и паразитология. Първите директори съответно на Инситута по морфология и Института по сравнителна патология са акад. Асен Иванов Хаджиолов, и акад. Ксенофонт Иванов, чиито годишнини честваме в настоящата 2013 година – 110 години от рождението на акад. Асен И. Хаджиолов и 115 години от рождението на акад. Ксенофонт Иванов. През годините директори на Института по морфология са били акад. Асен Иванов Хаджиолов и проф. Иван Горанов, на Института по клетъчна биология и морфология – акад. Асен Асенов Хаджиолов и чл.-кор. Йордан Йорданов, на Инстиута по експериментална морфология и антропология – чл.-кор. Йордан Йорданов. Централната лаборатория по регенерация се е ръководела от чл.-кор. Георги Гълъбов и проф. Стефан Манолов, а Институтът за изучаване на мозъка – от проф. Людмил Гицов

и проф. Виола Бурнева. Директори на Института по обща и сравнителна патология и ИЕПП са били акад. Захари Младенов, проф. Никола Сотиров, чл.-кор. Олга Полякова, проф. Иларион Янчев, доц. Светослав Иванов и проф. Илия Банков. Основател на Централната лаборатория по хелминтология през 1954 г. е проф. Константин Матов, която в последствие се ръководи от акад. Иван Василев и чл.-кор. Олга Полякова.

Включен в направление „Биомедицина и качество на живот", днес Институтът по експериментална морфология, патология и антропология с музей-БАН се очертава като уникален по своята тематика институт, в който се провеждат комплексни фундаментални и научно-приложни изследвания в областта на хуманната и ветеринарната медицина, в това число морфология, клетъчна биология, патология и антропология, имащи съществена роля при решаване на важни здравни и демографски проблеми.

В областта на експерименталната морфология и патология се провеждат изследвания за изясняване на патогенезата на социално-значими заболявания- дегенеративни, ракови, автоимунни заболявания и инфертилитет. С важно клинично значение е идентифицирането на биомаркери и разработване на иновативни подходи и методи, приложими в диагностиката на тези заболявания. От значение за ветеринарната медицина са дейностите по бързата идентификация и характеризиране на нововъзникнали актуални болести по животните от заразно и паразитно естество, които са съпроводени с големи стопански загуби.

От фундаментално значение са изследванията върху клетъчните и молекулярни механизми на действие на ендогенни и екзогенни фактори (физични, химични агенти, хормони и цитокини) върху нервната, половата и кръвната тъкан. Изучават се молекулярно-генетичните механизми на канцерогенезата, механизмите на имунния отговор в норма и патология, сложните механизми на взаимодействие на системата паразит-гостоприемник, като биоиндикатор за актуалното състояние на околната среда.

В института са изолирани няколко оригинални онкогенни ретровирусни щамове – като резултат от дългогодишна работа върху проблема за левкоза по бозайници и птици и тяхната уникалност е потвърдена от чуждестранни лаборатории. Създадени са клетъчни линии с потенциално приложение в биотехнологичната промишленост – за тестване на биопрепарати и лекарствени средства. Разработен е комплексен подход за оценка безвредността на химични съединения лекарствени средства, храни и биологично активни вещества с природен произход.

В областта на медицинската паразитология се извършват комплексни изследвания върху епидемиологията, патогенезата, имунитета, клиничните прояви и диагностикака на паразитози при домашни и диви животни. Особено място се отделя на трихинелозата – една от най-разпространените опасни зоонози. Разработват се и се изпитват нови методи и средства за профилакти, терапия и контрол на паразитози, в т.ч съвременни високоефективни противопаразитни средства.

В областта на макроморфологията, каквато е антропологията се провеждат мащабни изследвния на съвременното население на Република България и на живялите в миналото хора по нашите земи. Успешно е реализирана Националната програма „Антропологична характеристика на българския народ в края на XX век. В областта на физическата антропология зе извършват мащабни антропометрични изследвния на живото население на България, с оглед на здравословното му състоянииe и трудов потенциал. Предоставят се антропометрични нормативи за физическото развитие на деца и подрастващи, което е особено актуално днес, от гледна точка на проблема със затлъстяването в ранна възраст. Разработват се антропометрични маркери за диагностика на различни заболя-

вания. В приложен аспект се предоставят данни от значение за ортопедията и травматологията.

Характеризиране на антропогенетичната идентичност на българите в миналото и днес е мостът между антропологията на живото население и палеоантропологията. Антропогенетичната характеристика на българите, обект на медиинен интерес, предоставя генетични доказателства за развитието на българския, респ. европейския генофонд в еволюцията.

Националният антропологичен музей (НАМ) е уникална научна, културна и образователна институция, открит през 2007 г. на основата на постоянната антропологична екзпозиция „Човекът в миналото" създадена през 1997 г. Музеят представя оригинални пластични реконструкции на главата по черепа на бележити българи и оригинални експонати, резултат от дългогодишни изследвания на костни останки, намерени при археологически разкопки в нашата страна. Пътуващият вариант на НАМ гостува в регионалните мезуи в страната като популяризира и съхранява националното наследство и утвърждава музейното дело.

Както става ясно от изложеното до тук научно-изследователската проблематика на ИЕМПАМ е в съответствие с националните и европейски приоритети – „здраве", „безопастност на храни", „околна среда", „културно-историческо наследство". Тематиката е в съзвучие със стратегическите направления и функционални приоритети на БАН:

1. „НАУКАТА – ОСНОВНА ДВИГАТЕЛНА СИЛА ЗА РАЗВИТИЕ НА НАЦИОНАЛНАТА ИКОНОМИКА И ОБЩЕСТВО, БАЗИРАНИ НА ЗНАНИЯ"
2. „НАУЧЕН ПОТЕНЦИАЛ И ИЗСЛЕДОВАТЕЛСКА ИНФРАСТРУКТУРА – ЧАСТ ОТ ЕВРОПЕЙСКОТО ИЗСЛЕДОВАТЕЛСКО ПРОСТРАНСТВО" – **Програма 2.3.** „Качество на живота и интердисциплинарни изследвания на човека и живата природа"
3. „НАЦИОНАЛНАТА ИДЕНТИЧНОСТ И КУЛТУРНОТО РАЗНООБРАЗИЕ В ЕВРОПА И СВЕТА" – **Програма 3.2.** „История на българските земи, България и българите"

Днес научно-изследователската дейност в Института е организирана в 4 секции – „Експериментална морфология", „Патология", „Паразитология" „Антропология с НАМ". В Института работят 90 учени и специалисти с висше образование, от които 6 професори, 18 доценти, 16 гл. асистенти и 19 асистенти. Доктори на науките са 5 и 36 учени са с образователната и научна степен „доктор". **Младите учени и специалисти са близо 40 %**. В условия на финансова криза за БАН и научните изследвания в България ИЕМПАМ се развива успешно през последните три години, с нарастваща научна продукция, защитени дисертации и кариерно израстване. В този период са публикувани над 500 статии и са отбелязани близо 800 цитирания. Защитени са 15 докторски тези. Научно-изследователската дейност за последните 3 тодини е финансирана по линия на ЕС – 3 проекта по ОП „Човешки ресурси – 2 проекта, от НФНИ – 30 проекта, от чуждестранни договори – 13 в т.ч 8 по ЕБР. Успешно международното сътрудничество се извършва с престижни научни центрове и университети във Великобритания, Германия, Австрия, Италия, Русия, Литва, Латвия, Румъния, Египет в рамките на ЕБР и междуинститутски договори.

- В национален мащаб ИЕМПАМ развива широко сътрудничестно с: **Висши училища** – Медицинските и Ветеринарно-медицински факултети в страната (София, Пловдив, Варна, Стара Загора, Плевен), ВХТУ, Химически и Биологически факултет на СУ „Кл. Охридски", Биологически факултет на ПУ „П. Хилендарски",

- **Центрове и агенции** – Национален диагностичен ветеринарно-медицински институт, Национален център по заразни и паразитни болести, Национален онкологичен център, БАБХ. В последните седмици се оформи сътрудничество с Регионалните академични центорве в Плевен и Русе.
- **Университетски болници и Клиники** – Александровска болница (Кожна клиника, Ендокринологична, Урологична клиника), СБАЛ „Свети Наум", АГБАЛ „Света София", БАЛ „Доверие".
- **Фирми** – Витанеа ООД, Биовет Пещера

И не на последно място искам да отбележа ползотворното сътрудничество на ИЕМПАМ с други институти от **Академията** –институтите по Невробиология, Микробиология, Молекулярна биология, ИБИР, ИБЕИ, ИОНХ, ИОЦФХ, Институт по Полимери, Институт по Електроника, Институт по биомеханика, Институт по Археология.

Институтът популяризира научните постижения на цялата морфологична научна общност в България чрез периодичното издание на английски език – Acta morphologica et anthropologica, започнала своя път още през 1953 г. като Известия на Института по морфология. Институтът е организатор на националните морфологични дни и съорганизатор на националния конгрес на Българското анатомично дружество. Отпечатват се и две книжки годишно на Сборниците от работните срещи, организирани от института – Workshop по експериментални модели и методи в биомедицинските изследвания и Workshop по биологична активност на металите, синтетични и природни продукти.

И така – много или малко са 60 години? Много са от гледна точка на постигнатото – основание за гордост и удовлетоворения в днешния ден – за 60 годишната си история в Института са защитени над 160 докторски тези и 35 дисертации за доктор на науките. Отпечатани са 65 монографии и книги. Убедено можем да кажем, че с научно-изследователския си потенциал ИЕМПАМ заема достойно място в БАН и сред научната общност у нас и в чужбина. Въпреки предизвикателствата на времето Институтът успява да привлича и задържа младите си кадри. В днешния празничен ден тук се срещат мъдростта на опита на поколения изтъкнати учени и оптимизмът на младостта. Ще ги пренесем във времето с надежди за бъдещето. Ще има трайна следа след нас и ще оставим ИЕМПАМ в добри ръце.

19.11.2013 г.
София

Проф. дбн Нина Атанасова
Директор на ИЕМПАМ-БАН

Congratulatory letters from Institutes of BAS

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
ИНСТИТУТ ПО БИОРАЗНООБРАЗИЕ И ЕКОСИСТЕМНИ ИЗСЛЕДВАНИЯ
1113, София, ул. Ю. Гагарин № 2 ☎ (02) 8736137 факс: (02) 8705498, iber@iber.bas.bg; www.iber.bas.bg

До проф. д.б.н. Нина Атанасова
Директор
Институт по експериментална морфология, патология и антропология с музей при БАН

Уважаема проф. Атанасова,

От името на ръководството и колектива на Института по биоразнообразие и екосистемни изследвания при БАН Ви поздравявам по случай честването на 60-годишнината от основаването на Института по експериментална морфология, патология и антропология с музей при БАН.

Изминалите години на творческа изследователска работа и подготовка на висококвалифицирани кадри утвърдиха вашия институт като един от основните центрове на биомедицинските науки в България и забележим научен център в международен план.

Желая на Вас и на вашите колеги много здраве, творчески успехи, пълноценна изследователска работа в полза на науката и обществото, както и общественото признание, което вашето научно звено напълно заслужава!

19 ноември 2013 г.

С уважение:

проф. д-р Вълко Бисерков
Директор

ДО
ДИРЕКТОРА,
УЧЕНИТЕ И СЛУЖИТЕЛИТЕ
НА ИНСТИТУТ
ПО ЕКСПЕРИМЕНТАЛНА
МОРФОЛОГИЯ, ПАТОЛОГИЯ И
АНТРОПОЛОГИЯ С МУЗЕЙ

ПОЗДРАВИТЕЛЕН АДРЕС

УВАЖАЕМА ПРОФ. АТАНАСОВА,
УВАЖАЕМИ КОЛЕГИ,

ПОДНАСЯМ СЪРДЕЧНИТЕ ПОЗДРАВЛЕНИЯ НА УЧЕНИТЕ И СЛУЖИТЕЛИТЕ ОТ ИНСТИТУТ ПО БИОЛОГИЯ И ИМУНОЛОГИЯ НА РАЗМНОЖАВАНЕТО „АКАД. КИРИЛ БРАТАНОВ" - БАН ЗА ВАШАТА 60- ТА ГОДИШНИНА С МНОГО РАДОСТ И ВДЪХНОВЕНИЕ.

РАДОСТ ЗА ТОВА, ЧЕ ВСЕ ОЩЕ НИ ИМА, ЧЕ ВСЕ ОЩЕ УСПЯВАМЕ ДА ИЗПЪЛВАМЕ ДЕСЕТИЛЕТИЯТА СИ СЪС СЕРИОЗНА РАБОТА И ПОСТИГНАТИ ЗНАЧИМИ РЕЗУЛТАТИ ВЪВ ВРЕМЕ- ТРУДНО ЗА БЪЛГАРСКАТА НАУКА.

ВДЪХНОВЕНИЕ ОТ ТОВА, ЧЕ В РАБОТАТА НИ ПО НАПРАВЛЕНИЕ „БИОМЕДИЦИНА И КАЧЕСТВО НА ЖИВОТ" ИМАМЕ ТОЛКОВА СИЛЕН, КОРЕКТЕН И СЕРИОЗЕН ПАРТНЬОР. ДОКАЗАХТЕ ГО НЕ САМО С ВАШАТА РАБОТА, НО И С ВОЛЯТА ДА ПРЕВЪРНЕТЕ ФОРМАЛНОТО ОБЕДИНЕНИЕ В ИСТИНСКО ЕДИНСТВО, ИМЕНУВАНО ИЕМПАМ.

ДРАГИ КОЛЕГИ,

С ВАС ДЕЛИМ ЕДИН ОБЩ ДОМ, КОЕТО НИ ДАВА ПРИВИЛЕГИЯТА НА БЛИЗОСТТА ДА ВИЖДАМЕ, СЛЕДВАМЕ И РЕШАВАМЕ ОБЩИТЕ СИ НАУЧНИ ЗАДАЧИ, НО И ДА ЗНАЕМ, ЧЕ ИМАМЕ ВЗАИМНАТА СИ ПОДКРЕПА И СИГУРНОСТ.

БЛАГОДАРИМ ВИ И ЖЕЛАЕМ ЗДРАВЕ, СИЛА, УСТОЙЧИВОСТ, МНОГО, МНОГО ЧОВЕШКО ЩАСТИЕ И ТВОРЧЕСКО УДОВЛЕТВОРЕНИЕ!

ДИРЕКТОР:
ПРОФ. Д.Р.Д. КАЧЕВА, ДВМН

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
ИНСТИТУТ ПО ФИЗИОЛОГИЯ НА РАСТЕНИЯТА И ГЕНЕТИКА
Директор: 02-8728170; Зам. Директори: 02-9792611 или 02-9746228 вътр. 348
Ел. поща: ifrg@bio21.bas.bg Интернет: http://www.ifrg-bg.com/
Адрес: ул. "Акад. Г. Бончев", Бл. 21, 1113 София

ПОЗДРАВИТЕЛЕН АДРЕС

ДО КОЛЕКТИВА НА ИНСТИТУТА ПО ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ, ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С МУЗЕЙ БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

Уважаеми колеги,

За мен е голяма чест и удоволствие да ви приветствам по повод 60-години от основаването на Института по експериментална морфология, патология и антропология с музей при Българска академия на науките.

През изминалите години вие изградихте с много знания и всеотдайност Институт – синоним на професионализъм и академичност. Високата квалификация на вашия колектив завоюва заслужено уважение и признание в българската и международна научна общност със забележителни успехи в областта на експерименталната морфология, патология и антропология. Научните и практически разработки на учените от Института намират всеобщо признание и ние се отнасяме с уважение към всеотдайния ви труд и искрено се радваме на вашите постижения.

Пожелаваме ви по-нататъшни творчески успехи и плодотворна дейност за развитието на българската наука и просперитета на българските учени!

Честит празник!

Директор:

София
19.11.2013 г.

Професор Снежанка Дончева
Директор на Института по физиология
на растенията и генетика - БАН

До Директора на Института по експериментална морфология, патология и антропология с музей при Българска академия на науките

ПОЗДРАВИТЕЛЕН АДРЕС

Уважаема г-жо Директор,

Скъпи колеги и гости,

Ръководството и колективът на Института по микробиология "Стефан Ангелов" най-сърдечно поздравява учените и всички служители на Института по експериментална морфология, патология и антропология с музей при Българска академия на науките по случай 60 годишнината от неговото основаване, 115 години от рождението на академик Ксенофонт Иванов и 110 години от рождението на академик Асен Хаджиолов!

Тези забележителни годишнини, които ИЕМПАМ отбелязва днес измерват неговия завоюван авторитет и са знак за достойно извървян път, изградени традиции и бъдеще, изпълнени с високо чувство на отговорност и професионализъм от неговите основатели, всички днешни и утрешни учени – изследователи и служители.

В днешния тържествен ден академичното семейство на Института по микробиология „Стефан Ангелов" споделя радостта Ви, защото нашата обща благородна мисия е една – достойно отстояване на знанията и творческите умения, утвърждаване на научните ценности, които са по-трайни и значими от трудното ни всекидневие.

Пожелаваме Ви много здраве, творчески дух, ползотворна работа, високо самочувствие, нови достижения и успехи в научната дейност, за да докажем достойното място на България в европейската и световната наука.

Честит юбилей!

19 ноември 2013 г *Директор на ИМикБ-БАН:*
София *проф. д-р Христо Найденски, двмн*

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

ИНСТИТУТ ПО МЕХАНИКА

София 1113, ул. "Акад. Г. Бончев", бл. 4, тел: +359 2 973 31 40, факс:+359 2 870 74 98, E-mail:director@imbm.bas.bg

ДО
Директора на ИЕМПАМ-БАН
проф. Нина Атанасова, дбн
ТУК

ПОЗДРАВИТЕЛЕН АДРЕС

Уважаема госпожо Директор,

Уважаеми учени, преподаватели и служители на Института по експериментална морфология, патология и антропология с музей към БАН,

За мен е чест да Ви поздравя от името на Ръководството на Института по механика към БАН и лично от мое име по случай Вашия празник – 60 годишнината от създаването на ИЕМПАМ.

През тези години, благодарение на високия си професионализъм и плодотворната си работа, Вашият институт и научна колегия се утвърди като един от най-престижните научно-изследователски центрове в България и завоюва солиден и безспорен авторитет със своите фундаментални и научно-приложни изследвания в областта на хуманната и ветеринарната медицина, в това число морфология, клетъчна биология, патология и антропология, както и в областта на обучението на студенти, докторанти и пост-докторанти.

Пожелавам на колектива на ИЕМПАМ нови творчески успехи!

Директор:.....
/проф. дтн В. Кавърджиков/

19.11.13 г., гр. София

ИНСТИТУТ ПО НЕВРОБИОЛОГИЯ
БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
Директор: Проф. д-р Рени Калфин
Ул. "Акад. Г. Бончев", бл. 23, София 1113
Тел. 979-21-51; Факс 8719-109
E-mail: neurobiology@bio.bas.bg URL: http//www.bio.bas.bg/neurobiology

Поздравителен Адрес

Скъпи колеги и приятели
от Института по експериментална морфология,
патология и антропология с музей

Вашият съсед - Института по Невробиология
при Българска Академия на Науките
ви поздравява с 60-годишния ви рожден ден.
Искрено се радваме на вашите успехи
и ви пожелаваме непрекъснато да ги умножавате.

Желаем ви все така с непресъхващ ентусиазъм
да разпространявате светлината на знанието,
крепко здраве, творческо вдъхновение
и добри, щастливи дни.

ЧЕСТИТ ПРАЗНИК !

Директор:
(Проф. д-р Рени Калфин)

ИНСТИТУТ ЗА ЯДРЕНИ ИЗСЛЕДВАНИЯ И ЯДРЕНА ЕНЕРГЕТИКА при БАН

БЪЛГАРСКА АКАДЕМИЯ на НАУКИТЕ

ПОЗДРАВИТЕЛЕН АДРЕС

Уважаемо Ръководство и скъпи колеги от Института по експериментална морфология, антропология с музей-БАН,

Ръководството и служителите на Института за ядрени изследвания и ядрена енергетика при БАН най-сърдечно Ви поздравяват по случай 60-тата годишнина от основаването на вашия институт.

Извършваните от Вас фундаментални и научно-приложни изследвания в областта на хуманната и ветеринарна медицина, паразитологията, разработването на диагностични методи за дегенеративни, ракови и автоимунни заболявания имат съществена роля при решаване на важни здравни проблеми и проблеми на околната среда.

Пожелаваме Ви още по-голями успехи във вашето научно поприще!

Директор на ИЯИЯЕ:

Доц. д-р Димитър Тонев

гр. София, 19.11.2013 г.

БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ
Институт по биофизика и биомедицинско инженерство

1113 София, ул. Акад. Г. Бончев, бл. 21
Тел.: 02-9712264, Факс: 02-9712493, , URL: www.http://biomed.bas.bg/

ПОЗДРАВИТЕЛЕН АДРЕС

от
ИНСТИТУТА ПО БИОФИЗИКА И БИОМЕДИЦИНСКО ИНЖЕНЕРСТВО ПРИ БЪЛГАРСКАТА АКАДЕМИЯ НА НАУКИТЕ
до
ИНСТИТУТА ПО ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ, ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С МУЗЕЙ ПРИ БЪЛГАРСКАТА АКАДЕМИЯ НА НАУКИТЕ

Уважаема госпожо Директор,
Уважаеми колеги,

Имам удоволствието от името на всички учени и служители на Института по биофизика и биомедицинско инженерство при БАН и лично от мое име да поздравя, Вас и всички учени и служители на Института по експериментална морфология, патология и антропология с музей по повод на неговата 60-та годишнина.

Вашия Институт извоюва заслужен авторитет сред биологическите институти на БАН, развивайки медикобиологическата наука на едно високо ниво и подготвяйки висококвалифицирани млади научни кадри.

Пожелавам Ви здраве, висок дух, воля и компетентност, както на Вас така и на всички ваши колеги, в името на доброто бъдеще на Института, Българската академия на науките и на българската наука.

ЧЕСТИТ ПРАЗНИК!

чл.-кор. Андон Р. Косев
Директор на Института по биофизика
и биомедицинско инженерство при БАН

Congratulatory letters from Universities

МЕДИЦИНСКИ УНИВЕРСИТЕТ - ВАРНА
„Проф. д-р Параскев Стоянов"
Ул."Марин Дринов" 55, Варна 9002, България
Тел. : 052/ 65 00 57, Факс: 052/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

MEDICAL UNIVERSITY - VARNA
"Prof. Dr. Paraskev Stoyanov"
55, Marin Drinov Str., 9002 Varna, Bulgaria
Tel.: +359 52/ 65 00 57, Fax: + 359 52/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

Варна, 11.2013г.

ДО
Настоящите и бивши служители на
Института по експериментална морфология,
патология и антропология с музей (ИЕМПАМ)
Проф. д-р Нина Атанасова, дбн, Директор на ИЕМПАМ

Уважаеми колеги,

Честит Шестдесетгодишен юбилей!

Шестдесет години са и много, и малко. Те са малко в сравнение с един човешки живот, но те са преминали в служба на науката и просвещението, а това е много!

Вече шест десетилетия учените, преподавателите и служителите на ИЕМПАМ превеждат през дебрите на знанието своите млади учени, докторанти и дипломанти, като споделят своя човешки и професионален опит. Многобройни са научните ви успехи, във всички съвременни области на биомедицинската наука. Те са публикувани и цитирани в престижни международни издания и монографии. Постиженията ви имат пряко отношение към човека и могат да служат в клиничната практика.

Скъпи колеги и приятели, невъзможно е в едно кратко приветствие да се отрази всичко, което сте постигнали. На всички вас желая крепко здраве и нека следващите години ви радват с нови успехи и постижения.

Приемете нашите най-искрени пожелания за здраве, професионални и лични успехи!

Нека се множат поколенията, за които Вие ще бъдете както пример, така и опора!

Проф. д-р Антон Тончев, дмн, и целия
Колектив на катедрата по анатомия, хистология и ембриология
Медицински университет „Проф. д-р Параскев Стоянов" - Варна

МЕДИЦИНСКИ УНИВЕРСИТЕТ - гр. ПЛОВДИВ
MEDICAL UNIVERSITY - PLOVDIV

ПОЗДРАВИТЕЛЕН АДРЕС

До
Институт по ИЕМПАМ, БАН
По случай 60-сет годишен юбилей
– 19 ноември 2013 г.

УВАЖАЕМА ПРОФЕСОР АТАНАСОВА
УВАЖАЕМИ ЧЛЕН КОРЕСПОНДЕНТ ЙОРДАНОВ,
УВАЖАЕМИ ЧЛЕНОВЕ НА РЪКОВОДСТВОТО,
УВАЖАЕМИ УЧЕНИ ОТ ИНСТИТУТА ПО ИЕМПАМ, БАН!

За мен е особено удоволствие и чест от името на академичната общност на Медицински Университет-Пловдив да отправя сърдечните поздравления и благопожелания по повод честването на *60-тата годишнина от основаването на Института по Експериментална морфология, патология и антропология с музей.*

През всичките години на своята дълга история учените от Института извършват упорито и неуморно фундаментални и приложни изследвания в областта на морфологичните науки.

Приветствам Вашата инициативност и търсещ дух, които личат в множеството разработвани научни проекти на национално и международно ниво.

Като Зам. Ректор по качество и акредитация, но и като академичен преподавател в системата на висшето медицинско образование, искам да изразя личната си и на колегите благодарност за факта, че едно от основните направления в дейността на Института е подготовката и обучението на млади научни кадри. Грижата за формирането на знаещи и квалифицирани учени трябва да бъде приоритет, особено в назряващата криза за млад научен потенциал. Позитивен потенциал, който вие и България зная, че имаме. Поздравявам Ви, че през годините качеството и престижът на Вашата научна продукция непрекъснато се повишава и продължава да бъде на много високо ниво, доказателство за което са многобройните цитирания от учените по света.

В светлината на юбилея желая от сърце авторитетът на Института по Експериментална морфология, патология и антропология с музей сред научните среди да расте, да се разширява присъствието му в обществото и, което е най-важното, да се утвърди достойното място на българския учен!

Честита и светла 60-та годишнина!
Нека всички да мислим за националните интереси и цели!
Нека доброто да води делата ни!

ПРОФ. Д-Р СТЕФАН СИВКОВ,
ЗАМ. РЕКТОР ПО КАЧЕСТВО И АКРЕДИТАЦИЯ

Уважаема професор Атанасова,

Уважаеми дами и господа,

В развитието на всеки институт, в неговото минало и настояще има паметни дати, моменти и събития, които оставят своите трайни отпечатъци. Такъв е днешният ден. През 60-годишната дейност на Института по експериментална морфология, патология и антропология с музей в него се провеждат множество научно-приложни и фундаментални изследвания в такива важни области на медико-биологичното познание като клетъчната биология, морфологията, патологията и антропологията.

С високата си научна продукция Вашият инситут е утвърден научен център в областта на морфологията и патологията с достойно място в националното и международното научно пространство.

Пожелаваме на колегите от ИЕМПАМ много здраве и нови, още по-високи творчески успехи.

ЧЕСТИТ 60 -ГОДИШЕН ЮБИЛЕЙ!

ЧЕСТИТ ПРАЗНИК!

Декан на Биологическия факултет
на СУ"Св. Климент Охридски":

/доц. д-р Мариела Оджакова/

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ДО КОЛЕКТИВА
на ИЕМПАМ - БАН

УВАЖАЕМИ КОЛЕГИ,

Ръководството и колективът на Факултета по Химия и Фармация на СУ „Св. Кл. Охридски" най-сърдечно Ви поздравяват във връзка с честването на 60 години Институт по експериментална морфология, патология и антропология с музей при БАН.

Приемник на работните групи от Института по експериментална морфология и антропология с музей и от Института по експериментална патология и паразитология, Вашият колектив и до днес провежда компетентно важни фундаментални и приложни изследвания в областта на хуманитарната и ветеринарната медицина, свързани със здравни и демографски проблеми. В потвърждение на приноса Ви към българското общество е и Националният Антропологичен Музей – уникална за България научна, образователна и културна институция.

През целия 60-годишен период много колеги от Вашия Институт имаха и имат тясно сътрудничество с преподаватели и изследователи от Факултета по химия и фармация на СУ както в областта на научната проблематика и практическите въпроси, така и в обучението на докторанти, за чието израстване в перспективни научни работници е от съществено значение актуалността и значимостта на проектите, разработвани от Вас.

Пожелаваме Ви здраве и творчески успехи за поддържане на висококвалифицирания научен потенциал, който притежавате, за обогатяване на научната инфраструктура, за задълбочаване на международното сътрудничество и дългогодишните традиции в обучението на специалисти.

Уверени сме, че ползотворното сътрудничество със СУ „Св. Кл. Охридски" ще продължи и в бъдеще.

ЧЕСТИТ ЮБИЛЕЙ !

19.11.2013
София

ДЕКАН:
чл.-кор. проф. дхн Тони Спасов

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ДО ДИРЕКТОРА
НА ИНСТИТУТА ПО
ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ,
ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С
МУЗЕЙ – БАН
ПРОФ. Д-Р НИНА АТАНАСОВА, ДБН

УВАЖАЕМА ПРОФЕСОР АТАНАСОВА,

Академичният състав на Факултета по ветеринарна медицина при Лесотехническия университет – София изказва своето задоволство и съпричастност във връзка с честването на 60-годишнината на Института по Експериментална морфология, Патология и Антропология с музей.

За нас е чест и изключителна професионална гордост да си припомним богатата творческа биография на незабравимите професори – академик Ксенофонт Иванов и академик Асен Хаджийолов, които са тясно свързани с историята на Вашия Институт.

Значителни са успехите на колектива на ИЕМПАМ в научноизследователската дейност, които са световно известни. Със задоволство отбелязваме нашето двустранно сътрудничество в научноизследователската дейност. За нас е чест, че изтъкнати Ваши учени участват в обучението на студентите по ветеринарна медицина и в подготовката на докторанти.

Уважаеми колеги,

Ние желаем на Института по Експериментална морфология, Патология и Антропология с музей високи професионални успехи. Надяваме се, че сътрудничество ни в научната и учебната дейност ще се издигне до нови по-високи нива, които съвременното развитие на науката и образованието изискват.

Високи творчески успехи и новаторски постижения!

Честит юбилей!

19.11.2013 г.

Декан на ФВМ:
/Проф. д-р Богдан Аминков, двмн/

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До Директора и колектива на
Институт по експериментална
морфология, патология
и антропология с музей към БАН
София

ПОЗДРАВИТЕЛЕН АДРЕС

Уважаема проф. Атанасова,
Уважаеми колеги,

От името на Ректорското ръководство и академичната общност на Тракийски университет - Стара Загора най-сърдечно поздравяваме колектива на ***Института по експериментална морфология, патология и антропология с музей към БАН по случай***

60 – годишния юбилей!

Високо ценим Вашите постижения и научния Ви принос в българската наука. Приветстваме учените от Института и сме сигурни, че Вашия опит и научни изследвания във фундаменталните и научно-приложни изследвания в областта на хуманната и ветеринарната медицина, в това число морфология, клетъчна биология, патология и антропология имат важен принос за решаване на здравни и демографски проблеми. 60- годишната история на Института е доказателство за традиция и уважение към делото на създателите и научните постижения на всички, които работят в Института по експериментална морфология, патология и антропология.

От перспективата на човешкия живот, 60 години са зряла, улегнала възраст – възраст, натрупала богат капитал - опит, знания и мъдрост. Днешният празник е повод за високо самочувствие, удовлетвореност от развитието на българската наука и стремеж за отстояване на ценностите, които основателите на Института са ни завещали.

Пожелаваме Ви благополучие, вдъхновен труд и нови успехи, за да пребъде Вашия институт - люлка на българската наука и дух.

ЧЕСТИТ 60 ГОДИШЕН ЮБИЛЕЙ!

19.11. 2013 г.
Стара Загора

ПРОФ. ДСН ИВАН СТАНКОВ
РЕКТОР НА ТРАКИЙСКИ УНИВЕРСИТЕТ

До проф. Нина Атанасова, дбн
Директор на института по
експериментална морфология,
патология и антропология с
музей при БАН

ПОЗДРАВИТЕЛЕН АДРЕС

Уважаеми проф. Атанасова,

Позволете ми от името на академичната общност на Ветеринарномедицинския факултет при Тракийския университет град Стара Загора да Ви поздравя с 60-годишнината от създаването на Института по експериментална морфология, патология и антропология с музей при БАН. Като приемник на Института по експериментална морфология и антропология и Института по експериментална патология и паразитология обединеният научен институт днес е водещ център в областта на експерименталната морфология и патология, който с реализираните научни проекти достойно е защитил своето авторитетно място в международното изследователско пространство.

Научно-преподавателският колектив на Ветеринарномедицинският факултет към Тракийския университет приема тази 60-годишнина и като свой празник. Основанието за това се крие в обстоятелството, че в историята на нашите институции се вплита мащабното дело на такива учени - изследователи и преподаватели като акад. Ксенофонт Иванов, акад. Иван Василев, акад. Захари Младенов и др. Дългогодишната традиция и практика е взаимното сътрудничество и подпомагане между учените от института и академичната общност на Ветеринарномедицинския факултет и атестация за това са общите задачи, които е имало да се решават или тепърва предстоят с цел повече обществена полезност. Вярваме, че предизвикателствата, пред които сме изправени днес всички, ангажирани с наука и образование, ще можем да преодолеем само чрез такава действена колаборация при реализиране на модерни и необходими иновативни проекти.

От името на академичната общност на Ветеринарномедицинския факултет в Стара Загора пожелаваме на научния колектив на Института по експериментална морфология, патология и антропология с музей при БАН да съхрани традициите и извоюваният авторитет и да постигне нови творчески завоевания и постижения с които нашия народ и нация да се гордее.

ДЕКАН:.....
(Доц. Михни Янцканов, двмн)

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

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Morphology

Pili gemini – a common phenomenon of uncommon presentation and familial background

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Abstract: A 28-year-old man with diffuse alopecia and hair loss is presented. Confronting examination of the other male family members defined the diagnosis of familial pili gemini. Thus, a peculiar genetic item to a dermatological condition that appears with a rather unusual presentation is described. Further observations and genetic studies are needed to define the scientific significance of this phenomenon.

Key words: pili gemini, familial background

Introduction

The first description of multiple hairs is given by Flemming in 1883 [4]. The phenomenon was seen on the beard and for a long time attributed only to this location. Hair shafts dysplasia is admitted to be a consequence of splits of the follicular germen [8]. Currently, confusion on the clinical picture and pathogenesis of the various forms of hair shafts anomalies exist [1].

Herein, a familial case of different-sized hairs with single cuticles, growing from the same follicular matrix and emerging through a single pilary canal is discussed.

Case report

A 28-year-old male sought treatment of diffuse alopecia and hair loss, accentuated in the last few months. He had an elder brother with the same symptoms. Their father had hair loss and baldness since the age of 40. They all complained of permanent dandruff

and greasy hair. On physical examination the hairs in the frontal region of the scalp looked three or four times thicker than average. A magnifying glass revealed closely bundled hairs present in one follicle. They grew as a whole, surrounded by a common sheath. On dermoscopy two hair shafts came out of a single follicular opening (Fig. 1). Confronting visits of the other family members proved the same clinical picture. Histology taken by the scalp of our patient revealed two hair shafts converged towards the base, without fusion, arising from subdivided bulb surrounded with a common sheath (Fig. 2). Transverse sections showed two hairs surrounded by own cuticles, enclosed in the outer root sheath. The diagnosis of presumably autosomal dominant familial pili gemini of the scalp was suggested. Recommendations on appropriate daily care were provided.

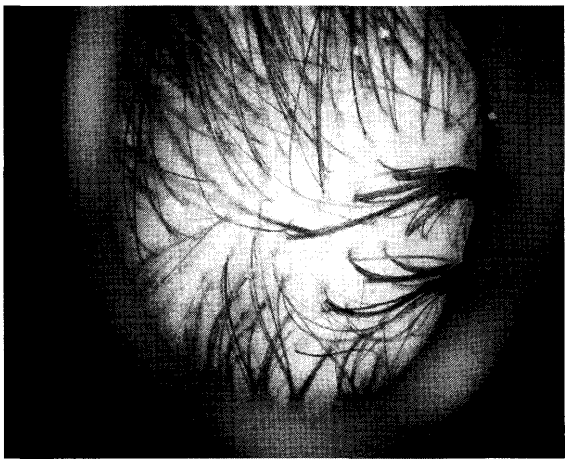

Fig. 1. Dermoscopy findings of two hair shafts coming out from a common follicular opening

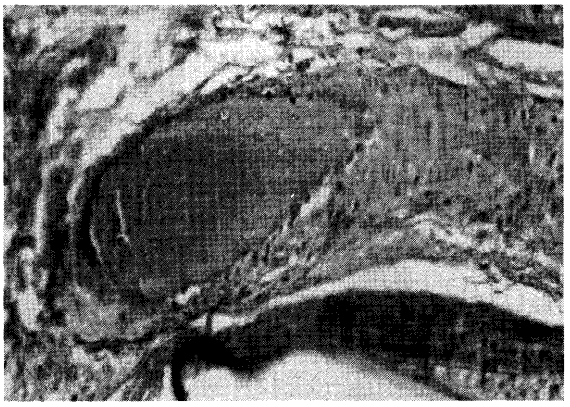

Fig. 2. A subdivided hair bulb with two hair shafts converged towards the base, without fusion, surrounded with a common sheath (HE, × 400)

Discussion

The hair has the most elaborately balanced structure with even minor functional deviations forming various aberrations [10]. The basic rules of hair morphogenesis are often break and on gross inspection of the skin incomplete hairs are present in one follicle or a bundle of hairs protrude from the follicular mouth [9]. Multiplicity of hairs may result either from remnants of old hairs in the follicle as an exaggeration of the normal cyclic activity of the papilla (trichostasis spinolosa) [12] or of splitting of the papilla matrix during the anagen of the hair to form compound follicle[7].

Double-tipped kinetic papilla produces two different-sized hair shafts with separate cuticles emerging through a single pilary canal [8]. This condition is known as pili gemini. The phylogenetic arrangement of hairs in groups of three or five gives the very common phenomenon of compound hairs – several hairs contained in the same follicle[2]. Two main mechanisms have been incriminated to cause the condition. According to Koelliker [5] accessory follicles grow down from the neck of the central one, while Rabl [11] favored the view of secondary merging of the superficial parts of independent follicles. This second opinion is widely recognized as the main reason for appearing of tufted hair folliculitis, a condition that is probably a consequence of staphylococcal scalp infection[3]. The most important differentiation of pili gemini and compound hairs is the level of hair follicle units merging [1]. The superficial merging comes out with tufted hair folliculitis, while matrix papilla tip merge forms two separate hair shafts with own cuticles, defining the pili gemini phenomenon.

Contra version exists as for the epidemiology and pathogenesis of hair shaft anomalies. Pili gemini can be observed both in hair dysplastic conditions such as cleidocranial dysostosis [7] and trichorinophalangeal syndrome, and in normal hair. According to Pincus, who first described the condition in 1951[9], the occurrence of the anomaly is really uncommon. The author pointed out that minor aberrations which furnish the basis for actual subdivision of the hair shaft usually affect the beard region and most often split the tip of the papilla. Thus, a complete subdivision of the hair shaft can result only if the split extends down to the neck of the papilla, at the site of inner rooth sheath formation. Therefore, the labiality of the hair matrix seems to play the most important role in the pathogenesis of multiple hairs. If the papilla tip split maintains during the whole anagen phase, hair shafts remain separate and form pili Gemini [13]. When the same papilla changes its shape repeatedly, it can produce hair shafts with bifurcations at irregular intervals defining the pili bifurcati. There is then no absolute distinction between the completely divided multiple hairs and the partly merged composite hairs [3]. Therefore, pili bifurcati can be considered a special case of pili gemini.

We presented a family with pili gemini that affect the scalp region. The location is not often described, which can be due to either low incidence or overlooking of the phenomenon. The signs of seborrheic dermatitis are probably secondary to male-pattern hair loss, seen in our patients. Of great interest is the familial background, which seems to be of autosomal dominant trait. To date, there were no other observations and genetic investigations in this area.

This anecdotal case report is presented to revive further knowledge of a well-known hair shaft anomaly presented at an unusual genetic background and atypical localization. Thus, we dare add a peculiar genetic item to a dermatological condition that appears with a rather unusual presentation. Further observations and genetic studies are needed to define the scientific significance of the phenomenon described.

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Serum antiganglioside IgG and IgM antibodies to GD1a in rat models of acute and prolonged lithium intoxication

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In the past years it is considered of critical importance to establish the significance of serum IgG and IgM anti-GD1a antibodies as potential biomarkers for neuronal damage in different neuropathies and neurodegenerative disorders. Although lithium salts are known to cause substantial neurodegeneration, the serum levels of anti-GD1a antibodies have not been studied in this type of intoxication yet. In this study, serum levels of IgG and IgM anti-GD1a antibodies were determined in rat models of acute and prolonged intoxication with LiCl using the enzyme-linked immunosorbent assay (ELISA) method. In both types of intoxication, serum antiganglioside IgG and IgM anti-GD1a antibodies titers were not elevated significantly to show that the blood-brain barrier in rats following Li treatment is not damaged. The results point out that IgG and IgM anti-GD1a antibodies cannot serve as serum markers for Li intoxication. Obviously, the acute or prolonged Li toxicity studies in rats cannot be used as models of progressive neuropathies.

Key words: serum IgG and IgM anti-GD1a antibodies, ELISA, acute lithium intoxication, prolonged lithium intoxication, rat

Introduction

Lithium is extensively used in psychiatric practice for the prevention and treatment of manic-depressive disorders. However, neurotoxicity of lithium salts within therapeutic doses has been reported in patients manifested by transient or persistent neurological deficits. Although those conditions are mostly transient and reversible, there is growing evidence that lithium can induce long lasting neurological sequelae [2, 4, 7, 9]. Side effects of Li generally correlated with the patient's serum level and often involve the central nervous system (CNS). Severe neurologic sequelae may occur in patients who take overdoses [8].

Gangliosides are a family of acidic glycosphingolipids highly concentrated in the nervous system, where they represent about 10% of the total lipid content. The ganglioside spectra of normal blood plasma are remarkably stable, but show pronounced changes in pathological conditions. GD1a is one of the major central nervous system neuronal ganglioside fractions. In our previous studies, a considerable increase of serum GD1a ganglioside was determined in both human cases and animal models of multiple sclerosis (MS – neurodegenerative multifactorial disorder with an autoimmune component). Autoantobodies against gangliosides GM1 or GD1a are associated with acute motor axonal neuropathy and acute motor-sensory axonal neuropathy. That is why over the past few years it is of critical importance to establish the clinical significance of serum IgG and IgM anti-GD1a antibodies as potential biomarkers for the diagnosis, classification, disease activity and prediction of clinical courses in antiganglioside antibody-mediated or other types of neurodegenerative disorders [5].

Although lithium salts are known to cause substantial neurodegeneration, no immunological studies about the possible involvement of serum IgG and IgM anti-GD1a antibodies in rats under the models of Li intoxication have been performed thus far.

The aim of the present study is to follow up the changes in serum IgG and IgM anti-GD1a antibodies in rat models of both acute and prolonged lithium chloride intoxication. The results are expected to elucidate the possible predictive value of those antibodies for lithium salts intoxication, as well as the extent of blood-brain barrier damage caused by Li-salts.

Materials and Methods

Mature Wistar rats (four-month-old) were subjected to acute lithium intoxication by a single dose of lithium chloride (250 mg/kg body weight, 0.2 ml dosing volume in saline, i.p.). Treated animals were sacrificed 24 hours following the administration under light anesthesia [9]. Healthy aged rats (eighteen-month old) were injected with the same volume of saline and used as controls.

Seven-month-old adult Wistar rats were subjected to a prolonged Li intoxication by receiving four administrations of lithium chloride with a quarter of the acute dose (250 mg/kg body weight) in the course of eight days (0.2 ml dosing volume in saline, i.p.) [4]. Animals were sacrificed under light anesthesia 24 hours after the last Li administration.

Three series of sera were obtained from the rats under the above experiments of acute and prolonged lithium intoxication. Isolation of serum antigangliosides antibodies was performed by the enzyme-linked immunosorbent assay (ELISA) method of Mizutamari [6] with slight modifications, as described before [1, 5]. Four independent analysis and quantification at various dilutions were conducted for each group and for control (no-Li) rats. The optical density (OD) was measured and read spectrometrically at 490 nm in ELISA reader Tekan Sunrise. The antigangliosides antibodies in the rat sera with Li-acute and Li-chronic intoxication, as well as healthy aged controls were calculated. The Student test was used to determine statistical differences between the groups using $p<0.05$ as the level of confidence.

Results and Discussion

Different studies show a significant increase of serum GD1a ganglioside in both human cases and animal models of multiple sclerosis [1, 5] as well as in other neuropathies [6]. The increase of titers of serum IgG and IgM anti-GD1a antibodies is usually con-

sidered as an indicator for neurodegeneration and blood-brain barrier damage. On the other hand, both acute and prolonged intoxications with lithium salts are shown to cause major pathomorphological changes in many regions of rat brain [4, 9] detected by the method of silver-copper impregnation for neurodegeneration [3]. Li is known to cross the blood-brain barrier leading to impairment of neuronal processes and neuronal death. However, it is not known yet whether the blood-brain barrier could be destructed as a result of the harmful action of Li on brain.

In our experiment, no statistically significant changes in the titers of serum IgG and IgM anti-GD1a antibodies were found (Fig. 1, Fig. 2). Optical density of the sera taken from Li-intoxicated animals did not exceed x ± 2 SD of the healthy controls to show a lack of abnormal antiganglioside antibodies values in both experimental and control animals' sera.

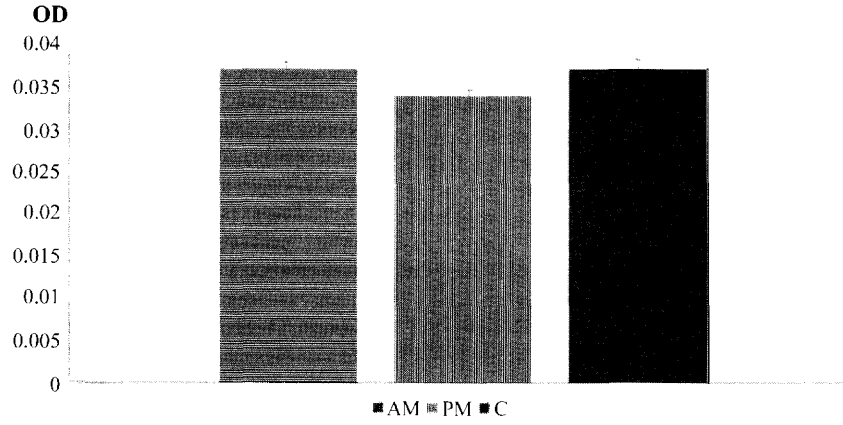

Fig. 1. Optical density (OD) of the titer of serum antiganglioside IgG antibodies to GD1a in rats subjected to acute model (AM) and prolonged model (PM) Li intoxication in comparison to control rats (C)

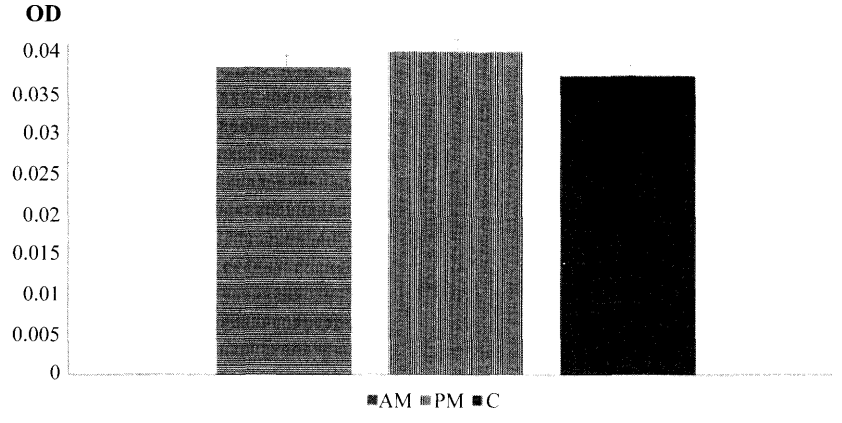

Fig. 2. Optical density (OD) of the titer of serum antiganglioside IgM antibodies to GD1a in rats subjected to acute model (AM) and prolonged model (PM) Li intoxication in comparison to control rats (C)

Legend: OD – optical density; AM – acute model; PM – prolonged model; C – control rats

In view of these results, it seems logical to conclude that lithium induced toxicity does not damage the blood-brain barrier. On the other hand, the lack of increased serum GD1a antiganglioside IgG and IgM antibodies titers suggests that rat models of acute or prolonged lithium intoxication cannot be used for the studies of highly advanced neuropathies, since the blood-brain barrier is not compromised.

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NOS positive mast cells in the pelvic urethra of male pigs

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With regard to our thorough research on mast cells in the pelvic urethra of male pigs, the aim of the present study was to obtain more data about the histochemical features of these cells by finding out whether they were positive for nitric oxide. The incentive of the study was the key role of nitric oxide (NO) and the closely related isoenzymes of nitric oxide synthase for a number of physiological and pathological events in the animal body. The study of NOS would also contribute to obtaining more information for the innervation of the organ in this animal species.

Key words: nitric oxide (NO), mast cells, pelvic urethra, pig.

Introduction

It is known that nitric oxide synthases (NOS) are haemoproteins which catalyse the oxidation of L-arginine and L-citrulline to nitric oxide. The synthesis of its isoenzymes is regulated by specific genes. Three isoforms are known, two of which are cell-bound – the neuronal and endothelial (nNOS and eNOS) and the third – free (iNOS). All three NOS are used as diagnostic markers of various vascular disorders, pathological deviations in the function of endocrine and exocrine glands (Quesada et al. 2002). NOS expression was established by Kawamoto et al. (1998) in the epithelium of human nasal mucosa, nasal glands, nerve fibres and the endothelium. Persson et al. (1998) assayed the possible co-presence of nitric oxide (NO) and acetylcholine in the major pelvic ganglia in rats by immunohistochemistry with antiserum against NO and acetylcholine esterase (AChE). Data from similar studies in the urethra of female pigs have demonstrated the presence of nitrergic, peptidergic and acetylcholine esterase-positive nerves in its distal part. Further, immunoreactivity to catecholamines containing various peptides, as well as immunoreactive nerves were established in the muscle layers of the urethra, in the propria, ureter and near the blood vessels (Crowe et al. 1989, Persson et al. 1995, Vodenicharov et al. 2005).

The important role of nitric oxide for a number of physiological and pathological events in the animal body, and the lack of data about NOS-positive mast cells in the pelvic urethra of domestic pigs motivated the present study aimed at completing the available information about the histochemical features of mast cells and the innervation of this organ in pigs.

Material and Methods

The specimens (pelvic urethras) were obtained from 12 healthy male Belgian Landrace pigs, 6–8 months of age, weighing 90–110 kg, slaughtered at the licensed slaughterhouse for a meat consumption of Dimes 2000 Ltd in compliance with all Bulgarian legislative norms. Immediately after the slaughter, the material was fixed by immersion in 10 % neutral formaldehyde (Merck, Darmstadt, Germany) for 48h. Further, the material was dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin. Cuts 5-6 μm thick were treated according to the routine ABC-HRP method with antigen unmasking in 0.01 M citrate buffer, pH 6.0 (Atanassova et al. 2005). Initially, they were treated with 3% (v:v) hydrogen peroxide (H_2O_2) in methanol and then, blocked with Normal Swine Serum with 5% BSA (Sigma Chemical A3425, St Louis, MO, USA). The primary universal antibody – rabbit anti-nitric oxide synthase universal (N-217, Sigma-Aldrich, Chemie Gmbh, Germany) for detection of the three NOS isoforms: neuronal, endothelial and inducible; was applied diluted 1:100 overnight at 4°C. The subsequent incubation was done with Swine Antirabbit Biotinylated IgG (DAKO E0353 Glostrup, Denmark) and ABC-HRP (DAKO; K0355, Glostrup, Denmark). The reaction was developed with DAB (liquid DAB+Substrate-Chromogen System) (DAKO; K3468, Glostrup, Denmark) and controlled under microscope, and afterwards was stopped in water. Then followed contrast staining with Harris' haematoxylin, dehydration and covering with Pertex mounting medium (CellPath plc). Negative controls were run without the primary antibody or after preabsorption with immunogenic peptide at a ratio of 1:10.

Results and Discussion

The light microscopy demonstrated expression of nitric oxide synthase in almost all structures of the pelvic urethral wall. In mucosal epithelial cells, positive expression was observed only in the cytoplasm, but not in the nuclei (Fig. 1).

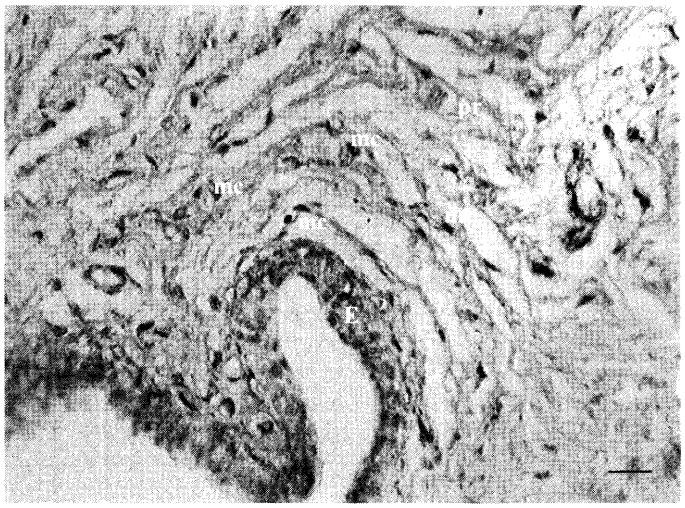

Fig. 1. Expression of nitric oxide synthase in epithelial cells (E) of the mucosa, as well as in mast cells (mc), located into the propria (pr). Bar = 20 μm

Immunohistochemical reactivity was observed also in secretory cells of glandular lobules of the prostate. From the studied arteries and veins of a various calibers, the strongest expression was observed in the endothelium of arteries (Fig. 2).

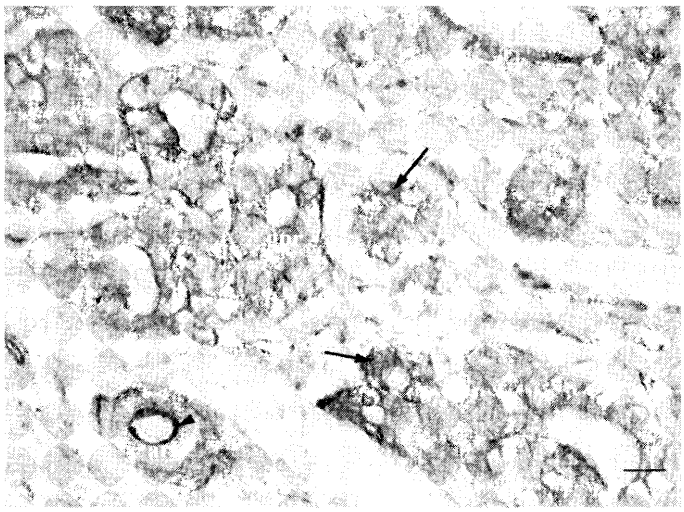

Fig. 2. Positive reaction in secretory cells (arrows) of glands (gpr), and in the endothelium (arrowhead) of the arteriole (art). Bar = 20 μm

A well expressed reactivity was also observed in the cytoplasm of striated muscle cells of M. urethralis (Fig. 3).

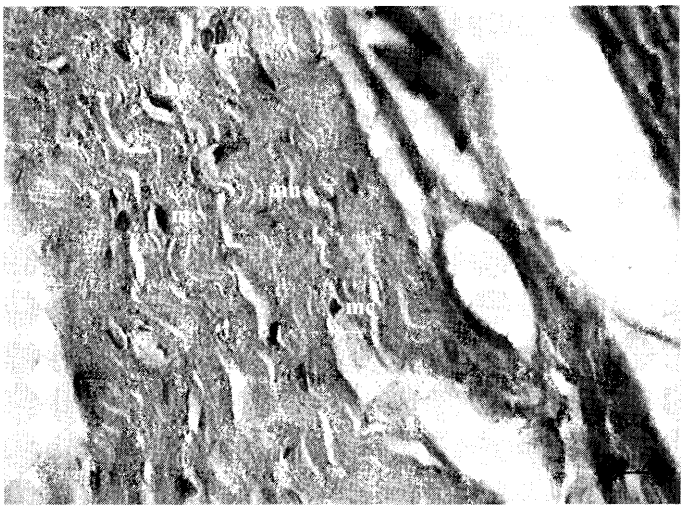

Fig. 3. Positive reaction in the cytoplasm of muscle cells (mu). Localisation of NOS-positive mast cells in M. urethralis (mu). Bar = 20 μm

The presence of NOS-positive mast cells was also detected in the different layers of the pelvic urethra. These cells were observed in the propria, mainly in vicinity of vessels from the microcirculatory vascular bed and near the organ's epithelium. Some mast cells were found out around the urethral glands. NOS-positive mast cells were also found in the connective tissue layers of *M. urethralis*. NOS expression was demonstrated in small and larger blood vessels, located in the urethral muscle and the connective tissue of the pelvic urethra. The strongest expression was detected in intimal endothelial cells, and a less strong one – in the adventitia. There were also numerous NOS-positive mast cells in the perimysium, among the muscle cells. Our findings are in agreements with data reported in female pigs and guinea pigs (Crowe et al. 1989, Werkstrom et al. 1998). In our experiment, NOS reactivity was also exhibited by the glandular epithelium of the disseminate part of the prostate gland and its excretory ducts. The re acted epithelial cells of the excretory ducts outlined clearly the pattern of their arrangement and their direction towards the urethral lumen.

In conclusion, the presented data allowed supporting the opinion of some researchers about the importance of investigating the distal urinary tract and the innervation patterns of *M. urethralis* and the pelvic urethra, as the domestic pig is an appropriate experimental model for studying pathological alterations in men. The results could be used in patients with complications following surgery of the urinary bladder or the urethra or after vertebral column injuries.

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Sex and Age Differences of Neurons Expressing NOS Immunoreactivity in the Pag of Male and Female Rats

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Expression of the enzyme nitric oxide synthase (NOS) was studied in the periaqueductal gray matter (PAG) of male and female rats during postnatal development at 30, 60 and 90 days of age. NOS-immunoreactive neurons were located in the dorsolateral (dlPAG), lateral and ventrolatelal (vlPAG) longitudinal subdivisions. Morphometric analysis revealed sexual dimorphism in the density of NOS-immunopositive neurons in the vlPAG of 30 days old prepubertal, 60 days old pubertal and 90 days old rats. Females showed numerous NOS-immunopositive neurons than males. The present results suggest that sex differences in the number of NOS-immunopositive neurons in the vlPAG may be related to epigenetic effects of gonadal hormones in the postnatal development.

Key words: PAG – NOS, postnatal development, sex differences, rat

Introduction

The midbrain periaqueductal gray (PAG) plays a modulatory role in a variety of behaviors including antinociception, reproduction, fear and anxiety, aggression and vocalization and sex differences are modulated by both the organizational and activational effects of gonadal steroids [5]. It is described to possess four longitudinal cell-rich columns - dorsomedial (dmPAG), dlPAG, lateral and vlPAG subdivisions, which serve as distinct anatomical modules for the specific functions [see 10]. The PAG integrates input from the limbic forebrain (including the amygdala) and the diencephalon with ascending input from the dorsal horn [^{2}] and projects to the rostral ventromedial medulla (RVM). The RVN in turn projects to the dorsal horn of the spinal cord and elicits the antinociceptive effects of opiates, as well as sex differences in opioid analgesia are modulated by effects of gonadal steroids [5]. Despite the critical role played by the PAG–RVM system in the spinal response to noxious stimulation, very little is known about the control exerted by brain stem descending fibres during postnatal development [2]. One set of primary factors that contribute to brain sexual differentiation are steroid hormones that are produced of the gonads and act directly in the developing brain. There are several ways to categorize the molecular mechanisms that drive brain deve-

lopment with or without sexual differentiation. One class of molecules that control gene expression is transcription factors, second class is effector molecules, which control and contribute to signaling from one cell to another. The potential molecular effector is nitric oxide (NO), which is a product of the enzymatic conversion of L – arginine to citrulline and is produced by three forms of NOS – neuronal (nNOS), inducible (iNOS), and endothelial (eNOS). NO plays many roles in development as well as adulthood. NO helps cell migration, cell proliferationand survival, which are all important factors for sexual differentiation [see 3].

In the light of these issues, the arm of the present study was to determine the density of NOS -immunopositive neurons in the PAG during postnatal development of the brain in male and female rats.

Material and methods

Nine female and 9 male Sprague-Dawley rats were used to study the localization of NOS immunoreactivity in the developing PAG. Intact animals were classified into 3 age groups: 30 days old, prepubertal rats, 60 days old, pubertal rat and 90 days old, young postpubertal rats. Animals were anaesthetized with thiopental (40 mg/kg body weight). Transcardial perfusion was performed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2. The brains were removed from the skulls and postfixed for 1 h in the same fixative. Afterwards, brains were washed in 0.1 M phosphate buffered saline (PBS) overnight at 4°C. Coronal sections (40 μm thick) were cut on a freezing microtom (Reichert-Jung, Germany). Sections were made at three PAG levels: cranial PAG - between bregma - 5.3 and bregma - 6.3, middle PAG - between bregma - 6.3 and bregma - 7.3 and caudal PAG - between bregma - 7.3 and bregma -8.3 [6]. Free-floating sections were preincubated for 1 h in 5% normal goat serum in PBS. Afterwards, incubation of the sections was performed in a solution of the primary antibody for 48 h at room temperature. We used a monoclonal anti-nNOS antibody (Sigma, St. Louis MO, USA) in a dilution of 1:1000 according to instructions of the manufacturers. After rinsing in PBS, sections were incubated with biotinylated anti-mouse IgG (Vector Labs. Inc. Burlingame, Calif., USA, dilution, 1:500) for 2 h. Sections were washed in PBS and incubated in a solution of avidin-biotin-peroxidase complex (Vectastain Elite ABC reagent; Vector Labs., Burlingame Calif., USA; dilution 1:250 in PBS) for 1 h. This step was followed by washing in PBS and then in 0.05 M Tris-HCl buffer, pH 7.6, which preceded incubation of sections in a solution of 0.05% 3,3¢-diaminobenzidine (DAB, Sigma) containing 0.01% H_2O_2 for 10 min at room temperature for the visualization. Sections were collected in Tris-HCl buffer 0.05 M, pH 7.6. In control sections, no significant staining was observed under the control conditions. Morphometric analysis was performed by capturing images of PAG through a 40 objective using a microanalysis system Nikon photomicroscope ECLIPSE 80i (digital camera DXM 1200C and the measured area of 0.360185 mm^2). Data the entire drawings were entered.

Results and discussion

Areal staining patterns on coronal sections of across the rostrocaudal axis in PAG subdivisions at levels of +5.3 to + 8.3 mm from bregma [6] were analyzed (Fig. 1).

The principal findings were as follows. First, immunostaining of the NOS immunoreactivity showed a striking specific pattern of neuronal profiles in dlPAG, vlPAG

and around aqueductus cerebri (AC) in male and female rats (Fig. 2). The distribution of the NOS-immunoreactive neurons in the PAG generally coincided with that observed in previous studies [1, 8]. Most of NOS-immunoreactive neurons are medium size ovoid, fusiform to multipolar or small rounded neurons arrenged in dlPAG, vlPAG and around AC (Fig. 2), a phenomenon reported [1, 4].

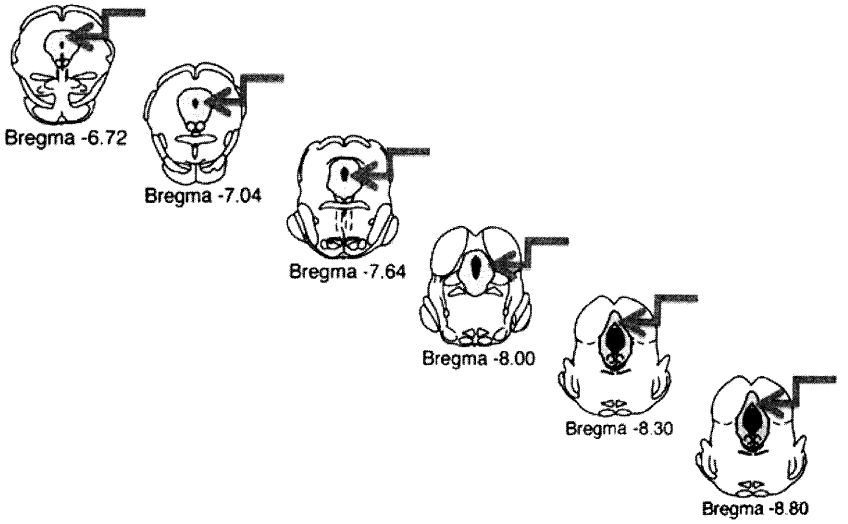

Fig. 1. Sections were made at three PAG levels: cranial PAG – between bregma – 5.3 and bregma – 6.3. middle PAG – between bregma – 6.3 and bregma – 7.3, caudal PAG – between bregma – 7.3 and bregma – 8.3

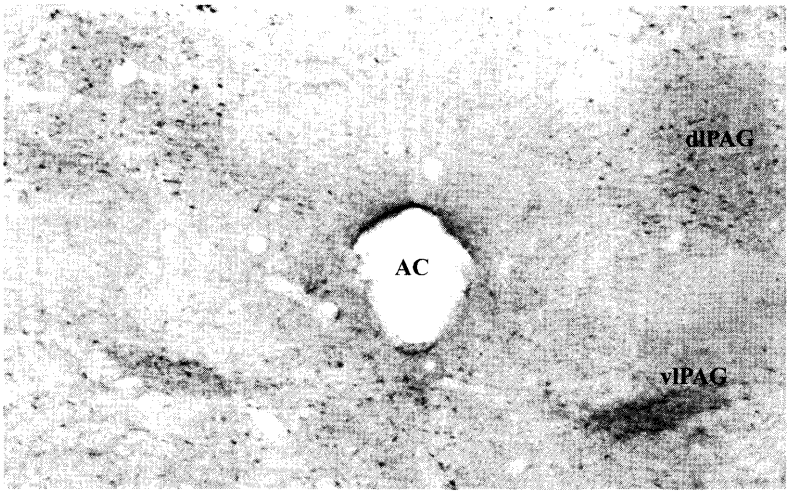

Fig. 2. NOS – immunopositive neurons are located in dl PAG, vlPAG and around aqueductus cerebri (AC). × 4

Second, the average density of NOS-immunoreactive neurons per μm^2 in the vl-PAG of female rats was greater than in males of the tested age groups (Fig. 3. Females showed a greater density of NOS-immunoreactive neurons than males and increased with age in both sexes. The average density of NOS-immunoreactive neurons in the dlPAG of male and female rats were similar in all age groups ($P>0.1$; Fig. 4). However, NOS-immunoreactive neurons showed a increase in number per μm^2 during aging in both sexes (Figs. 3, 4).

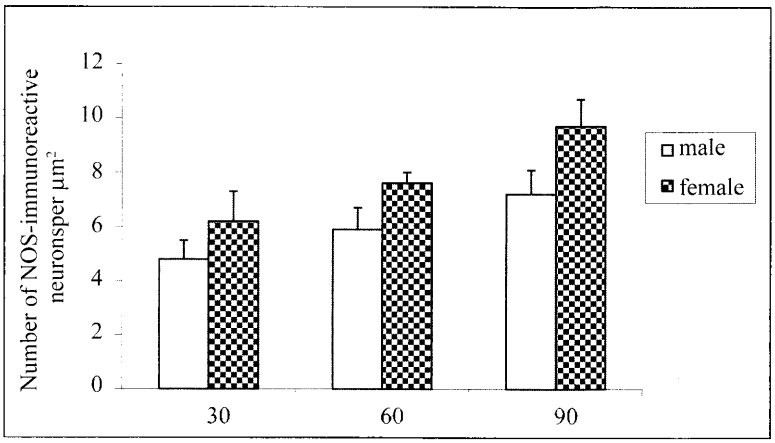

Fig. 3. The average density of the NOS-immunoreactive neurons in the vlPAG of female irats is greater than in male rats at 30 days, 60 days and 90 days male rats. There is a statistically significant increase in the neuronal density from female to male rats ($P < 0.05$).

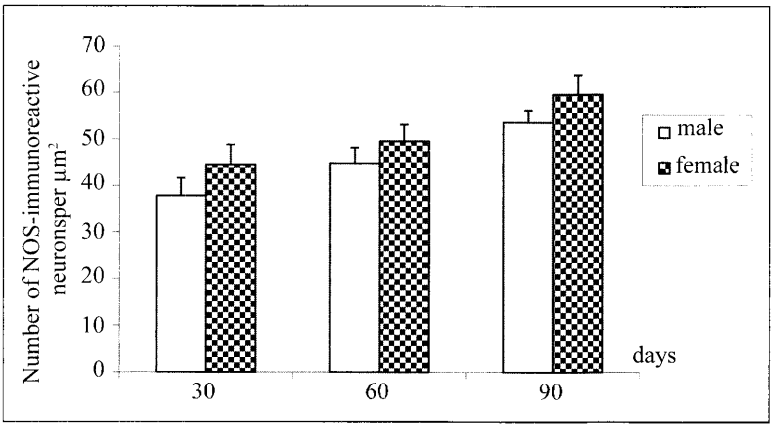

Fig. 4. The average density of the NOS-immunoreactive neurons in the dlPAG of female is greater than in male rats at 30 days, 60 days and 90 days male rats. There is not a statistically significant increase in the neuronal density from female to male rats ($P < 0.1$).

Third, these results suggest that sex differences in the density of NOS-immunoreactive neurons in the rat vlPAG is related to epigenetic effects of gonadal hormones during early stages of development and undergo additional modifications in later stages.

This conclusion corresponds to results that showed such a correlation between androgens and expression of different neuroactive substances in various brain regions [7, 9].

In summary, our morphometric study reveals that sex-dependent differences in the density of NOS-immunoreactive neurons of the postnatal vlPAG is established in all postnatal ages. These new data emphasize the need to examine NOS immunoreactivity in neurons in postnatal PAG after experimental manipulation of the hormonal balance.

Acknowledgments. The computer-assisted measurements were carried out in collaboration with Mrs. D. Brazitsova.

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Improved IEF method for the separation of proteins

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Abstract: This work is a continuation of our efforts towards developing an improved IEF method for the separation of proteins. Studying the electrochemical reactions occurring on the electrode surface we found that an unusually high electrode current is registered during the early phase of the experiment. We turned our attention to this side effect in an attempt to find a way for effective suppression of the electrolysis of water, which in its turn will bring about a decrease of the current. It was observed that the addition of gelatin to the electrode solutions suppresses the magnitude of the current flowing through the system, which allows the IEF system to approach steady state for a shorter time. In addition we separated the electrode solutions by Nafion® membranes, which selectively restrict the processes of migration and diffusion in the whole electrophoretic system. Under these newly developed conditions the ampholyte pH gradient is strongly affected, behaving as a carrier with very low ionic strength, in which the magnitude of the current flowing through the system rapidly decreases. Thus the isoelectric focusing can be carried out closer to steady state, obtaining sharp protein separation for a considerably shorter run time.

Key words: isoelectric focusing, protein separation, utilization of the electric current

Introduction

A number of studies have revealed that there is a deviation from the idealized model on which Svensson's IEF theory is based [1, 19]. Recently several theoretical treatments of steady state electrolysis and related processes have been published [20, 2, 3, 12, 26, 22, 25]. The theoretical model elaborated in these papers includes the consideration of chemical reactions between the electrolyte subspecies that produce an electric current, with the participation of hydrogen and hydroxide ions. It has long been recognized that there is a certain instability associated with carrier ampholyte pH gradients. It is reflected in the progressive flattening of the gradient in the neutral region (plateau phenomenon) and cathodic, anodic or symmetrical drifts, causing a gradual loss of the basic and/or acidic end of the pH gradient. Through a computer simulation elaborated by Mosher, Thormann and Bier [13, 14] it was revealed that the gradients develop because

a diffusional mass flux is needed to balance the net electrophoretic flux. These authors concluded that the fluxes are due to the ampholytes not being isoelectric, in the sense that the concentrations of their positively and negatively charged species are not equal. Employing phosphoric acid and sodium hydroxide as electrode solutions and varying the concentrations of the solutions, they concluded that "the loss" of the respective extreme regions of the pH gradients mainly depends on the correlation between the initial concentrations of the acid and the base. Applying the concept of the "moving reaction boundary" Pospichal et al. [21], Cheng-Xi Cao [4, 5] and Cheng-Xi Cao et al. [6, 7] use a mathematical approach to describe the transport of particles during IEF. Recently a series of self-coordinated processes occurring under conditions of isoelectric focusing were studied thoroughly [16, 17, 18]. It was established that under electric field a two-way process begins where the migration of the ions produced by water electrolysis is accompanied by diffusion of charge-compensating particles, originating from the electrode solutions and/or some constituents of the carrier matrix. As a result a non-ampholyte (primary) pH gradient is generated in PAG, which can be routinely measured. Because of the continuous flow of ions and particles the primary pH gradient changes with time bringing about a continuous change of the ampholyte pH gradient. It was established that irrespectively of the type of electrode solutions employed when the electrophoresis is carried out in a power mode the current gradually decreases, tending to reach a minimal value and the ampholyte pH gradient is relatively stable [17]. The same correlation was observed for the yield of water ions, which decreases during the process, following a non-linear relationship similar to that of the current. Our experience shows, that while in the electrophoretical system there are conditions allowing the electrolysis of water, i.e. water is present in the system, the current cannot reach a value of zero, the immediate consequence of which is that steady state can be approached, however it cannot be attained. In this context, any substance present in the electrode solutions that can decrease the current will be suitable to bring the system nearer to steady state for a shorter duration. Recently we managed to achieve this by introducing gelatin into the electrode solutions. This result prompted us to extend our studies in this direction by employing the ion exchange membrane Nafion® to restrict the mass transport and electron conduction in the electrophoretic system and to study how it affects the IEF of proteins.

Nafion®

Nafion® is a poly(tetrafluoroethylene) based ionomer, which was developed by Dr. Walther Grot at DuPont in the late 1960's by modifying Teflon® [15]. The ionic properties of Nafion® are created by adding sulfonic acid groups, a chemical with very strong ionic properties, into the bulk polymer matrix. Thus Nafion® combines the physical and chemical properties of its Teflon base material with ionic characteristics. It is found effective as a membrane for proton exchange by permitting hydrogen ion transport while preventing electron conduction [9, 10, 11, 24, 27].

Experimental

Materials, Equipment and Isoelectrophoretic Conditions

Polyacrylamide gel slabs (250×120×2 mm) were prepared using 5% gel concentration and 3% degree of cross-linkage. All of the reagents used for the self-preparation of polyacrylamide gels (PAG) were "puriss" and "for electrophoresis" grade from

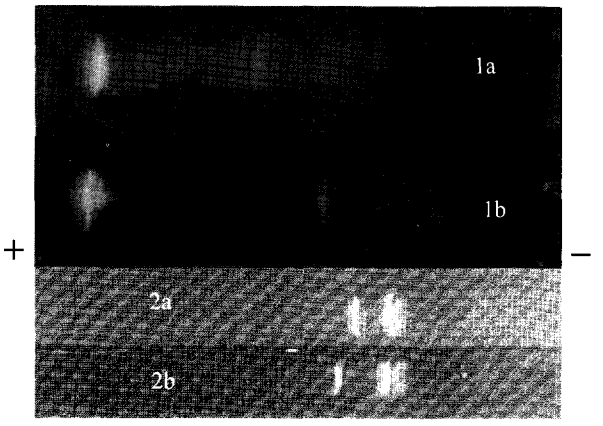

Fig. 1. Effect of gelatin on IEF of a Protein standard mixture (1a, 1b) as well as a hemoglobin standard (2a, 2b). Two types of electrode solutions were employed: 0.1 M phosphoric acid and sodium hydroxide (a) and 0.1 M phosphoric acid and sodium hydroxide in the presence of 1% gelatin (b). PAG was prepared in the presence of carrier ampholytes. The maximum preset voltage is reached 30 min faster when gelatin is introduced into the electrode solutions, and then the ectrophoresis was continued for two hours at 800 V. The focused protein bands have an almost identical separation and sharpness, however, their position along the gel differs, which is caused by the pH gradient drift. Running conditions: 800 V, 20 mA, 15 W. The letters denoting the electrophoregrams correspond to the electrode solutions employed

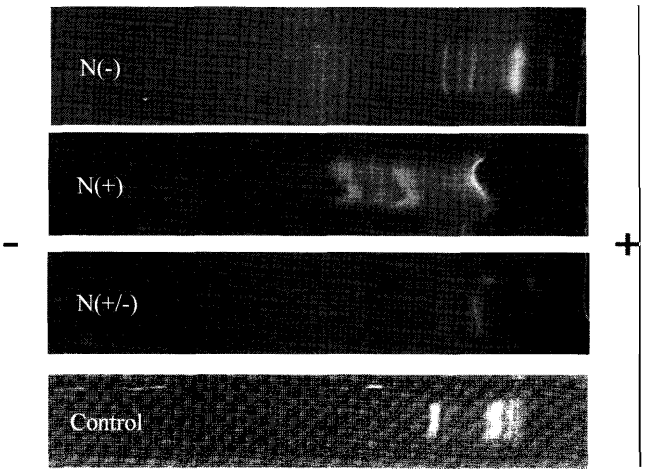

Fig. 2. Effect of Nafion® on IEF of a Protein standard mixture. The electrophoresis was carried out under electrophoretic conditions as in Fig. 1. The focused protein bands have an almost identical pattern, however their position along the gel differs, which is caused by the use of Nafion®. Best result as regards the separation of protein bands and their sharpness was obtained when Nafion® was used to separate only the cathode electrode solutions ( ). As an exception, when Nafion® was used to separate both electrode solutions (N+/-) the proteins are not separated at all

"Fluka" (Germany). 2.2 mL of carrier ampholytes (CA) "Ampholyte high-resolution 3-10" (catalogue No. 39878), Fluka & Riedel, The Sigma-Aldrich Family (USA), per 60 mL gel were introduced, followed by 20 mg ammonium persulfate and 0.06 mL TEMED. Solutions of 0.1 M phosphoric acid (Merck, Darmstadt, Germany) and sodium hydroxide (Reanal, Budapest, Hungary) or distilled water alone (pH=6.75) were used as electrode solutions. The total volume of each electrode solution was 100 mL and was bubbled with argon prior to use for about 15 minutes. In some instances 0.01%, 0.1% or 1% (m/v) gelatin (for electrophoresis, type A, G8150, Sigma) or Triton X-100 (CAS number 9002-93-1, laboratory grade, Sigma Chemical) were added to the electrode solutions. As a separator between the electrode solutions and the gel we used Nafion® 117 perfluorinated membrane, thickness 0.007 in. (catalogue No. 939), which was purchased from Aldrich, USA. As protein standards we used 5 μL 5% (*m/v*) solution of Protein Test Mixture 9 ("wide-range" pI-Marker Proteins), purchased from Serva Electrophoresis GmbH, Heidelberg, Germany (catalogue No. 39206) as well as a hemoglobin standard (Sigma, St. Louis, MO, USA). Staining and destaining procedure of the gel was performed according to the method described by Righetti and Drysdale [23]. Electrophoresis was performed under argon and was carried out using a Pharmacia ECPS 3000/150 Power Supply (Uppsala, Sweden) and an LKB 2117 Multiphor (Uppsala, Sweden) apparatus cooled by running water at a temperature of about 10°C. Platinum electrodes (thin platinum wire – 0.3 mm in diameter, 26 cm length) hanging on a plastic plate (LKB, Sweden) were immersed to the bottom of both electrode solution reservoirs, where the electrode strips were soaked in the corresponding electrode solution. The strips were connected to the gel ends by Whatman 3MM chromatographic paper. To separate the electrode solutions, respectively the electrode strips from the gel, a Nafion® sheet was superimposed on the gel surface selectively: on the anode side (N+), on the cathode side (N-) or on both sides of the carrier gel (N+/-) simultaneously.

The power supply was set to the limiting values of 800 V, 20 mA and 150 W. The duration of the process was read from the moment when the voltage reached the limiting value of 800 V.

Results and discussion

1. Physicochemical influence on the electrode current

Studying the electrochemical reactions occurring on the electrode surface and taking into consideration their relationship with the electrode current we reached the conclusion that under the conditions of IEF the abnormal maximum, which is always registered, is analogous to the same phenomenon observed in polarography. In polarography the abnormal jump of the current is suppressed by addition of small amounts of certain substances like the non-ionic detergent Triton X-100 or gelatin. In this paper we turned our attention to the analogous side effect observed by us, which is registered for both electrode currents in electrophoresis in an attempt to find a way for effective suppression of the electrolysis of water, which in its turn will bring about a decrease of current.

2. Influence of gelatin added to the electrode solutions on the electrode current

We studied the influence of gelatin contained in the electrode solutions on the current flowing through the electrophoretic system. The obtained data show that the decrease of the anode current is proportional to the concentration of gelatin dissolved in the electrode solutions of distilled water. Furthermore, the decrease of the anode current leads to a corresponding increase of the cathode current when the concentra-

tion of gelatin is in the range of 0.01% – 0.1%. However, when the highest feasible 1% concentration of gelatin was employed there was a considerable lowering of both currents. We can now propose that the current flowing through both electrodes can be decreased efficiently by adding 1% gelatin to the electrode solutions, so that steady state is attained for a shorter run time.

3. Influence of Triton X-100 added to the electrode solutions on the electrode current

We studied the influence of 0.01%, 0.1% or 1% concentrations of Triton X-100 introduced in the electrode solutions on the electrode current. Contrary to the results obtained with gelatin containing electrode solutions, in the case of Triton X-100 we found a reciprocal relationship. The magnitude of the current was very high throughout the process.

4. IEF of proteins in the presence of gelatin and Triton X-100 into the electrode solutions

To further verify the influence of gelatin added to the electrode solutions consisting of phosphoric acid and sodium hydroxide, we carried out IEF of a standard protein mixture. For the purposes of comparison we used two types of electrode solutions – the first contained 1% gelatin and in the second gelatin was omitted. It was established that the maximal preset voltage is reached 30 min faster when gelatin is present in the electrode solutions, as compared to the case when gelatin is absent. Electrophoresis was continued for two hours after the maximal preset voltage was reached when the process was interrupted and the electrophoregrams were compared. As can be seen on Fig. 1 the focused protein bands have a very similar separation concerning the number of separated bands, however, their position along the gel is different. Obviously, the presence of gelatin in the electrode solutions brings about suppression of the electrolysis of water, which results in a reduction of the amounts of hydrogen and hydroxide ions liberated in the electrode solutions.

The same electrophoretic system was studied, where gelatin was replaced with 0.01%, 0.1% or 1% concentrations of Triton X-100. Carrying out IEF in the presence of 1% Triton X-100 we observed a most considerable prolongation of the time for which the voltage reaches the preset limiting value, approximately 90 min. In addition we observed that the electrophoresis was accompanied by a considerable transport of water toward the anode, which caused a swelling of the carrier gel. Under these conditions the anodal proteins precipitate, thus compromising the electrophoretic separation.

5. IEF of proteins in the presence of Nafion® as a separator

IEF was carried out with and without Nafion® as described in Experimental. The corresponding results are presented on Fig. 2. A general observation is that the pH gradients are shifted to the more alkaline pH values in respect to Control, where Nafion® is omitted. The maximal shift was observed in two cases: when Nafion® was used to separate both electrode solutions and in the case when Nafion® is applied only on the cathode domain of the carrier. In the case when Nafion® is used to separate only the anode electrode solution the shift is higher in the more acidic region of the gel, but towards the cathode the same pH gradient deviates less from the Control pH values. This result undoubtedly shows that Nafion® selectively restricts the mass transport between the electrode solutions, thus influencing the distribution of the carrier ampholytes along the gel. To verify the expected influence of Nafion®, used to separate the electrode solutions consisting of phosphoric acid and sodium hydroxide, we carried out IEF of a standard protein mixture. For the purposes of comparison four experiments were carried out: without (Control) and with Nafion®. It was established that the maximal preset voltage is reached fastest (for about 150 min) in the cases when Nafion® was used to separate both electrode solutions and when it is on the cathode side of the gel. In the Control,

however, the maximal preset voltage is reached about 30 min slower than "the fastest" runs, under the conditions described above. When Nafion® is used to separate only the anode, the maximal preset voltage is reached slowest (for about 300 min). Electrophoresis was continued for two hours after the maximal preset voltage was reached, then the process was interrupted and the electrophoregrams were compared. As can be seen on Fig. 4 the focused protein bands have an almost similar separation concerning the number of separated bands, however their position along the gel is different. As a result of electroosmosis, on the electrophoregram, which is obtained when Nafion® is used to separate only the anode electrode solution, we observe wavy-shaped protein bands. Undoubtedly the best result was obtained when Nafion® is placed on the cathode domain of the carrier. Concerning the best separation obtained, the presence of Nafion® in the cathode domain of the carrier apparently restricts the migration of hydroxide ions, while the migration of hydrogen ions from the anode is unlimited.

Concluding remarks

The core of the present work is to stress the importance of the electrode solutions for the entire IEF process, which so far appears to be overlooked. In this paper we offer a novel modification of the IEF method, allowing the electrophoresis to be carried out closer to steady state, obtaining sharp protein separation for a considerably shorter run time. This has direct bearing to the improvement of the results obtained in 2-dimensional polyacrylamide gel electrophoresis and thus to proteomics as well. In our opinion the results reported here are a further contribution toward the elucidation of the role of the electrode solutions in the isoelectrophoretic process. In this context a newly discovered method, intended to obviate water electrolysis and related processes occurring on the electrodes under condition of electrophoresis, was recently published [8]. Unfortunately, this study is beyond the scope of our paper, but it is a starting point to make IEF applications not to be a daunting task.

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Stage-specific expression of p63 in rat germ cells – marker of meiotic phase of spermatogemesis in normal and experimental conditions

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Abstract: P63 protein isoforms are found in adult male and female urogenital organs and mammary glands and they are essential for male and female reproduction. Data about expression of p63 protein in the main testicular cells during pre and postnatal periods is quite limited. The present paper aimed to follow cellular localization and distribution of p63 in germ cells during development of the testis and in adulthood in normal and experimental conditions. Our study revealed stage specific pattern of expression of p63 proteins in spermatocytes later than middle pachytene stage of meiosis during the cycle of the seminiferous epithelium. Our data demonstrated that p63 is developmentally regulated in the testis and possibly changed with apoptotic and mitotic activity of germ cells. P63 is suggested to have clinical importance playing a role in preventing testicular lesions as apoptosis provides a mechanism for removing incorrectly differentiated gonocytes, which are thought to give rise to germ cell tumors.

Key words: P63 protein, spermatocytes, meiosis, germ cell, spermatogenesis

Introduction

The p53 family includes the three genes p53, p63, and p73. They have a modular structure consisting of the transactivation (TA), the DNA-binding (DBD), and the oligomerization domain. All three genes regulate cell cycle and apoptosis after DNA damage. However, despite a remarkable structural and partly functional similarity among p53, p63, and p73, mouse knockout studies revealed an unexpected functional diversity among them. P63 and p73 knockouts exhibit severe developmental abnormalities but no increased cancer susceptibility, whereas this picture is revealed for p53 knockouts. However, the existence of p53-like and p53-inhibitory versions of TP73 and TP63 genes, plus intimate functional cross-talk among all family members, endows these genes with both tumor suppressor and oncogenic roles [9].

The p53/p63/p73 family members are capable of interacting in many ways that involve direct or indirect protein interactions, regulation of same target gene promoter and regulation of each other's promoters. Although the proteins and their isoforms are

expressed at various levels depending on tissue type and developmental stage, the presence of an isoform at low levels does not necessarily mean it is insignificant [11]. The p53 family members and their isoforms can bind differentially to promoters and it may well prove that the ratio between isoforms is an important cell fate determinant. The changes upon stimuli of the balance and interactions between the isoforms are likely to be fundamental to our understanding in the transition between normal cell cycling and the onset of tumour formation.

Expression of p63 is absolutely essential for limb formation and epidermal morphogenesis including the formation of adnexa (teeth, hair, mammary and prostate glands, and sweat and lacrimal glands). The p63-null animals have defects of the apical ectodermal ridge and they show severe limb truncations or absence of limbs and absence of skin, teeth, mammary, lachrymal or salivary glands and craniofacial [2, 8]. The animals do not survive beyond a few days postnatally. Similar defects are found in children affected by ectrodactyly, ectodermal dysplasia and facial clefts (EEC syndrome) and recently an autosomal dominant rare mutation in p63 gene has been shown to be responsible for this syndrome.

The human and mouse p63 genes expressed as two major types: full-length proteins containing the TA domain and ΔN proteins missing the TA domain. Each of them was express at least three alternatively spliced C-terminal isoforms (α, β, γ). P63 containing the transactivation domain (TAp63) and amino-deleted p63 isoforms (ΔNp63) exert distinct (often opposite) functions on stemness, cycle arrest, mobility and invasion (epithelial–mesenchymal transition) and senescence. TAp63 induces cell death and cell cycle arrest with tumor-suppressor features, whereas ΔNp63 exerts oncogenic properties and is generally overexpressed in cancer. TAp63 and ΔNp63 (and their ratio) regulates chemosensitivity that is of clinical importance for cancer diagnosis and prognosis [9]. Generally, the more aggressive metastatic tumors lose p63 expression, suggesting that p63 loss accelerates tumorigenesis and metastatic spread. Correspondingly, disruption of p63 in squamous cell lines results in upregulation of genes associated with increased invasiveness and metastasis in tumors. This suggests that p63 is a marker of epithelial tumors such as ductal carcinoma in situ of the breast or prostatic intraepithelial neoplasia [4].

In postnatal epidermis, p63 expression is restricted to the nuclei of basal cells of normal epithelia (skin, esophagus, tonsil, prostate, urothelium, ectocervix, and vagina) and to certain populations of basal cells in glandular structures of prostate, breast, and bronchi [8]. In the female reproductive tract, all six splice variants of p63 were expressed in cervical/vaginal epithelium, oocytes in ovary and in a subset of epithelial cells in the ampulla of oviduct. Moreover, an antibody specific for ΔN forms detected proteins only in cervical/vaginal epithelium but not in the uterus, ovary and oviduct whereas TA splice variants were detected in oocyte.

In contrast, testicular germ cells were unreactive for ΔN or α isoforms, but reactive with anti-pan-p63 antibodies. This confirms that ΔNp63 isoforms are expressed in squamous/ basal epithelial and myoepithelial cells, while TAp63 forms are expressed in germ cells. Protein for α-isoforms was expressed in squamous epithelial tissues and oocytes. These expression patterns suggest functional differences in p63 isoforms in adult male and female urogenital organs and mammary gland [7, 12, 13, 14, 15].

Data about expression of p63 protein in the main testicular cell during pre and postnatal periods is quite limited. In addition, androgens are known to be essential for initiation of meiosis during puberty and testosterone suppression induced neonatally by DES or GnRHa inhibit meiotic differentiation of spermatocytes. In this respect the **aim** of the present paper is to follow cellular localization and distribution of p63 in germ cells during development of the testis and in the course of the first spermato-

genic wave in normal and experimental conditions. Our study is focused on the expression of p63 during the cycle of the seminiferous epithelium and on stage specific pattern of the p63 protein.

Materials and methods

Animals: Wistar rats, bred and maintained under standard conditions. We used experimental model for manipulation of neonatal hormonal environment by treatment with DES-10 μg and paraffin embedded tissue samples were provided by the Centre for Reproductive Health in Edinburgh. Briefly, the testes and epididymides with the vas deferens attached were fixed for ~5h in Bouins then transferred into 70% ethanol before being processed for 17.5 h in an automated Leica TP1050 processor and embedded in paraffin wax. Sections of 5μm thickness were cut and floated onto silane coated slides dried at 50°C overnight before being used for morphological and immunohisochemical studies.

Immunohistochemistry: Unless otherwise stated, all incubations were performed at room temperature for 30 min. Sections were deparaffinised and rehydrated. Antigen retrieval procedure was applied by pressure-cooking for 5 min in 0.01M Citrate buffer, pH 6.0 at full pressure. At this stage and after all subsequent steps, sections were washed twice (5 min each) in Tris-buffered saline (TBS; 0.05M Tris-HCl, pH 7.4, 0.85% NaCl). Endogenous peroxidase activity was blocked by immersing sections in 3% (v/v) H_2O_2 in methanol. To block non-specific binding sites, sections were incubated for 30 min. with normal rabbit serum. Primary mouse monoclonal anti p63 antibody (sc0586 Santa Cruz Biotech, USA) was used at dilution 1:500 and sections were incubated overnight at 4°C in a humidified chamber. Biotinylated secondary anti-mouse IgG antibody (Dako) was used at 1:500 dilution in blocking mixture followed by incubation for 30 min. with avidin-biotin conjugated to horseradish peroxidase (ABC-HRP; Dako) diluted in 0.05M Tris-HCl, pH 7.4. Immunostaining was developed using 3,3′-diaminobenzidine (Liquid DABplus; Dako). All sections were then lightly counterstained with hematoxylin. The intensity of immunostaining was scored on an arbitrary scale ranging from negative (-) through weakly positive (+) to intensely positive (++++).

Results

Our immunohictochemical studies on embryonal day 21.5 did not found any expression of p63 proteins in the fetal rat testes. The negative large gonocytes (prespermatogonia) are seen in the center of seminiferous cords. The similar negative reaction was observed in the testes on postnatal day 8th and differentiating spermatogonia that actively proliferate are located on the basal membrane of the cords.

First faint expression of p63 proteins appeared on day 15th in the nuclei of single pachytene spermatocytes adluminally located. On day 18th more immunopositive spermatocytes at stage middle pachytene were seen in the seminiferous tubules. Germ cells in earlier stages of meiosis (leptotene and zygotene) are negative.

Strong immunoreactivity of p63 was evident on day 25th and some stage specificity can be seen as four type tubules can be distinguished based on the different association of germ cell types (Fig. 1a). Spermatocytes at stage late pachytene are more intensively stained compared to the spermatocytes at stage middle pachytene.

In the adult rat testes spermatogenesis is complete and fourteen stages of cycle of the seminiferous epithelium are present. Stage specific pattern of expression of p63 proteins is obvious and reaction is confined to the primary and secondary spermatocytes in the

tubules from middle (VII-VIII) to late stages (IX-XIV) (Fig. 1c). Early pachytene spermatocytes in stages I-VI are negative for p63. Primary spermatocytes at middle pachytene stage of meiosis are intensively stained. Strong immune-reactivity continues in late pachytene spermatocytes in stages IX-XII of spermatogenic cycle. Primary spermatocytes at diplotene stage in XIII stage of the cycle are less immune-reactive that pachytene germ cells. Weak expression can be seen in the nuclei of secondary spermatocytes in stage XIV. More advanced postmeiotic germ cells, spermatids do not express p63.

The testes from rats treated neonatally with DES showed suppressed spermatogenesis manifested by dramatic reduction in germ cell number, especially evident for primary spermatocytes on day 18th and day 25th. In seminiferous tubules form 25 day old DES treated testes single middle pachytene spermatocytes can be seen that exhibit strong immuno-expression for p63 comparable to that in controls (Fig. 1b). In adult DES treated testes of spermatocytes were less intensively stained compared to the controls (Fig. 1d).

Paraffin sections from ductus deferens of 18 day old control rats were used as positive control where strong expression is shown in basal epithelial cells (Fig.1e). For validation of the DES treatment sections form ductus deferens of 18 day old DES treated rats were used where lack of p63 and altered basal cells differentiation were reported in our previous study (Fig. 1f) [1].

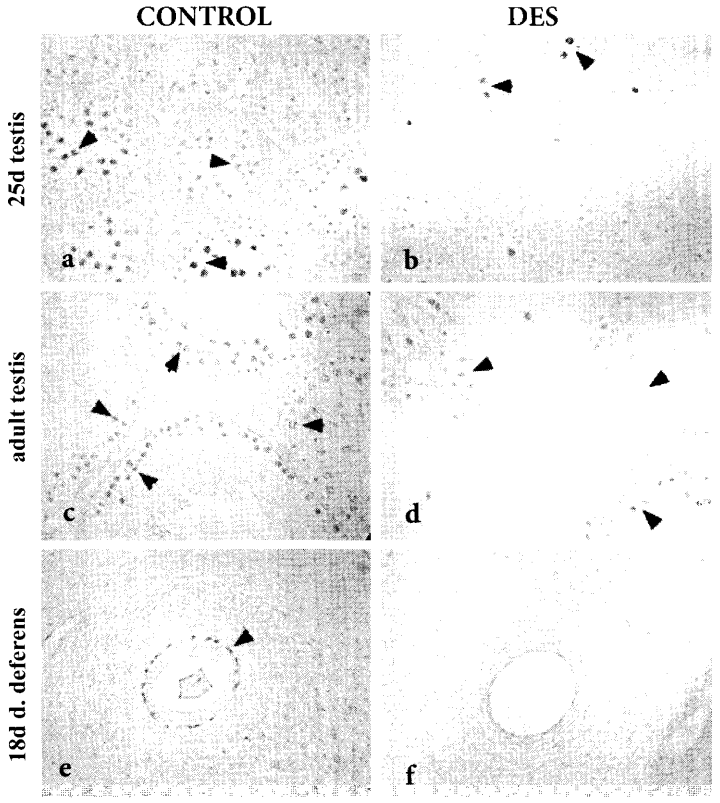

Fig. 1. Immunoexpression of p63 in the germ cells (spermatocytes) of control rat and DES treated testes on day 25 (a, b) and in adulthood (c, d). Positive controls from ductus deferens from control and DES treated rats on day 18 (e, f). Note basal epithelial cells are labelled

Semi-quantitative and schematic presentation of immunoexpression of p63 proteins during the stages of spermatogenic cycle is shown on is shown on table 1 and fig. 2.

Table 1. Semi-quantitative immunoexpression of p63 in the stages of spermatogenic cycle

Stages of the cycle	I-VI	VII-VIII	IX-XII	XIII	XIV
Type of spermatocyte	early pachytene	middle pachytene	late pachytene	diplotene	secondary spermatocytes
Intensity of immune reaction	-	+++	+++	++	+

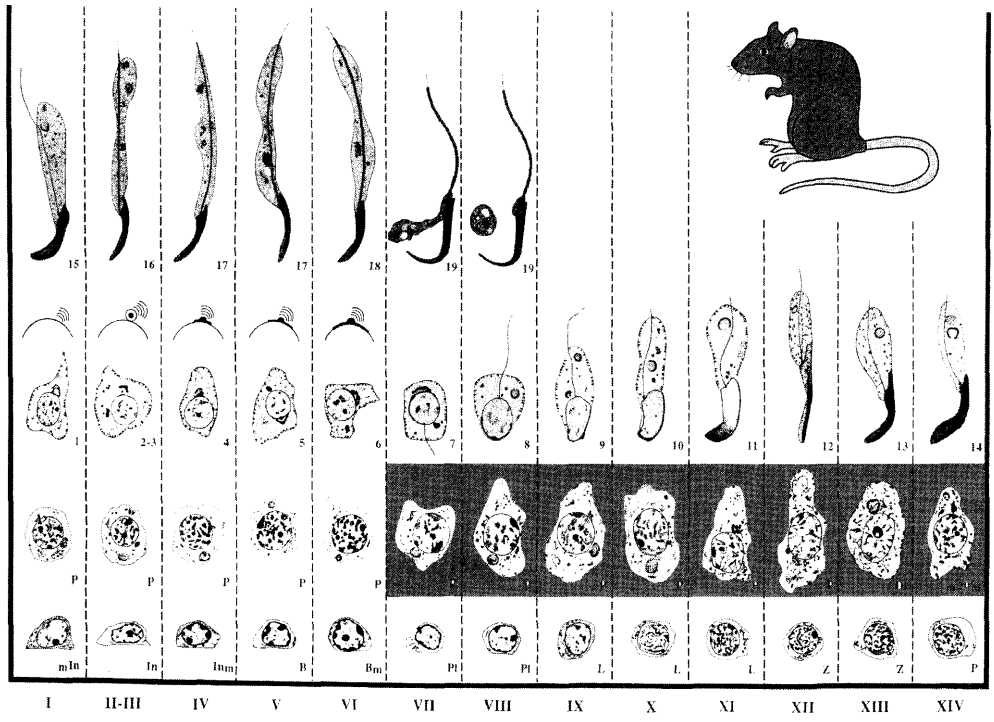

Fig. 2. Schematic presentation of immunoexpression of p63 during spermatogenic cycle

Discussion

All the three member of p53 family were expressed in the mouse testis [12]. In the mammalian testis, it has been shown that p53 plays important roles in the regulation of germ cell apoptosis and meiosis. P53 is expressed in spermatocytes demonstrated by immunohistochemistry and *in situ* hybridization [18]. The knockout of p53 gene results in increased number of abnormal gametes due to suppression of spontaneous apoptosis [19]. In the testis, however, participation of p63 to DNA damage-induced apoptosis has not proved yet.

Immunohistochemical studies by Hamer et al. [5] revealed presence of p73 in the cytoplasm of spermatogonia, spermatocytes, residual bodies, as well as in the nuclei of spermatocytes and round spermatids. In contrast to the p73 -/- mice, in which no structural abnormalities were found in reproductive organs of either male or female by histology, the function of p63 in spermatogenesis is obscure, since p63 null mice born with severe developmental defects and die soon after birth [12].

In our study we used an anti-p63 antibody (4A4 Santa Cruz Biotechnology, California, USA) raised against the amino terminus of ΔNp63 (amino acids 1-205). Since 15-205 amino acid region of ΔNp63 is a DNA binding domain which coincides with 111-301 amino acids of Tap63, this antibody reacts with all six p63 variants of mouse, rat and human origin in Western blotting and immunohistochemistry.

Our developmental study demonstrated specific reaction for p63 protein in the nuclei of meiotic germ cells (spermatocytes) and is in concern with data by Hayashi et al [6] in rat and by Nakamuta and Kobayashi [13] in mice. As expression of p63 in primary spermatocytes at early puberty coincides with appearance of Notch 1 and its ligand Jagged 2 [6], p63 was suggested to governs the balance between development, differentiation and apoptosis of germ cells through the Notch signaling system and p53 target genes. Moreover, our detailed observation on the expression of p63 during the cycle of seminiferous epithelium provide new data about stage specific localization of p63 protein in primary spermatocytes from middle pachytene till diplotene stage of prophase I of meiosis and in secondary spermatocytes, as well. On day 25 (mid puberty) four type/stages of seminiferous tubules can be distinguished where different intensity of immune reaction was found. In adult testes we observe expression of p63 in stages VII–XIV of the spermatogenic cycle. Nuclear localization of p63 proteins at specific stages of spermatogenesis suggests their involvement in the regulation of cellular function during spermatogenic cell differentiation. On the other hand p53 is also expressed in spermatocytes [18]. Since TAp63 can transactivate p53-response genes and induce apoptosis, and the localization of p63 in developing testis was coincided with those of p53, TAp63 might induce the transcription of genes required for the cell cycle regulation or apoptosis of germ cells synergistically with p53 [13].

In our study we did not found any reaction of p63 in mitotic dividing germ cells -spermatogonia of developing and adult testes and they confirm observations in rat and mice by the authors mentioned above [6, 13]. However, Nakamuta and Kobayashi [12, 14] provide data for early expression of p63 since embryonal day 8.5 (e8.5) in primordial germ cells in hindgut to e11.5 in genital ridge that continues later in fetal male and female gonads. An important role of p63 in migration of germ cells and their colonization to the gonads is suggested. There are no differences between males and females as for the role of p63 in primordial germ cells before the germ cells are determined their different developmental fates to the testis or ovary. As opposite events occur in the fetal male and female gametogenesis specific pattern of p63 expression in mouse fetal gonads are found. In the fetal testes p63 was seen in the proliferating prespermatogonia from e12.5 to e18.5 and then protein expression declines and diminishes during quiescent period prior to resumption of germ cell development after birth. In contrast, germ cells in fetal ovary enter meiosis before birth, and arrest at the prophase of the first meiotic division, which do not complete until a few hours before ovulation. Moreover p63 protein is confined to the oocytes of primordial and primary follicles and expression is lost as follicles develop [16]. Despite the significant differences between male and female gametogenesis, there is temporal and spatial expression of p63 protein in germ cells involving early fetal events and resumption of cell cycle progression at puberty. Hence, an important role for p63 in cell cycle control and in regulation of germ cell development/meiosis is suggested.

Our study on developing and adult rat testes does not find any localization of p63 in postmeiotic stages of spermatogenesis – round spermatids as it was reported in mice by Nakamuta and Kobayashi [13]. This discrepancy could reveal some species specificity in expression of p63 proteins.

A study by Petre-Lazar et al. [16] followed ontogeny of each p63 mRNA isoforms during testis development to demonstrate correlation between their expression and gonocyte activity (proliferation/apoptosis versus quiescence). As p63γ mRNA and protein are strongly expressed in quiescent gonocytes, the γ isoforms appears to be the determining factor in these processes, rather than the balance between p63 N-terminal isoforms (TA and ΔN). P63 is suggested to be involved in spontaneous apoptosis in the germ cell lineage. There are many pro-apoptotic factors that are up-regulated by Tap63γ in different models and the Bcl2 and the Notch families may be also involved in apoptosis of postnatal germ cells.

As p63-/- mice died at birth Petre-Lazar et al [16] performed *in vitro* studies using tissue fragments of fetal testes from p63 -/- and p63 +/+ mice. Invalidation of p63 resulted in an increase number of gonocytes during the culture period of 3 days due to a decrease in spontaneous apoptosis. Lack of p63 also caused abnormal morphology of germ cells (giant cells) that was found in p63 +/- adult male mice. These giant germ cells are reported in rat neonatal testes after treatment with phthalate (DBP) [3] as well as in human testicular carcinoma in situ which is thought to originate from the abnormal differentiation of fetal gonocytes, possibly after exposure to estrogens or xenoestrogens [17]. The potent synthetic estrogen, diethylstilbestrol also has been reported to perturb p63 expression in the Mullerian duct [7] and in basal cells of developing rat epididymis and ductus deferens [1].

In conclusion, our results demonstrated that p63 is developmentally regulated in the testis as well as throughout the spermatogenic cycle and possibly changed with apoptotic and mitotic activity of germ cells. P63 is suggested to have clinical importance playing a role in preventing testicular lesions as apoptosis provides a mechanism for removing incorrectly differentiated gonocytes, which are thought to give rise to germ cell tumors.

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Expression of carbohydrate-binding proteins in culture medium from MCF-7 cells treated with metal complexes of the cholic acid

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Abstract: Levels of N-Ac-ß-D-mannosamine-binding proteins were higher in the culture medium from MCF-7 (human adenocarcinoma cells) treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$ as compared their levels in the culture medium from non-treated cells. We also found higher levels of D-galactosamine- mannose- and galactose-binding proteins in culture media from tumor cells treated with $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to their levels in the culture media from non-treated cells. D-glucosamine-binding proteins were down-regulated after treatment with all of the metal complexes. Treatment of MCF-7 with $La(Chol)_3\ 2H_2O$ led to higher levels of N-Ac-D-glucosamine- and D-mannosamine-binding proteins in the culture medium, compared to non-treated cell. D-fucose-binding proteins were up-regulated in cell culture treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to their expression in non-treated cells.

Key words: carbohydrate-binding proteins, MCF-7 adenocarcinoma, metal complexes.

Introduction

Carbohydrate-binding proteins (CBPs) play important role in the processes of malignant transformation and metastasis in a variety of tumor cells. Qualitative and quantitative changes in the expression of intracellular and cell surface galectins have been correlated with transformation and metastasis of tumor cells [7]. Carbohydrate-binding proteins with specificities other than galactose are expressed in many tumor cells. However their role in tumor cell biology is not as clear as the role of galectins. Fucose-binding proteins are expressed in rhabdomyosarcomas [2] and human epithelial tumor [3]. Liver metastases of three other types of primary tumors showed a tendency towards preferential expression of additional fucose-binding proteins [6]. Secretion of these carbohydrate-binding proteins was not followed up, but such secreted receptor could participate in

cell adhesion phenomena through binding to terminal fucose residues on the complex type N-linked glycans. Mannose–binding proteins were found in human teratocarcinoma cells [4]. Specific anti-carbohydrate immunotherapy of Guerin tumor cells correlate strongly with their proliferation index [1]. Metastatic lesions to lung from three different types of primary tumors revealed tumor-associated mannan-binding proteins [6]. Spontaneous strongly metastatic variants (ESb) of a murine lymphoma contained additional sugar receptors for N-acetylglucosamine. In another model system derived from the murine mastocytoma cell line P815×2A, biochemical analysis of the liver-metastasizing variant P815×2B revealed additional characteristic acetylgalactosamine- and maltose-specific binding proteins [5].

Materials and Methods

Synthesis of metal complexes of cholic acid: A solutions of 10 ml containing 0.5 mM of $Cu(CH_3COO)_2 H_2O$, $Co(NO_3)\ 6H_2O$, $La(NO_3)\ 6H_2O$ were added to 10 ml 1 mM solution of sodium cholate ($C_{24}H_{39}O_5Na$). The resulting mixture was stirred and heated for 1 hour. Formed precipitates were filtered, washed with water and dried over P_4O_{10}.

Culturing and treatment of MCF-7 cells: MCF-7 (human breast adenocarcinoma) cells were routinely grown as monolayer cultures in a combination of E-199 and Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 mg/ml). The culture was maintained at 37 °C, 5% CO_2 in a humidified atmosphere. At the 24th h cells from monolayers were washed and covered with media modified with 100 mg/ml of the compound examined.

Haemagglutination experiments: Agglutination assays were done in microtitter U plates using serial two-fold dilutions of cell culture samples. For sugar inhibition studies, 1 M of the corresponding sugars were added in place of the 0.15 M NaCl and preincubated with the lectin source for 30 min at room temperature.

Results

N-Ac-β–D-mannosamine-binding proteins: Levels of N-Ac-β–D-mannosamine-binding protein were of the same order in MCF-7 cells treated $Co(Col)_2.2H_2O$ and non-treated cells. The highest levels of expression were found in the culture medium from MCF-7 cells treated with $Cu(Chol)_2.4H_2O$, whereas tumor cells treated with $La(Chol)_3\ 2H_2O$ complex expressed an intermediate levels of N-Ac-β–D-mannosamine-binding proteins, see Fig 1.

D-galactosamine-binding proteins: CBPs with specific to D-galactosamine in treated MCF-7 cells were upregulated compared to non-treated cells, see Fig. 1. Among treated cells the ones treated with $La(Chol)_3\ 2H_2O$ secreted the highest levels of D-galactosamine CBP in the culture medium.

D-mannose-binding proteins: We found higher levels of D-mannose CBPs in treated cells compared to non-treated ones, see Fig. 1. Tumor cells treated with $La(Chol)_3\ 2H_2O$ complex had the highest levels of D-mannose-binding proteins in their culture media when compared to levels of these CBPs in culture medium from tumor cells treated with $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ complexes.

D-glucosamine-binding proteins: We found that levels of D-glucosamine specific carbohydrate-binding proteins were lower in culture media from MCF-7 cells treated with $Cu(Chol)_2.4H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to the levels of these CBP in culture medium from tumor cell treated with the $Co(Chol)_2.2H_2O$ complex, see Fig. 1. Culture media from tumor cells treated with $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$ had lower levels of D-glucosamine-binding proteins, as compared to their levels in non-treated cells.

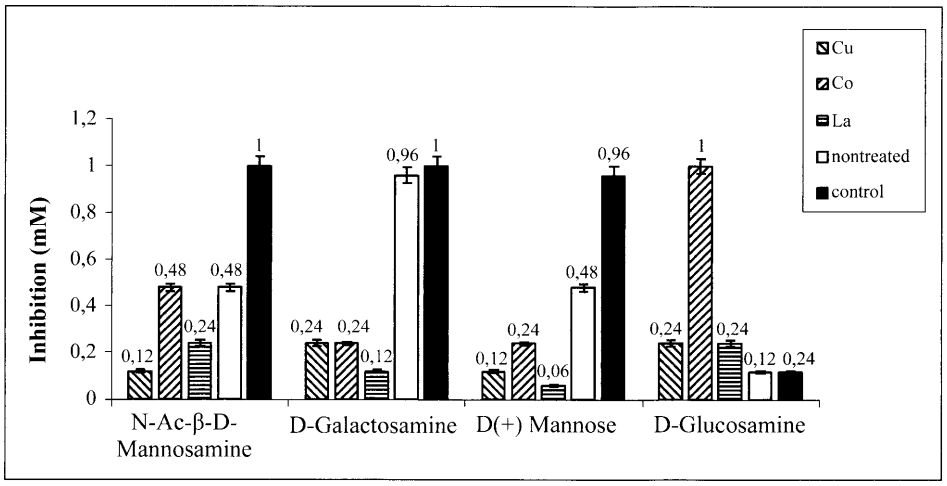

Fig. 1. Inhibition of haemagglutination with N-Ac-β-D-mannosamine, D-galactosamine, mannose and D-glucosamine between rat erythrocytes and culture media from MCF-7 breast adenocarcinoma cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$ (Cu), $Co(Chol)_2.2H_2O$ (Co) and $La(Chol)_3\ 2H_2O$ (La) complexes of the cholic acid. Culture media from non-treated cells (nontreated), only culture media (control).

N-Ac-D-glucosamine-binding proteins: N-Ac-D-glucosamine CBPs were upregulated in treated MCF-7, as compared to their levels in the control, see Fig. 2. Culture media from MCF-7 tumor cells treated with 100 μg/ml $La(Chol)_3\ 2H_2O$ had higher levels of this carbohydrate-binding protein, as compared to tumor cells treated with $Cu(Chol)_2.4H_2O$ and $Co(Col)_2.2H_2O$.

Galactose-binding proteins: We found that the levels of carbohydrate-binding proteins (CBPs) with specificity towards galactose were higher in culture media from MCF-7 cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$ and $La(Chol)_3\ 2H_2O$, as compared to their levels in culture medium from cells without treatment, see Fig. 2. The highest levels of expression of this CBP were detected in the tumor cell treated with $Cu(Chol)_2.4H_2O$.

Fucose-binding proteins: D-fucose CBPs were upregulated in treated cells, compared to non-treated, see Fig. 2. MCF-7 cells treated with $La(Chol)_3\ 2H_2O$ had the highest secretion of fucose-binding proteins in the culture media.

Mannosamine-binding proteins: Mannosamine-specific carbohydrate-binding proteins were found to be down regulated in MCF-7 cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$, $Co(Chol)_2.2H_2O$, as compared to the levels of these CBPs in non-treated cells, see Fig. 2. $La(Chol)_3\ 2H_2O$ treated cells had the highest expression of mannosamine-binding proteins.

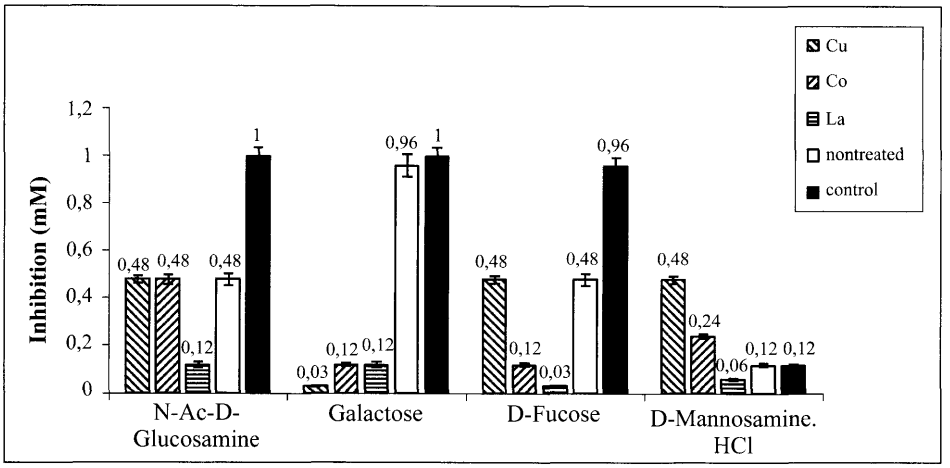

Fig. 2. Inhibition of haemagglutination with N-Ac-D-glucosamine, galactose, D-fucose and D-mannosamine. HCl between rat erythrocytes and culture media from MCF-7 breast adenocarcinoma cells treated with 100 μg/ml $Cu(Chol)_2.4H_2O$ (Cu), $Co(Chol)_2.2H_2O$ (Co) and $La(Chol)_3$ $2H_2O$ (La) complexes of the cholic acid. Culture media from non-treated cells (nontreated), only culture media (control)

Discussion

Carbohydrate-binding proteins (CBPs) are usually up-regulated in many tumor cell lines. CBPs are involved in biological events concerning tumor behavior such as homo- and heterotypic cell adhesions and adhesion to the extracellular matrix (ECM). The aim of our study was to evaluate expression and secretion of carbohydrate-binding proteins in culture media from MCF-7 adenocarcinoma cells treated with metal complexes of the cholic acid. To our knowledge there are no data on the influence of cholic acid and its metal complexes on expression of tumor associated carbohydrate-binding proteins.

We followed up expression of carbohydare-binding proteins with different specificities in Guerin tumor cells treated with metal complexes of the cholic acid. For N-Ac-β–D-mannosamine-binding proteins we found that $Cu(Chol)_2.4H_2O$ complex is most potent effector for upregulation of these proteins. On the other hand D-galactosamine-binding proteins were upregulated after treatment with $La(Chol)_3$ $2H_2O$ complex. Similar to D-galactosamine-binding proteins, D-mannose-binding proteins were also upregulated after treatment with $La(Chol)_3$ $2H_2O$ complex. Levels of expression of D-glucosamine-binding proteins were higher after treatment with $Co(Chol)_2.2H_2O$ complex. Upregulation of N-Ac-D-glucosamine-binding proteins was also observed after treatment of tumor cells with $La(Chol)_3$ $2H_2O$ complex. Expression of Galactose-binding proteins, on the other hand, was affected by all three of the investigated metal complexes. Treatment with $La(Chol)_3$ $2H_2O$ complex of cholic acid led to upregulation of Fucose-binding proteins and Mannosamine-binding proteins.

In conclusion we can say that $La(Chol)_3$ $2H_2O$ complex of cholic acid is the most potent effector leading to upregulation of five of all 8 investigated carbohydrate-binding proteins. Following-up expression of CBPs can be helpful to assess treatment of tumor cells. Combined with measuring of the proliferation index expression of these proteins can be used to follow effectiveness of anti-cancer drugs.

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In vitro-cultivation of human oral mucosa epithelial cells and tissue explants as a modern method for applications in therapy of limbal stem cell deficiency. A pilot study

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Possibilities for application of oral mucosa epithelial cells and tissue explants for development of novel therapeutic strategies in ocular limbal stem cell deficiency were examined. For this goal, because of the proved expression of limbal epithelial stem cell markers, epithelial stem cells and tissue explants from human oral mucosa were *in vitro*-cultivated in appropriate laboratory conditions. Techniques for substrate adhesion of the isolated cells and tissue explants from human oral mucosa on glass or plastic lamella, previously treated with poly-L-Lysine, with gelatine and with Fetal Calf Serum (FCS), respectively, were tested. All cells were characterized on the basis of their morphological characteristics: shape, presence or appearance of mitotic figures, as well as confluence and adherence on the substrate used. Formation of both adherent and non-adherent cell sheets, consisting of cells with different morphology and maturation degree, was observed. Future experiments in this direction should be connected mainly with cultivation of oral mucosa tissue explants and epithelial cells, on a bio-membrane in its role of appropriate biological substrates, as well as with proof of specific markers in them, about eventual possibilities for future applications in construction of implants for the needs of reparative ophthalmology.

Key words: human oral mucosa, epithelial stem/progenitor cells, tissue explants, *in vitro*-cultivation.

Introduction

The concept of limbal stem cells (LSCs) has been imposed from the combined presence in them of markers for cell differentiation (as Keratins K3, K12, Connexin Cx43, etc.), as well as of stem cell markers (Keratin K19, ABCG2, protein p63, Vimentin, Nestin, Integrins 1 and α9, Enolase) [6, 16-18]. Those cells are localized in the so named ***limbus*** (***Limbus corneae***). In the normal ocular surface it has been characterized as covered of highly specialized cells [2-4, 7, 9, 15, 16]. Respectively, the improvement and development of novel therapeutic strategies is necessary in the treatment of limbal stem cell deficiency (LSCD) [8, 13-17], which could be a result of *Stevens-Johnson* syndrome (SJS), ocular cicatricial pemphigoid, as well as different types of mechanical, physical

and/or chemical injury [11]. As a potentially hopeful method in this aspect, the application of oral mucosa epithelium as a source of epithelial stem cells, has been discussed [2, 8, 13-17].

Cultivated autologous oral mucosal epithelial transplantation has been characterized as successful tissue-engineering technique for generation of autologous epithelial cells and/or tissue explants for therapeutic practice, and, in particular, in reconstructing the ocular surface in different cases of LSCD [11, 14, 20]. Analogically to the normal ocular surface, the normal oral cavity has been found to contain several different types of stratified squamous epithelia, including as nonkeratinized, parakeratinized and orthokeratinized [10, 20]. The longevity of epithelial cell cultures, derived from normal, nonpathologic oral mucosa, has been described as dependent of the length of time in culture or of the number of passages and population doublings [10].

In this direction, the main idea was connected with initial studies on development of novel methods for laboratory cultivation of tissue explants and cells from human oral mucosa, for eventual effective and safe treatment in different cases of LSCD.

Materials and Methods

Different combinations of the growth media Dulbecco's Modified Minimal Essential Medium (DMEM) and Ham's or of DMEM and F12 were used. Those media mixtures were supplemented with 10% Fetal Bovine Serum (FBS) and antibiotic mixture (100 UI/ml Penicillin, 0.25 mg/ml Streptomycin and 0.25 mg/ml Amphotericin-B). Subsequently, L-Glutamine, 10 ng/ml Epidermal Growth Factor (EGF - Sigma-Aldrich), 5 μg/ml Insulin, 0.4 μg/ml Hydrocortisone, 24 μg/ml Adenine, as well as 2% ml/ml conditioned cultural fluid of previously cultivated in it 3T3 feeder cells (fibroblasts from embryos of Balb/c experimental mice), were added. The isolated cells and tissue explants from human oral mucosa were seeded directly on plastic or glass lamella, previously treated with poly-L-Lysine, Gelatine and/or FCS, respectively, which were put in appropriate dishes for cultivation with liquid growth media, and incubated at 37°C, in incubator with 5% CO_2 and 95% air humidity. The so prepared cultures of cells and tissue explants were observed as native preparations by inverted light microscope (Leica), supplied with mega-pixel CCD-camera.

Results and Discussion

Because of the proved expression of some markers, also indicated in limbal stem cells [3, 6, 10, 11, 14, 15, 19, 20], epithelial cells from oral mucosa were analogically *in vitro*-cultivated.

Cells with different morphology and in different stages of proliferation and maturation were observed, which could be confirmed by the established changes in their shape – round, oval or polygonal, respectively (Fig. 1). Probably, those signs depend on the stage of cell differentiation: round and/or oval cells (Fig. 1A, B), cell sheets, composed mainly of undifferentiated cells with round and/or oval shape (Fig. 1B) and clusters, composed mainly of more differentiated polygonal cells and small amounts of early epithelial progenitors (Fig. 1D, E). These features were observed in use of the three different types of substrates for seeding of the cells.

In seeding of tissue explants from human oral mucosa, gradual separation of smaller tissue fragments, composed mainly of undifferentiated cells with round and/or oval form, could be seen (Fig. 2). Here again these characteristics were present inde-

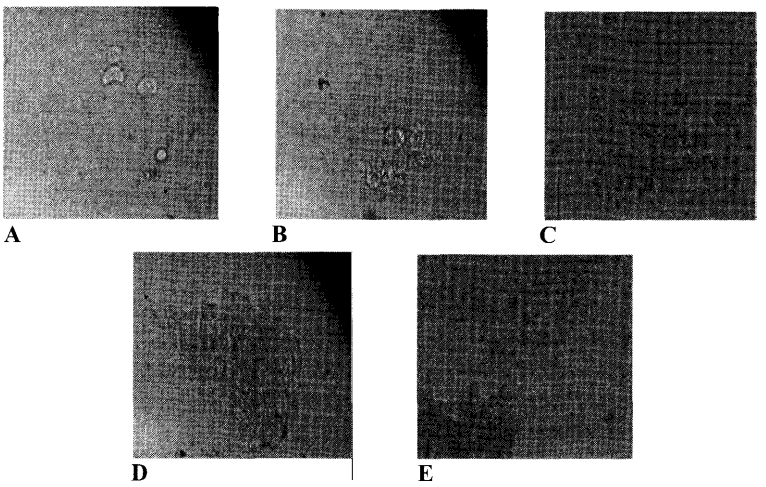

Fig. 1. Human oral mucosa epithelial cells in different phases of proliferation and differentiation: in early phases of differentiation, characterizing with round and oval shape (A) and (B); cell sheets, composed of many actively proliferating early cell progenitors in different sub-stages, characterizing with round and oval shape, but only few amounts of more differentiated cells with polygonal shape could be seen (C); cell sheets, composed mainly of mature epithelial cells with polygonal form (D) and (E) (Native preparations)

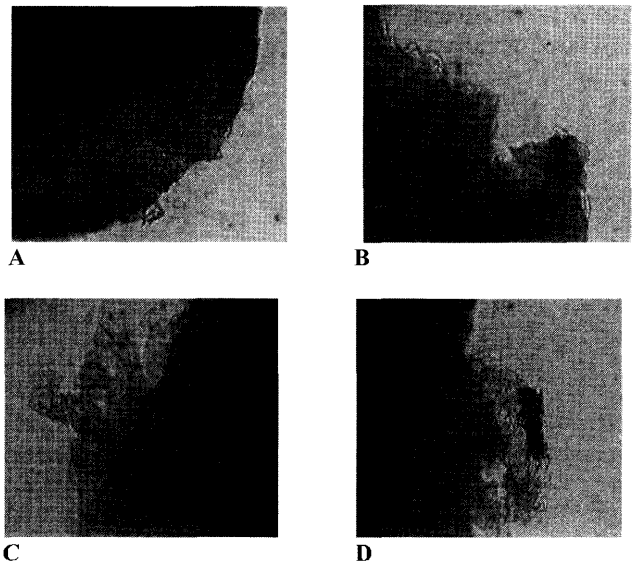

Fig. 2. Tissue explants from human oral mucosa epithelium on different hours from their direct seeding in liquid growth medium: Gradual separation of smaller tissue fragments, composed mainly of undifferentiated cell progenitors cells with round and oval form, could be seen (Native preparations)

pendently of the used substrate. Those our results were in agreement with the literature findings about the proved different types of stratified epithelia in the oral mucosa zone (Fig. 2A) [10]. The noticed increase in the sub-populations from the separate cells in the tissue explants in the time was accepted as a proof for their strong proliferation capacity (Fig. 2B-D).

Conclusion

In *in vitro*-incubation of cells and tissue explants from human oral mucosa, cells with different shape and morphology, in different stages of proliferation and differentiation were noted. A proof for their strong proliferation capacity was the observed increase in the cell sub-populations.

Future studies, connected particularly with proof of limbal stem cell markers in the so cultivated tissue explants and epithelial cells from oral mucosa, but also of techniques for their laboratory cultivation on appropriate substrates for the needs of reparative ophthalmology, are necessary.

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Treatment of the *Graffi* Tumor in Hamsters Using Plasmonically Activated Gold Nanoparticles

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Summary: Local application of heat is a well-known concept in therapeutic medicine that has been explored extensively for the treatment of cancer and other conditions. This study has been designed to determine the photothermal properties of plasmonically heated gold nanoparticles (GNPs) in vivo, using experimental animal model - solid myeloid *Graffi* tumor in hamsters. Combining cytochemical, biochemical and histopathological methods we found that combination of GNPs (40 nm and 100 nm) and laser treatment with different characteristics of the laser beam resulted in localized heating and causing local destruction of the tumor tissue, prolonged survival rate and mean survival time of the tumor bearing animals. This study demonstrates that GNPs are a novel class of photothermal agents which cause cell injury and death through conversion of absorbed light to thermal energy.

Keywords: gold nanoparticles, photothermal therapy, *Graffi* tumor

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Introduction

The revolution in cancer therapy has taken place by emerging use of laser light to achieve controlled and confined thermal damage in the tumor tissue. Laser is an optical source that emits photons in a coherent and narrow beam [1]. Noble metal nanoparticles have become very useful as agents for photothermal therapy of their enhanced absorption cross sections, which are four to five orders of magnitude larger than those offered by conventional photoabsorbing dyes. This strong absorption ensures effective laser therapy at relatively lower energies rendering the therapy method minimally

invasive. Irradiation with short laser pulses has been shown to lead to rapid heating of the particles and vaporization of thin layer of fluid surrounding each particle, producing microscopic explosions and bubble formation [3-5, 7-9]. Clusters formed by the assembly of gold nanoparticles enhance the bubble formation, causing more efficient cancer cell killing [8].

Aim

The aim of the present study is to elucidate the effects of local application of gold nanoparticles in combination with laser beam irradiation on parameters of the tumor growth and histopathological evaluation of the tumor tissue damage.

Materials and Methods

Golden Syrian hamsters, 2-4 months old, weighing approximately 100 g were purchased from a breeding base Oncology Center, Sofia. The animals were divided into experimental groups and were kept under standard conditions in individual plastic cages with free access to food and water. All studies were performed in accordance with the Guide for Care and Use of Laboratory Animals, as proposed by the Committee on Care Laboratory Animal Resources, Commission on Life Sciences and National Research Council. An experimental *Graffi* myeloid tumor was created and maintained monthly in vivo by subcutaneous transplantation of live tumor cells by method described by Toskova et al., 2008 [6]. Spontaneous regression in this experimental tumor model was not observed. The tumors were irradiated using Nd-YAG laser at λ = 532 nm, pulse duration τ p= 15 ns and repetition rate 1 Hz. Gold nanoparticles (GNP) with diameters of 40 nm and 100 nm (BBInternational, Cardiff, UK) were used as colloid solutions without surfactants, stabilizers or enhancers. Changes in tumor volume and mean survival time (MST) of tumor-bearing hamsters after combination laser/GNP therapy were followed. Untreated tumor-bearing and healthy animals were used as controls. Samples of tumor tissue were selected for histopathological studies. They were obtained from animals from each experimental group and were processed and stained with haematoxylin-eosin according to the standard histological technique. At the 72nd hour after treatment, experimental tumor bearing animals from different groups were euthanized. The solid tumors were dissected and selected parts were immediately fixed for 48 hours in 10% phosphate buffered formalin pH 7.2 (end formalin concentration was 3,8 – 4%), included in paraffin and cut in sections of 4 μm. Representative histological sections were stained with hematoxylin-eosin.

Some experiments were carried out aimed to clarify whether inhibition of proliferation of *Graffi* tumor cells takes place through apoptosis. For this purpose primary culture of *Graffi* tumor cells were cultured for 4 h on coverslips, then colloid gold was added to each sample in end concentration 10 μg/mL and cells were cultured for 24 hours to ensure the passive transport of the GNPs into the tumor cells. Cells were irradiated with Nd-Yag laser system with parameters of the laser beam depending on the requirements of the experiment. After two hours fluorescent analysis was performed. AO stains both viable and dead cells emitting strong green fluorescence, as a result of intercalation between the bases of double-stranded DNA and red-orange fluorescence after binding to single-stranded RNA [2]. In contrast, PI is a fluorochrome which does not stain viable cells with intact cell membrane. It stains the dead and late apoptotic cells with altered cell membrane permeability.

Results and discussion

Morphological changes in *Graffi* tumor cells treated with gold nanoparticles and irradiated with laser beam with different energies observed with fluorescent microscopy are shown in Fig. 1. The nucleus of the untreated *Graffi* cells showed homogenous fluorescence with no signs of segmentation and fragmentation. Cells treated with gold nanoparticles and irradiated exhibited different signs of early and late apoptosis. The obtained results support the claim that the combination of gold nanoparticles (40 nm and 100 nm) and laser irradiation induces death of *Graffi* tumor cells through apoptosis.

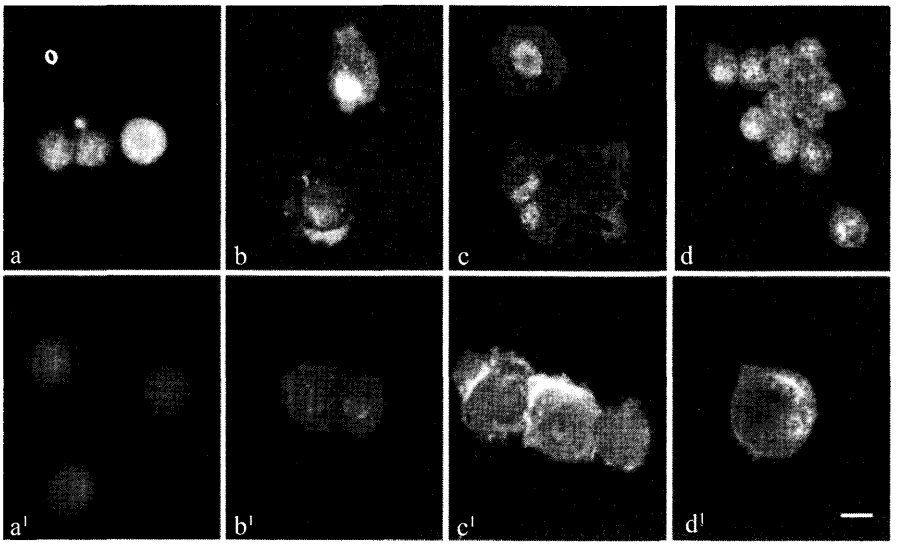

Fig. 1. Fluorescent micrography of *Graffi* tumor cells, stained with acridine orange and ethidium bromide. Bar = 20 μm

The combination of GNP and laser therapy on hamsters with *Graffi* tumor showed temporary positive effect on the metric parameters of the tumor growth, expressed in reduction of tumor volume and prolonged mean survival time (data not shown).

The results observed in native scanned histological preparations showed that at the 72nd hour after treatment (Fig. 2) a narrow zone of necrotic effect in the tumor tissue. This zone lays on the axis of action of the laser beam and is well pronounced when the tumor was treated with 40 nm gold nanoparticles, while in tumors treated with 100 nm nanoparticles this zone of destruction is much wider (Fig, 2A). At the 7th day after the treatment the zone of necrotic alterations in the tumor tissue is unclear due to the lateral growth of the tumor tissue, remained unaffected from the photodynamic therapy (Fig. 2B). In the cases with small tumor formations the neoplastic tissue was totally destructed (data not shown).

The treatment of *Graffi* tumor bearing animals with gold nanoparticles and laser irradiation induces pathomorphological changes in the zone of treatment, shown in Figure 3. These changes detected by pathohistological methods could be classified in the following zones:

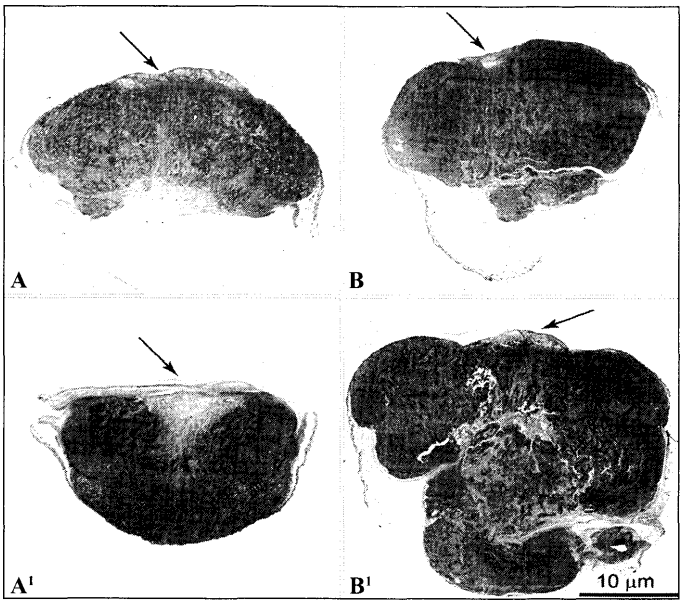

Fig. 2. Solid *Graffi* tumor in hamsters at 3rd (A, A¹) and 7th (B, B¹) day after combined treatment with nanoparticles and laser. (A, B) – Zone of tumoricide effect on the neoplastic tissue (arrow) after treatment with gold nanoparticles (40 nm) and laser (80 mJ/cm²) at 3rd and 7th day respectively; (A¹, B¹) – Zone of tumoricide effect on the neoplastic tissue (arrow) after treatment with nanoparticles (100 nm) and laser (80 mJ/cm²) at 3rd and 7th day respectively. Scanned native histological preparations

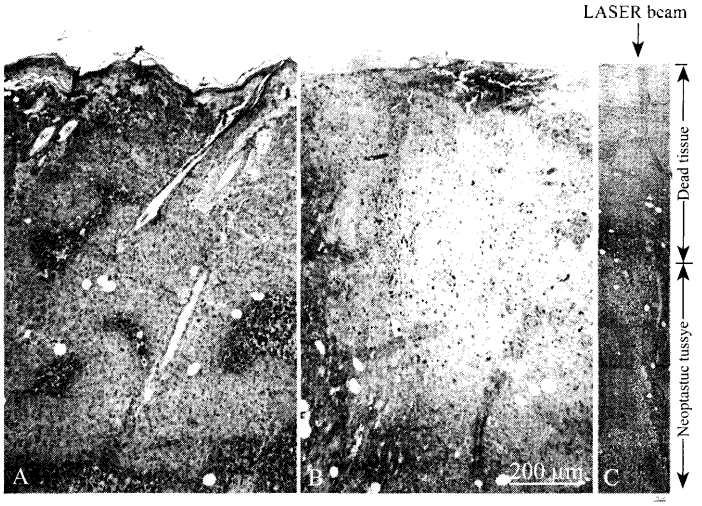

Fig. 3. Solid myeloid *Graffi* tumor in hamster, inoculated with gold nanoparticles (40 nm). (A) – untreated surface with intact structure; (B) – totally destructed skin and neoplastic tissue in laser treated tumor; (C) from top to bottom – skin, dead tumor tissue and viable neoplastic tissue. Hematoxylin- eosin

a. Superficial zone of total necrotic tissue (SZTNT) – necrotic tumor cells as cell debris with nuclear fragments or entirely lytic cells with pale nuclei (Fig. 4a);
b. Middle superficial zone of necrotic tissue (MSZNT) – lytic cells and basophilic agglomerations. (Fig. 4b);
c. Middle deep zone of necrotic tissue (MDZNT) – tumor necrotic tissue, hemorrhages (Hrrg) and inflammatory mononuclear cells (Mo) (Fig. 4c);
d. Zone of deep necrotic tissue (ZDNT) – necrotic tissue with inflammatory mononuclear cells (Mo) (Fig. 4d);
e. Zone of deep neoplastic tissue (ZDNeoT) and Zone of lateral neoplastic tissue (ZLNeoT) – neoplastic tissue with the specific characteristics of myeloid *Graffi* tumor in hamsters (Fig. 4e).

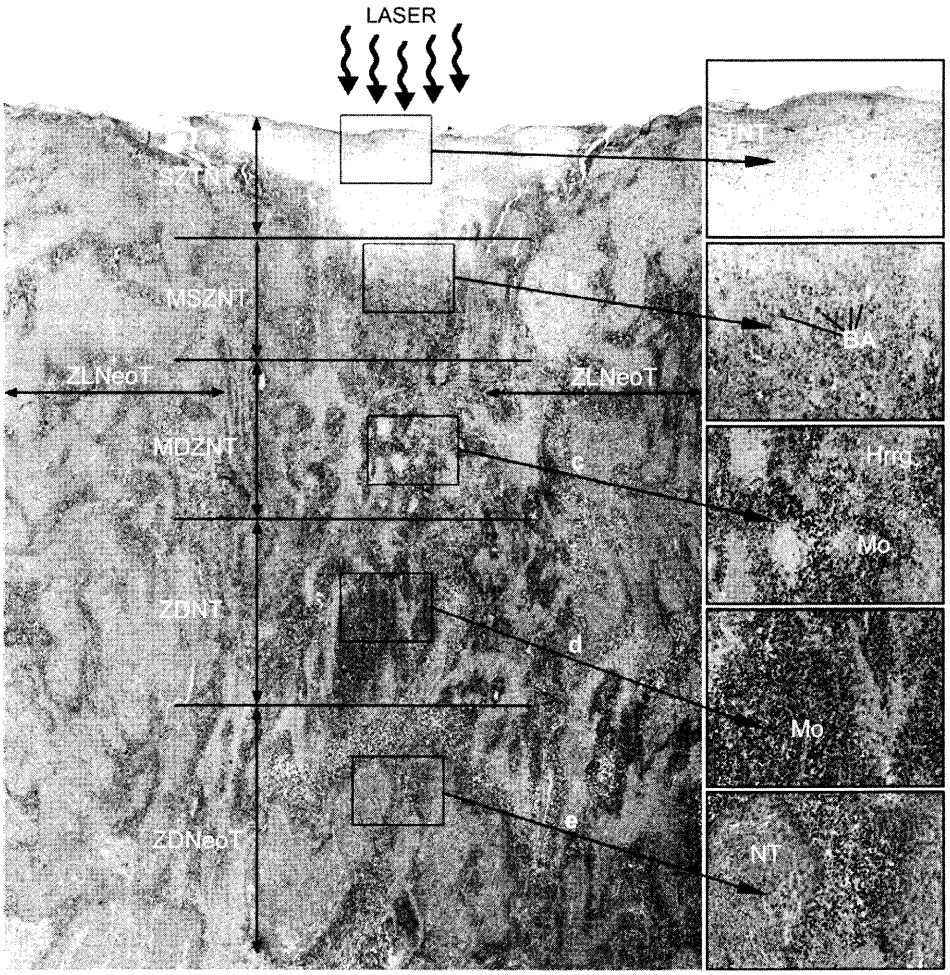

Fig. 4. Classification of the tissue lesions detected in solid myeloid *Graffi* tumor in hamsters after local treatment with gold nanoparticles (40nm) and laser irradiation: (a) SZTNT – Superficial zone of total necrotic tissue; (b) MSZNT - Middle superficial zone of necrotic tissue; (c) MDZNT – Middle deep zone of necrotic tissue; (d) ZDNT – Zone of deep necrotic tissue; (e) ZDNeoT and ZLNeoT – Zone of deep neoplastic tissue and Zone of lateral neoplastic tissue. Hematoxylin-eosin

The conducted studies showed that the combination of treatment with gold nanoparticles and laser effectively suppressed the tumor tissue growth and had temporary positive effects on the reduction of tumor cell mass within the solid tumors. Pathohistological studies clearly highlighted separate zones of nanothermolysis in the tumor tissue, which could help to improve the parameters of the nanoparticles and laser system in future experiments, aiming the optimal conditions for total destruction of the tumor cells in lateral and deep zones. These neoplastic cells remained viable which allowed the lateral tumor growth and explained the temporary inhibition on the tumor growth.

The results obtained showed that application of plasmonically activated gold nanoparticles for *in vivo* treatment of *Graffi* tumor in hamsters demonstrate considerable antitumor effect and have the potential to be used for local treatment of small solid tumors.

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Anthropology

Metrical characterization and bilateral asymmetry of human zygomatic bone (craniometrical study)

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Abstract: Zygomatic bone is situated in the upper and medial section of the facial part of the skull and is of great importance for its shape. Nevertheless, metrical data concerning dimensions, proportions and bilateral asymmetry of the zygomatic bone are not comprehensive enough. On the other hand, it is hard or completely impossible to compare data given from different authors. In this study a total of 125 adult male skulls were investigated to perform a detailed characterization of zygomatic bone as well as to evaluate the manifestation of bilateral asymmetry. Seven linear features were measured separately on both sides. Quantitative assessment of the bilateral asymmetry and converting the absolute asymmetry values into relative values was performed using the Index of Asymmetry (IA). According to our results, the left zygomatic bone is larger as a whole, while the right one is more projected with higher lateral surface and significantly wider frontal process.

Keywords: zygomatic bone, metrical characterization, bilateral asymmetry

Introduction

Zygomatic bone is situated in the upper and medial section of the facial part of the skull and is of great importance for its shape. The zygomatic bone forms the prominences of the cheek and separates the orbit from the temporal fossa. It articulates with the maxilla, the greater wing of the sphenoid, and the zygomatic processes of the frontal and temporal bones. It is irregular in shape and has three surfaces, two processes and five borders [18]. The strongly prominent and situated more parallel to the frontal plane zygomatic bones contribute to enlargement of the face breadth.

Conversely, when the bones are less prominent and situated more sagittal the face looks narrower and gracile [27]. The size and curvature of the bone varies greatly in different population, being smaller and flatter in Caucasian skulls and larger and more curved in Mongoloid race [1, 23, 24].

Knowledge about human skull asymmetry in normal dry specimens is useful as a parameter for medical and dentistry practice. The caliper direct method for evaluation of the bilateral asymmetry is a reliable technique used in anatomical and anthropological studies [17]. Fazekas & Kósa [5] recorded the length and width of the bone during fetal life. Moss, Noback & Robertson [13] also recorded length and height of the bone from 8 to 20 weeks but landmarks are not defined. Nevertheless, metrical data concerning the dimensions, proportions and bilateral asymmetry of the zygomatic bone are not comprehensive enough. On the other hand it is hard or completely impossible to compare data given from different authors. This difficult comes from insufficient standard dimensions for this bone and the various measurements between different craniometrical points used by the researchers. In this context the aim of the study is to perform a detailed metrical characterization of zygomatic bone as well as to evaluate the manifestation of bilateral asymmetry.

Material and Methods

A total of 125 male skulls from the ossuary at the National Museum of Military History, Sofia, were studied. The skulls belong to adult male individuals.

For quantification of craniofacial asymmetry are used measurements for the right and left sides separately. The differences between homologous measurements supply information about the dominant side. This method provides good information about side differences and local imbalance [7].

For the aim of this study were measured (in millimeters) bilaterally seven linear features between standard craniometrical points by Martin & Saller [11]:

1. Total height of zygomatic bone (*zm – fmt*). The linear distance between the landmarks *zygomaxillare* and *frontomalare temporale* (Fig. 1), sliding caliper.

2. Height of lateral (external) surface of zygomatic bone (*zm – ju*). The linear distance between the landmarks *zygomaxillare* and *jugale* (Fig. 1), sliding caliper.

3. Height of frontal process of zygomatic bone (*ju – fmt*). The linear distance between the landmarks *jugale* and *frontomalare temporale* (Fig. 1), sliding caliper.

4. Breadth of frontal process of zygomatic bone (*fmt – fmo*). The linear distance between the landmarks *frontomalare temporale* and *frontomalare orbitale* (Fig. 1), sliding caliper.

5. Arc of zygomatic bone, after Alekseev and Debetz [26]. The least distance on the zygomatic bone surface between the landmarks, in which the breadth of zygomatic bone is measured, tape.

6. Projection of zygomatic bone, after Alekseev and Debetz [26]. The greatest perpendicular, pulled down from the line of zygomatic bone breadth to the surface of the bone, coordinate caliper.

For more detailed metrical characterization and assessment of the bilateral asymmetry were included data for the breadth of zygomatic bone, obtained in our previous study on the same material [14]:

7. Breadth of zygomatic bone, chord – after Alekseev and Debetz [26]. The direct distance from the lowest point situated on temporozygomatic suture (at a transition from the lateral surface to the inferior surface of zygomatic arch) to the crossing point of zygomaticomaxillary suture with the lower rim of the orbit (Fig. 1), sliding caliper.

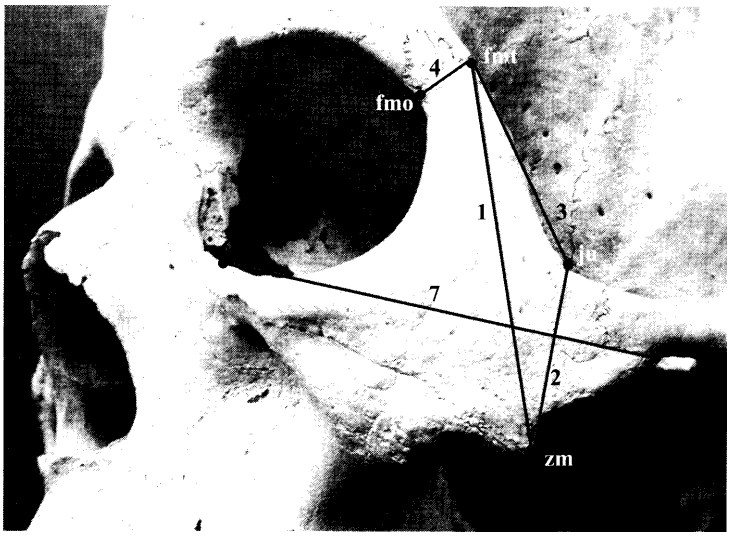

Fig. 1. Measurements of zygomatic bone: 1. total height of zygomatic bone (*zm – fmt*); 2. height of lateral surface of zygomatic bone (*zm – ju*); 3. height of frontal process of zygomatic bone (*ju – fmt*); 4. breadth of frontal process of zygomatic bone (*fmt – fmo*); 7. breadth of zygomatic bone, chord

In the present study any difference between the homologous distances of the right and left sides is considered as an asymmetry. Quantitative assessment of the bilateral asymmetry and converting the absolute asymmetry values into relative values were performed using Wolanski's index for intergroup comparison [21]. In this case, the index was used to evaluate the bilateral asymmetry and is referred to as Index of Asymmetry (IA): IA = $[2.(x_d - x_s).100]/(x_d + x_s)$, x_d – value of the measurement on the right side; x_s – value of the measurement on the left side.

The sign of the resulting IA value designates the direction of bilateral asymmetry; "-" signifies the left side priority and "+" signifies the right side priority. IA is calculated individually and the given data represent the mean from the individual values.

Results

The biostatistical data are presented in Table 1.
The differences between both sides are expressed in index units (IU). The lowest value found is -0,68 IU and the highest is 4,31 IU, which may be considered as significant. Moreover, four of all investigated measurements show left side dominance. The left zygomatic bone is higher (-0,68 IU), it is also wider, with larger arc (-0,54 IU) and chord (-0,18 IU), and with insignificantly higher frontal process (-0,02 IU). The rest three measurements show priority for the right side. The right zygomatic bone is more projected (0,25 IU), with relatively higher lateral surface (0,77 IU) and with significantly wider frontal process (4,31 IU). Standard deviations show that the homogeneity of each measurement is similar with exception for the projection and the height of the frontal process of zygomatic bone, which are more dispersed. Nevertheless, Student's t-test shows that the breadth of frontal process is the only measurement, which displays statistically significant bilateral difference at $p < 0,05$ with priority for the right side.

Table 1. Biostatistical data about the investigated linear features of zygomatic bone

No	Measurements of zygomatic bone	Right						Left						p	IA	
		n	mean	SEM	SD	min	max	n	mean	SEM	SD	min	max		IU	SD
1	Total height (zm-fmt)	125	46,96	0,27	3,04	38,50	54,50	125	47,28	0,28	3,11	39.00	55.00	0.41	- 0.68	3.01
2	Height (zm-ju)	124	27,54	0,25	2,78	18,00	37,00	125	27,32	0,25	2,74	19.00	37.50	0.52	0.77	3.77
3	Height of frontal process (ju-fmt)	124	25,40	0,20	2,27	20,00	32,00	125	25,38	0,20	2,27	20.00	30.00	0.95	- 0.02	5.05
4	Breadth of frontal process (fmt-fmo)	125	6,71	0,09	1,01	4,00	9,00	125	6,42	0,09	0,95	4.00	8.50	0.02*	4.31	1.50
5	Breadth - arc	122	58,56	0,45	4,94	44,00	70,00	125	58,92	0,43	4,82	47.00	70.00	0.56	- 0.54	1.15
6	Projection	123	10,54	0,15	1,66	6,50	15,00	125	10,52	0,15	1,67	6.00	14.50	0.94	0.25	6.15
7	Breadth - chord	123	53,13	0,35	3,89	40,00	63,00	125	53,22	0,33	3,67	43.00	63.00	0.86	- 0.18	0.2

* statistically significant difference at $p < 0,05$

Discussion

Working on skulls, Woo [22] found that the left zygomatic bone was predominant. In their monograph, Kadanov and Mutafov [28] cited Ludwig (1932), that the left side was larger than the right side and the left zygomatic bone was more projected forward compared to the right one. In accordance with their own data from metrical investigation of contemporary cranial series, Kadanov and Mutafov [28] established that the middle section of the facial part of the skull (both zygomatic bone and maxilla) was more often symmetrical in vertical direction compared to horizontal direction. They also concluded that the asymmetry was more often manifested in the breadths than in the heights of that part of cranium. According to our results, the asymmetry was established in the breadths as well as in the heights of the zygomatic bone, but it was manifested to the greatest extend in the breadths (breadth of frontal process 4,31 IU). In other studies it was established that the manifestation and degree of craniofacial asymmetry were most pronounced in the mid-facial section, i.e. in the morphological structure maxilla-zygomatic bone [9, 10]. Kadanov, Yordanov and Aleksandrova [29] figured out the fact, that left half of the facial part was narrower and the entrance to the left orbit was situated more higher compared to the right one. According to our results, the left zygomatic bone was larger as a whole, while the right one was with more massive frontal process and lateral surface and more projected. The different results of the researchers probably could be explained with the different methods used to determine the craniofacial asymmetry.

In general facial asymmetry can be summarized and divided into three main categories, 1 – congenital, originating prenatally; 2 – developmental, arising during growth with inconspicuous etiology; and 3 – acquired, resulting from injury or disease. Based on the craniofacial structures involved, facial asymmetry can be classified into dental, skeletal, soft tissue and functional components. Skeletal asymmetry may involve one bone or it may affect a number of skeletal structures on one side of the face [4]. Normal asymmetry in the area of the craniofacial skeleton can be directional or fluctuating in nature. Directionality can in principle be found in three dimensions: anteroposterior, cranio-caudal, and asymmetries in the left-right dimension. Fluctuating asymmetry is another type of asymmetry normally found in the craniofacial structures, where the side of the larger and smaller paired structure is randomly determined [15].

Perfect bilateral symmetry in the body is basically a theoretical concept that rarely exists in live organisms [2]. The human skull is definitely asymmetrical, this is not a matter of skull bones that differ individually from a symmetrical model, but the skull is asymmetric as a whole. Some dimensions of the skull bones are prominent on the right side and some on the left [3, 22]. Knowledge of quantitative normal cranial asymmetry in a population without pathology or functional disturbance is necessary to avoid malpractice [17].

There is no consensus in the literature on the degree, side and spatial localization of facial asymmetry [7, 25]. A mild degree of asymmetry is common in the face of normal and healthy individuals. The point where the "normal" asymmetry becomes "abnormal" cannot be easily defined [2]. According to Rossi, Ribeiro & Smith [16] the larger the asymmetry, the more the attention it has to be given because one may be nearer to a pathological condition. Nevertheless, there are no clearly defined limits to determine certain difference between homologous measurements of both sides as an asymmetry. Some authors considered that asymmetry existed when the means of the differences between the sides were statistically different from zero. Others used the Student's t-test for paired samples to consider the differences between the right and left sides as asymmetries or considered as asymmetry when the differences between measurements of the

right and left sides were 2 mm or larger. This limit was chosen arbitrarily and turns out to be variable because it depends on the skull size [16].

In general facial asymmetry affects the lower face more frequently than the upper face [4]. Severt & Proffit [19] reported frequencies of facial laterality of 5%, 36% and 74% in the upper, middle and lower thirds of the face. Furthermore, in the literature there is no agreement about the side of dominance. Using the different evaluation methods for assessment of craniofacial asymmetry, various conclusions were proposed by different researchers. However, it is difficult to compare these studies, since the methods, the measurements and the sample characteristics (sex, age, race) are very different [7]. Nevertheless, some authors conclude that the right side of the face has dominance over the left side [6, 8, 20, 22]. Others established that the left side of the face predominates over the right side [3]. According to Ferrario at al. [7] the two side of face showed significant differences in shape, but no differences in size. Moreover, the soft-tissue cover partly masks the underlying skeletal imbalances, and skeletal asymmetries less than 3% are not clinically discernible [6].

A number of explanations have been given for asymmetry causes, including genetic problems and environmental factors producing differences in the right and left sides [2]. According to Melnik [12], the organism does not favor identical growth of homologous bilateral structures. The difference in the degree of growth between the right and left sides may be caused by genetic factors, environmental factors, or a combination of the two factors. The expression of the craniofacial asymmetry can be related to heredity as well as to the functional activity of the skeletal muscular system, especially of the masticatory apparatus. Therefore, facial asymmetry has been associated with functional activities of the masticatory musculoskeletal system [16].

Because of the key role of zygomatic bone in the structure and aesthetical appearance of the face the evaluation of its bilateral asymmetry is of great importance to the morphologist, anthropologists, medics and in particular to the aesthetic surgeons. We believed that this study complements the knowledge of the zygomatic bone morphology and could be useful in further studies of the facial asymmetry manifestations.

Conclusion

The metrical characterization shows that the breadth of frontal process is the only measurement, which displays statistically significant bilateral difference with priority for the right side. According to IA data, the left zygomatic bone is higher; it is also wider, with larger arc and chord, and with insignificantly higher frontal process. The right zygomatic bone is more projected, with relatively higher lateral surface and with significantly wider frontal process.

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Anthropometrical characteristic of *cavitas glenoidalis* and *caput humeri*

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The study aims to be obtained morphometrical data for *cavitas glenoidalis* and *caput humeri* in order to assess the intensity of the sexual differences and study the metrical relationships between these bone structures. *Cavitas glenoidalis* is measured in 65 male and 65 female scapulae and *caput humeri* – in 65 male and 65 female humeri. Three metrical features of *cavitas glenoidalis* and three metrical features of *caput humeri* are measured. The sexual differences are assessed by Wolansky's index for inter-group comparisons. The metrical data are statistically analyzed by linear correlation analysis as well. According to the results, *cavitas glenoidalis* of male and female scapulae differs most strongly in its depth and *caput humeri* – in its diameters. The correlation matrices show well pronounced dependences between the metrical features of *cavitas glenoidalis* and *caput humeri* separately as well as between the features of both bone structures. However, the relationship between the depth of *cavitas glenoidalis* and the height of *caput humeri* is the slightest one.

Key words: cavitas glenoidalis, caput humeri, metric features, sexual differences, correlations.

Introduction

Cavitas glenoidalis is a shallow articular surface, which is located on the lateral angle of the *scapula* and forms the glenohumeral joint along with *humerus*. This joint accomplishes the greatest mobility of all joints in the body. That determined *cavitas glenoidalis* and *caput humeri* as objects of the current study in order to be obtained morphometrical data for them both to assess the intensity of the sexual differences and study the metrical relationships between these bone structures.

Material and methods

The anthropological investigation is performed on osteological material from archaeological excavations of medieval necropoles in the territory of Northeastern Bulgaria. *Cavitas glenoidalis* was measured in 65 male and 65 female *scapulae* and *caput humeri* – in 65 male and 65 female *humeri*. Only adult skeletons with preserved pairs of *scapu-*

lae and *humeri* were chosen for the analysis. Skeletal sex and age were determined by standard anthropological methods [7, 12, 13].

The anthropological investigation includes three metrical features of *cavitas glenoidalis* and three metrical features of *caput humeri*. Features with numbers in brackets are described by the classical methods of Martin-Saller:

1. Length of *cavitas glenoidalis* (12), LCG – the linear distance between the highest point on the higher margin of *cavitas glenoidalis* and the lowest point on its lower margins, sliding caliper;
2. Breadth of *cavitas glenoidalis* (13), BCG – the linear distance between the outermost points on the lateral margins of *cavitas glenoidalis*, sliding caliper;
3. Depth of *cavitas glenoidalis* (14), DCG – the linear distance from the deepest point of *cavitas glenoidalis* to the line connecting points between which is measured the length of *cavitas glenoidalis*, coordinate caliper;
4. Greatest transversal diameter of *caput humeri* (9), GTDCH – the linear distance between the outermost points on the lateral margins of *caput humeri*, sliding caliper;
5. Greatest sagittal diameter of *caput humeri* (10), GSDCH – the linear distance between the highest point on the higher surface of *caput humeri* and the lowest point on its lower surface, sliding caliper;
6. Height of *caput humeri*, HCH – the distance from the most prominent point of *caput humeri* to the line connecting points between which is measured the sagittal diameter of *caput humeri*, coordinate caliper.

The metric data are statistically analyzed using SPSS version 16,0. The established sexual differences are evaluated by the Student's t-test at $p<0,01$ and $p<0,001$. The quantitative assessment of sexual differences is made by Wolansky's index for intergroup comparisons [11]. The index is used to determine the sexual differences and is called Index for Sexual Differences (ISD): $ISD = 2.(x1 - x2).100/(x1 + x2)$, where x1 is the mean value of the feature in males and x2 is the mean value of the feature in females. The positive values of ISD show sexual differences in favour of the males and the negative ones – sexual differences in favour of the females.

The metrical data are statistically analyzed by linear correlation analysis. The strength of relationships is assessed by the scheme, published by Kalinov [14]: very low correlation ($r \leq 0,30$), low ($r = 0,31 \div 0,50$), moderate ($r = 0,51 \div 0,70$), high ($r = 0,71 \div 0,90$) and very high ($r \geq 0,91$). The significance of the correlations is evaluated at $P<0,05$ and $P<0,01$. The positive sign of correlation coefficient shows that an increase in the value of one variable indicates a similar increase in the value of the second variable. A correlation coefficient of less than 0 indicates a negative correlation.

Results and discussion

Basic statistics on the metrical features of *cavitas glenoidalis* and *caput humeri* are presented in Table 1. All investigated features of *cavitas glenoidalis* and *caput humeri* are larger in the male scapulae. The sexual differences are statistically significant at $p<0,001$ and the depth of *cavitas glenoidalis* in the left scapulae is the only one which shows a difference at $p<0,01$. The average of the length and breadth of *cavitas glenoidalis* in our study are greater than the values reported by Churchill et al. [3], Frutos [4] and Özer et al. [8], which measured these diameters in the male and female scapulae separately.

As far as the bilateral differences are concerned, *cavitas glenoidalis* and *caput humeri* are larger on the right side in both sexes. Our results for the asymmetry of *cavitas*

Table 1. Biostatistic data of measurements of male and female scapulae and humeri

Features	Side	Male			Female			Sexual differences		
		n	$\bar{x}$	SD	n	$\bar{x}$	SD	Absolute difference	t-test	ISD
Length of *cavitas glenoidalis* (12)	right	37	41,8	2,4	33	36,7	2,9	5,1	8,144***	13,0
	left	28	40,4	2,4	32	35,6	2,0	4,8	8,424***	12,6
Breadth of *cavitas glenoidalis* (13)	right	37	29,1	1,7	33	25,3	1,6	3,8	9,324***	14,0
	left	28	28,4	1,7	32	24,8	1,5	3,6	8,610***	13,5
Depth of *cavitas glenoidalis* (14)	right	37	5,0	0,9	33	4,2	0,6	0,8	4,465***	17,4
	left	28	4,7	0,8	32	4,1	0,8	0,6	2,708**	13,6
Greatest transversal diameter of *caput humeri* (9)	right	37	45,1	2,6	33	39,1	2,5	6,0	9,722***	14,3
	left	28	43,7	2,6	32	38,5	2,1	5,2	8,391***	12,7
Greatest sagittal diameter of *caput humeri* (10)	right	37	47,9	2,5	33	41,9	2,6	6,0	9,778***	13,4
	left	28	47,9	3,1	32	41,3	2,3	6,6	9,532***	14,8
Height of *caput humeri*	right	37	18,1	1,5	33	15,9	1,5	2,2	6,109***	12,9
	left	28	17,7	1,9	32	15,6	1,1	2,1	5,416***	12,6

* – $P < 0,05$; ** – $P < 0,01$; *** – $P < 0,001$

glenoidalis, especially about its length, differ from these ones obtained by Mamatha et al. [6], which show greater length of the cavity on the left side, but greater breadth on the right one. But yet Sato and Noriyasu [9] also established that *cavitas glenoidalis* and *caput humeri* are larger on the right side in both sexes. According to them, this appears to point to a wider range of motion in the right shoulder joint.

According to the ISD data in the right and left scapulae, the depth of *cavitas glenoidalis* shows the strongest sexual differences among the features of *cavitas glenoidalis*. It is followed by the breadth of *cavitas glenoidalis* and the last one is the length, which shows slightest sexual differences. The ISD data for the humeral features show that the strongest sexual differences in the right *humeri* are observed for the greatest transversal diameter of *caput humeri*, and in the left ones – for the greatest sagittal diameter. The height of *caput humeri* illustrates the slightest differences on both sides.

Correlations between anthropometric features of *cavitas glenoidalis* and *caput humeri* are presented in Table 2, Table 3, Table 4 and Table 5. The correlation matrices of both sexes show that most of the correlation coefficients are statistically significant, as the significance level in majority of the dependences is high ($P < 0,01$). The results of the comparative analysis of the dependences between the investigated features in both sexes show that only positive correlations are available among the statistically significant dependences.

Table 2. Significance, direction and degree of the correlations between anthropometric features of right male scapulae and humeri

Features	LCG	BCG	DCG	GTDCH	GSDCH	HCH
LCG	1			0.78**		0.25
BCG		1	0.38*	0.71**		0.46**
DCG			1	0.44**	0.09	-0.02
GTDCH				1	0.80**	0.49**
GSDCH					1	
HCH						1
Low degree			High and very high degrees		* P<0.05; ** P<0.01	

Table 3. Significance, direction and degree of the correlations between anthropometric features of left male scapulae and humeri

Features	LCG	BCG	DCG	GTDCH	GSDCH	HCH
LCG	1			0.76**	0.72**	0.46*
BCG		1	0.31*			0.50**
DCG			1	0.44**	0.36	-0.01
GTDCH				1	0.85**	0.47*
GSDCH					1	
HCH						1
Low degree			High and very high degrees		* P<0.05; ** P<0.01	

Table 4. Significance, direction and degree of the correlations between anthropometric features of right female scapulae and humeri

Features	L*CG*	B*CG*	D*CG*	GTD*CH*	GSD*CH*	H*CH*
L*CG*	1	0.84**	0.55**	0.76**	0.67**	0.50*
B*CG*		1	0.52*	0.73**	0.71**	0.62**
D*CG*			1	0.21**	0.15	0.17
GTD*CH*				1	0.86**	0.58**
GSD*CH*					1	0.81**
H*CH*						1
Low degree		Moderate degree		High and very high degrees		* P<0.05; ** P<0.01

Table 5. Significance, direction and degree of the correlations between anthropometric features of left female scapulae and humeri

Features	L*CG*	B*CG*	D*CG*	GTD*CH*	GSD*CH*	H*CH*
L*CG*	1	0.61**	0.05*	0.36*	0.26*	0.29
B*CG*		1	0.25*	0.30	0.26*	0.50**
D*CG*			1	0.14	0.37*	0.44**
GTD*CH*				1	0.35*	0.24
GSD*CH*					1	0.81**
H*CH*						1
Low degree		Moderate degree		High and very high degrees		* P<0.05; ** P<0.01

The features of *cavitas glenoidalis* in both sexes correlate with each other predominantly of a moderate degree. The relationship between the breadth and depth of *cavitas glenoidalis* in the male *scapulae* is an exception and the degree is "low" on the both sides. A different degree is also found in the relationship between the length and breadth of *cavitas glenoidalis* in the right female scapulae, but it is "high". According to Bukov et al. [1], the length and breadth of *cavitas glenoidalis* correlate of a high degree, and the dependences between these lengthwise features and the height of *cavitas glenoidalis* are of low and moderate degrees, which resembles to our results, although they have not studied male and female *scapulae* separately.

The correlations between the features of *caput humeri* in both right and left male humeri show that both diameters correlate of a high degree, the sagittal diameter and the height correlate of a moderate degree and the transversal diameter and the height – of a low degree. In the right female *humeri*, the sagittal diameter correlates with the other two features of a high degree, and the transversal diameter and the height correlate of a moderate degree. Bukov et al. [2] also established a high correlation between both diameters of *caput humeri*. The correlations in the left female bones are lower, and the transversal diameter depends on the other two features of a low degree; the sagittal diameter and the height correlate of a moderate degree. Similar results for correlations between features of *caput humeri* are reported in our previous study, devoted to the correlations between humeral length and features of the proximal humeral end [10].

The results obtained for the dependences between the features of *cavitas glenoidalis* and these ones of *caput humeri* are the most interesting in order to be studied the metrical relationships between these bone structures. The length and breadth of *cavitas glenoidalis* and both diameters of *caput humeri* in male bones correlate strongly and the degrees are "moderate" and "high". Such degrees are observed in the right bones of female skeletons. But in the left ones, a moderate degree is found only between the length and breadth of *cavitas glenoidalis* and the sagittal diameter of *caput humeri*; the correlations with the transversal diameter are of a low degree.

The length of *cavitas glenoidalis* and the height of *caput humeri* correlate with each other comparatively poorly. The breadth of *cavitas glenoidalis* and the height show slightly higher correlation coefficients, which are of a moderate degree or on the border between the categories "low" and "moderate".

The correlations between the depth of *cavitas glenoidalis* and both diameters of *caput humeri* are low or very low, and only the correlation with the transversal diameter in the male bones and this one with the sagittal diameter in the left female bones are statistically significant. The relationship between the depth of *cavitas glenoidalis* and the height of *caput humeri* is unexpectedly slightly, as in male bones it is even negative. Only in the left bones of female skeletons this dependence is a bit higher and statistically significant. A possible reason for this result is that *cavitas glenoidalis* and *caput humeri* do not fit perfectly to each other and there are many other structures (muscles, tendons, ligaments, bursae), which take part in the shoulder joint. *Cavitas glenoidalis* (with labrum glenoidale) covers only one quarter to one third of the surface of *caput humeri* and to keep the humeral head in close contact with the cavity a number of muscles blend with the capsule to form the rotator cuff [5].

The dimensions of *cavitas glenoidalis* and *caput humeri* as well as the relationships between their shapes are of great importance for the understanding of variations in normal anatomy and they could be considered in cases of shoulder arthroplasty.

Conclusions

Cavitas glenoidalis and *caput humeri* are larger in male skeletons. In both sexes the right *scapulae* and right *humeri* has greater *cavitas glenoidalis* and *caput humeri* respectively, than the left ones.

The comparative assessment of the sexual differences shows that *cavitas glenoidalis* of male and female scapulae differ most strongly in its depth, and *caput humeri* – in its diameters.

The correlation matrices show well pronounced dependences between the metrical features of *cavitas glenoidalis* and *caput humeri* separately as well as between the features of both bone structures. Nevertheless, the relationship between the depth of *cavitas glenoidalis* and the height of *caput humeri* is the slightest one. Remarkable bilateral differences are observed in the female correlation matrices and the correlations in the left female bones are an exception to the mentioned above.

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An unusual variety of double quadratus femoris muscle

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Quadratus femoris muscle muscle is one of the muscles of the gluteal region. It takes part in the external rotation in the hip joint. During routine dissection in the section hall of the Department of Anatomy and Histology in Medical University – Sofia we came across a very interesting variation of a double quadratus femoris. We saw an additional belly situated below the main belly. Two bellies are clearly divided medially, but laterally they merged. We made series of pictures and did literature. There are very few literature data describing variations of quadratus femoris muscle. The existing data in the literature are concernved the fusion of adductor magnus and quadratus femoris and in other cases the fusion of the inferior gemellus and quadratus femoris **[4]**. Sometimes quadratus femoris is absent **[1, 5]**. It may be replaced by an unusually thick obturator internus muscle or by a large inferior gemellus **[2, 4]**. Quadratus femoris muscle may be divided at its femoral insertion into two parts: one posterior, with the normal attachment, and the other anterior, with insertion onto the intertrochanteric crest **[3]**.

Introduction

Quadratus femoris muscle belongs to the muscles of the gluteal region with Glutaeus maximus, Glutaeus medius, Glutaeus minimus, Tensor fasciae latae, Piriformis, Obturator internus, Gemellus superior, Gemellus inferior and Obturator externus. The Quadratus femoris is a flat, quadrilateral muscle, situated between the Gemellus inferior and the upper margin of the Adductor magnus. It is separated from the last mentioned by the terminal branches of the medial femoral circumflex vessels. The Quadratus femoris starts from the upper part of the external border of the ischial tuberosity, and is inserted into the quadrate tubercle on the intertrochanteric crest and into the upper part of the quadrate line. A small bursa is often found between the front of this muscle and the lesser trochanter. The Quadratus femoris is supplied by the last lumbar and first sacral nerves. This muscle takes part in the external rotation in the hip joint. In some cases the muscle is absent.

Material and Methods

During routine dissection in February 2013 in the section hall of the Department of Anatomy and Histology in Medical University – Sofia we came across a very interesting variation of a double quadratus femoris. The quadratus femoris that presented anatomical variations was photographed using a Nikon Coolpix 995 camera with a 3,34 Megapixels. We made series of pictures and did literature.

Results

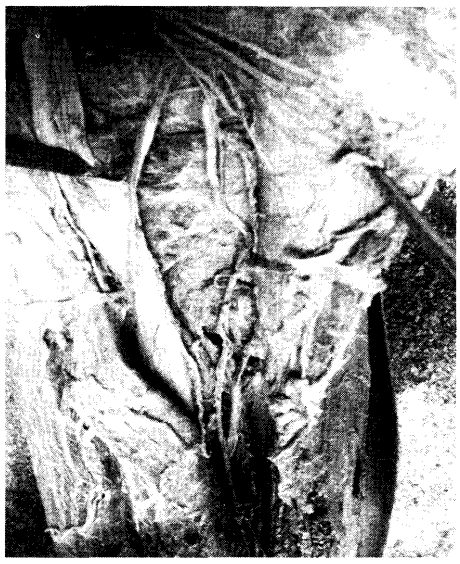

Fig. 1. The presence of an additional belly of quadratus femoris muscle situated immediately below to the main belly

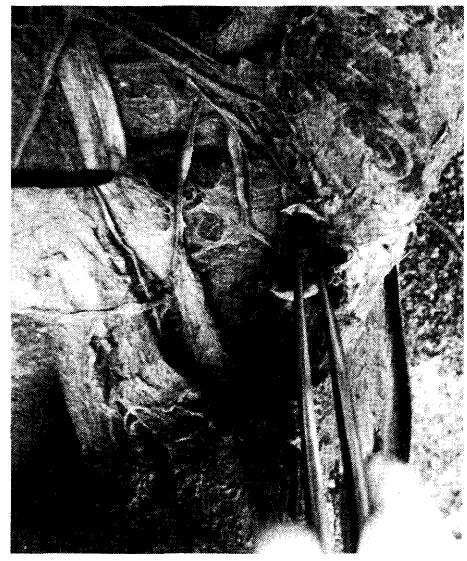

Fig. 2. The additional belly of quadratus femoris muscle started from ischial tuberosity and merged with the main belly

Fig. 3. Two bellies of quadratus femoris were clearly divided and their medial part was covered by the sciatic nerve.

Conclusion

We find very few literature data describing variations of quadratus femoris muscle. It is interesting to mention that existing data in the literature concerning the fusion of adductor magnus and quadratus femoris and in other cases the fusion of the inferior gemellus and quadratus femoris **[4]**. Sometimes quadratus femoris is absent **[1, 5]** or replaced by an unusually thick obturator internus muscle or by a large inferior gemellus **[2, 4]**. Quadratus femoris muscle may be divided at its femoral insertion into two parts: one posterior, with the normal attachment, and the other anterior, with insertion onto the intertrochanteric crest **[3]**.

In our case the additional belly is clearly divided from the main belly medially, but laterally two bellies are merged. On the other hand this additional belly could be considered as the part of adductor magnus muscle, irrespective of the above mentioned fact, that two bellies of quadratus femoris were clearly separated. That should be considered when performing a surgery in the gluteal area especially with rear access to the capsule of the hip joint.

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Review articles

Why vitamin D deficiency is thought to be a risk factor for multiple sclerosis?

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Geographic variation in the incidence of cause unknown multiple sclerosis (MS) supports the probability that environmental factors are involved in the etiology. Vitamin D inhibits the development of autoimmune diseases such as diabetes, rheumatoid arthritis, lupus and multiple sclerosis. Vitamin D for humans is obtained from sun exposure, food and supplements. Preliminary evidence suggests that persons with high circulating levels of vitamin D are at lower risk of MS, thus, vitamin D supplementation may reduce the risk of developing MS, also may reduce the relapse rate among patients with relapsing-remitting MS. The results of previous studies suggested that MS risk is related to vitamin D status at different ages, possibly starting in utero and extending through early childhood, adolescence and adult life. Independent data may argue for potential additional mechanisms associated with a 25-OH-D decrease immediately prior to disease manifestation. Here are referred evidence for the relationship between sun exposure, vitamin D, and the data of MRI in patients with multiple sclerosis.

Key words: vitamin D, vitamin D deficiency, multiple sclerosis

Vitamin D was named in 1922 by American biochemist Elmer McCollum (1879-1967), who performed experiments to understand the contents of fish liver oil. It was named "D" because it was the fourth substance he identified.

Vitamin D is a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium and phosphorus in our bones and aid in cell to cell communication throughout the body. Five forms of vitamin D have been discovered, vitamin $D_1 - D_5$. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements [14]. The body can also synthesize vitamin D (specifically cholecalciferol) in the skin, from cholesterol, when

sun exposure is adequate (hence its nickname the "sunshine vitamin") [3]. Vitamin D_3 is made in the skin when 7-dehydrocholesterol reacts with ultraviolet light at 270-300 nm wavelengths - peak vitamin D_3 production occurs between 295-297 nm. It is only when the UV index is greater than 3 that these UVB wavelengths are present. Frequent exposure of the skin to sunlight promotes sufficient vitamin D synthesis without the need for supplements, however, adults who have darker skin pigmentation or frequently wear sun protection during outdoor activities are often vitamin D deficient.

Vitamin D deficiency is prevalent in infants who are solely breastfed and who do not receive vitamin D supplementation and in adults of all ages who have increased skin pigmentation or who always wear sun protection or limit their outdoor activities. Vitamin D deficiency is often misdiagnosed as fibromyalgia. A new dietary source of vitamin D is orange juice fortified with vitamin D. The recommended adequate intakes vitamin D in the absence of exposure to sunlight is a minimum of 1000 IU vitamin D/d to maintain a healthy concentration of 25(OH) D in the blood [9].

Multiple sclerosis (MS) is an immune-mediated inflammatory disease that attacks myelinated axons in the central nervous system (CNS), destroying the myelin and the axon in variable degrees [6,13,17]. MS is considered to be multifactorial with an autoimmune component. There is growing evidence suggesting that hormones can affect and be affected by the immune system [5]. The hypothesis that there was insufficient vitamin D in the body, as a risk factor for developing MS, formed and developed for over 50 years [1]. Further experimental, epidemiological and genetic studies showed that the mediator between sunlight and immune system is likely to be vitamin D [2,8].

Low levels of vitamin D are associated with multiple sclerosis. Supplementation with vitamin D may have a protective effect, but there are uncertainties and unanswered questions. "The reasons why vitamin D deficiency is thought to be a risk factor for MS are as follows: I - MS frequency increases with increasing latitude, which is strongly inversely correlated with duration and intensity of UVB from sunlight and vitamin D concentrations; II - prevalence of MS is lower than expected at high latitudes in populations with high consumption of vitamin-D-rich fatty fish; III - MS risk seems to decrease with migration from high to low latitudes." A clinical trial sponsored by Charite University in Berlin, Germany, was begun in 2011, with the goal of examining the efficacy, safety, and tolerability of vitamin D_3 in the treatment of multiple sclerosis [2, 15, 16].

According to modern concepts, hormones such as prolactin and vitamin D, and more recently identified ones, such as leptin and gherlin, may be used to modulate the immune response and may also influence the course of MS [5]. The influence the course of MS has been a matter of controversy for a long time.

Results from epidemiological and clinical studies clearly suggest that changes in vitamin D serum concentrations are correlated with the magnitude of the risk of developing MS, the phases of relapsing-remitting MS and with gender differences in vitamin D metabolism. Experimental and clinical studies also have established that 25-hydroxy vitamin D (25(OH)D) and 1,25-dihydroxy vitamin D (1,25(OH)2D) exert an immunomodulatory effect in the CNS and peripheral organs of the immune system [18].

Issues that are discussed include the vitamin D serum concentration needed to suppress the aberrant immune response in MS patients; a subgroup of MS patients suitable for vitamin D treatment, the vitamin D being applied in optimally effective and safe dosage.

The majority of MS patients are deficient in vitamin D in the blood serum or failure of its consumption. It is also noted that during exacerbations of MS, vitamin D concentration in the blood is lower than during remission and exacerbation severity is inversely proportional to it. Furthermore, studies have shown that during the months of low irradiation, the number of multiple sclerosis exacerbation increases.

In animal experiments it was shown that calciferol is able to prevent the development of acute autoimmune encephalomyelitis (AAE - MS model), and reduces the severity of the clinical manifestations of the introduction to the advanced stage of the disease. UV irradiation and also the introduction of cholecalciferol is largely prevented the development of the AAE. In vitro studies have also shown that the protective effects of vitamin D appears to relate to the stimulation of cells that produce IL-10 (anti-inflammatory agent), reduction in the number of cells secreting interleukins 6 and 17 (a substance activating inflammation) and with increasing amounts of regulatory T cells (regulate the inflammation process) [2].

A study, participants were more than 7 million Americans, was conducted in the United States. It showed that the level of vitamin D in the blood at least 99.2 nmol/L reduces the risk of developing MS by 62 %, compared to individuals, having concentration of serum vitamin D less than 63.2 nmol/L. At the same time, this study showed that the concentration of vitamin D in the blood serum of healthy young people of the white race is an important risk factor for the development of their MS, regardless of place of birth and latitude of residence [2]. Professor G. Ebers said, "There is absolutely no problem with taking vitamin D up to 4000 IU/day." [10].

Although no significant association between high-dose vitamin D treatment and risk of MS relapses was found, the studies were limited by several methodological limitations [12]. Further larger, more prolonged studies are merited. Any randomized controlled trial assessing the effect on the relative risk of relapse of any formulation or dose of vitamin D, in participants with MS, was eligible [12].

Disease occurrence and progression are considered by some to be associated with low serum levels of vitamin D. Studies investigating vitamin D supplementation in MS patients have illustrated a noticeable improvement in the course of the disease [11].

Until recently, there has been a paucity of data from randomized controlled trials to establish clear cut beneficial effects of vitamin D supplementation during pregnancy. An overview of vitamin metabolism, states of deficiency, and the results of recent clinical trials conducted in the U.S. are presented with an emphasis on what is known and what questions remain to be answered [19].

Prior research evaluated the role of vitamin D deficiency as a risk factor for development of MS and as a modifier of its clinical course as well as of common symptoms of patients with MS, such as pain and depression. The interaction between IFN-β and vitamin D in terms of their combined efficacy was also previously studied both clinically and in an animal model, with yet conflicting results [7].

The present randomized, double-blind, placebo-controlled trial, though modest in its sample size, did not detect beneficial effects of vitamin D supplementation on IFN-β -related Flu-like symptoms in patients with MS, but did provide support to its immunomodulatory properties. Vitamin D appears to influence IL-17 secretion in IFN-β -treated patients in a dose dependent manner. While serum IL-17 was significantly increased after low dose vitamin D treatment, heterogeneous responses were noted after high dose vitamin D [7].

The findings are in-line with a series of clinical trials of vitamin D supplementation for patients with MS, which generally did not show added benefit in terms of clinical efficacy, but did show clues for improvement in markers of inflammation and related MRI findings, beyond the reported effects on disease prevention. Further large scale trials and meta-analyses of available data are needed to elucidate the role of vitamin D for immunocompetence and as part of the treatment armature of immune-mediated diseases as MS [7].

In a study involving 200,000 women, who for 30 years were under observation, it was shown that increased levels of vitamin D in serum are associated with a reduced

risk of multiple sclerosis. In women who took a daily dose of 10 mg vitamin D or more, the risk of MS decreased by 42%. Norwegian scientists have demonstrated that regular (more than three times per week) use of marine fish reduces the risk of MS in young people living in the Arctic Circle (high-risk zone MS).

As Zivadinov said "Sun exposure may have direct effects on MRI measures of neurodegeneration in MS, independently of vitamin D." [20].

For healthy individuals, serum vitamin D concentrations of 50-125 nmol/L (20-50 ng/mL) are generally considered adequate for bone and overall health, according to the Institute of Medicine. Serum vitamin D concentrations of 75-100 nmol/L (30-40 ng/mL) have been proposed as optimal for patients with MS. Achieving these levels may require the use of supplemental vitamin D in doses up to 3000 IU daily; maintaining these levels appears to require doses of 500 to 800 IU daily. The safety and effectiveness of vitamin D supplementation among patients with MS remains unclear [13].

The primary objective of the Golan's study was to test whether vitamin D supplementation may ameliorate IFN-β-induced Flu-like symptoms. Secondary objectives were to evaluate the safety and tolerability of vitamin D in two different regimens, to determine the extent it influences serum 25-OH-D and to assess the effect of vitamin D supplementation on IFN-β- treatment efficacy, determined by relapse rate and EDSS, as well as on the serum levels of cytokines associated with immune-mediated diseases such: IL17, IFN γ and IL-10, proposed to be associated with MS disease fluctuating activity [4,7].

Higher 25-OH-D serum levels were reported with lower risk to develop MS later in life. Likewise, prior to first clinical disease manifestation was associated with an increased risk for MS. The aim was to investigate both 25-OH-D serum levels and Immunoglobulin G (IgG) response against Epstein-Barr virus (EBV) before the first clinical MS manifestation in individuals who had donated blood prior to disease onset [4].

In some studies when considering vitamin D as a key environmental factors were not taken into account or excluded the effect of other proven risk factors (infection with the EBV, smoking) [2]. In the study of insolation is important to remember that the relationship between the amount of vitamin D formed and the level of insolation is not direct, it contribute to the presence of clothing, use of sunscreens, skin type and color, as well as the time of day. In addition, there are indications that the insolation has independent immunomodulatory effect of vitamin D. Thus further studies on possible interactions between different environmental factors and these factors' role in the disease pathogenesis are justified and necessary.

In conclusion, low vitamin D may be associated with clinical MS breakthrough within 2–3 years [4].

Therefore, until further high quality evidence is available, clinicians may wish to consider relevant MS guidelines on vitamin D supplementation when making decisions about the care of people with multiple sclerosis [11]. Adequately powered, multicentre trial with a focus on clinical as well as immunological and MRI outcomes that are meaningful to people with MS, and are able to provide insight into the benefits of vitamin D in people with MS, are still required.

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Aminopeptidase A in different diseases: a minireview

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Aminopeptidase A is an ectoenzyme, widely expressed in mammals and humans. It performs important physiological functions. One of these functions is to play a role in the control of blood pressure by converting angiotensin II to angiotensin III in the brain. Aminopeptidase A is involved in development of preeclampsia during pregnancy. It is expressed in many malignant neoplastic lesions and can serve as a biomarker for neoangiogenesis. The aim of the present review is to summarize the existing data about aminopeptidase A and its role for different diseases.

Key words: Aminopeptidase A, renin-angiotensin system, tumor biomarker, neoangiogenesis

Name and mammalian tissue distribution

Aminopeptidase A (APA, EC 3.4.11.7) was first identified in rat and guinea pig kidney sections where the enzyme catalyzes the hydrolysis of N-(α-L-glutamyl)-β-naphthylamide [7]. Subsequently, the enzyme was found to hydrolyze N-terminal aspartyl residues and was named Aminopeptidase A [7]. Since α-L-glytamyl derivatives are more efficiently hydrolyzed than are α-L-aspartyl derivatives, the enzyme is named glutamyl aminopeptidase, too. Different substrates had been used to determine the activity of Aminopeptidase A in preparations from different sources. That is why the enzyme has a lot of names – aspartate aminopeptidase, angiotensinase A, Ca^{2+}-activated glutamate aminopeptidase, membrane aminopeptidase II and the BP-1/6C3 antigen [22].

Tissue distribution of APA had been revealed by using immunohistochemistry [12]. In mammals, the highest APA levels had been detected in the intestinal brush border and kidney proximal tubules [27]. In the brain, APA is localized primarily in microvascular elements, the choroid plexus, and the ependymal lining [8]. The enzyme is expressed also in the capillary endothelium of all the studied organs.

Structure and regulation

APA is a membrane-bound zinc metallopeptidase [5]. The enzyme cleaves specifically the N-terminal glutamyl or aspartyl residues from peptide substrates, such as angiotensin II and cholecystokinin-8 [25]. The pH optimum of APA depends on the source and the peptide substrate and varies in the interval 7.0-8.0 [22]. The enzyme is composed of a small N-terminal cytoplasmic domain (17 residues), a 22-residue transmembrane domain and a large extracellular C-terminal domain that contains the active site [30]. Its activity is modulated by calcium ions. In the molecule of APA Ca^{2+}-binding site is situated in the immediate vicinity to the catalytic Zn^{2+} [33] to allow the correct orientation of the substrate in the enzyme active center. APA is sensitive to inhibition by metal chelating agents [4] and is completely inhibited by transitional metal ions such as Zn^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} and Cd^{2+} [22]. EC33 [(S)-3-amino-4-mercapto-butyl sulfonic acid] is a specific and selective inhibitor of APA. In vivo experiments of APA inhibition are usually made using RB150 – a dimmer of EC33 generated by creating a disulfide bond [2]. In contrast to EC33, this substance is able to cross the blood brain barrier. In the brain, disulfide bridge is degraded by reductases to release two molecules EC33, effectively inhibiting APA.

Aminopeptidase A in T and B cell development

APA is expressed on the pre-B and immature B cells [13]. The enzyme is also present on bone marrow-derived stromal cells and cortical epithelial cells of the thymus. A mouse model of BP-1 deficiency had been used to explore the physiologic role of APA in T- and B- cells maturation [13]. Those cells development appeared to be normal suggesting that APA is not essential for this process, possibly because the APA deficiency is compensated by other peptidases [22].

Regulation of blood pressure

APA as a therapeutic target for hypertension

APA is present in several brain nuclei containing nerve terminals and AT1 receptors involved in blood pressure regulation [14]. Those data suggest that the enzyme is an integral component of the brain RAS in humans and rodents and plays a role in blood pressure regulation [16]. Many experiments show that in both central and peripheral RAS APA is responsible for the conversion of angiotensin II (AngII) to angiotensin III (AngIII). Both AngII and III possess a similar affinity to AT1 receptors [31]. AngII is a principal effector peptide of RAS, which induces vasoconstriction and increases sodium and water retention leading to an increase in blood pressure [16]. AngIII exerts a tonic stimulation and affects the control of blood pressure [23]. Whereas AngII and AngIII are believed to be of almost equivalent importance in the maintenance of central blood pressure, AngII is the most important peripheral agonist acting on AT1 receptors [18].

Overacting of RAS is responsible for the development of hypertension [23]. Thus, spontaneously hypertensive rats exhibit RAS hyperactivity and a significantly higher APA activity than normotensive rats, suggesting the enzyme contribution to increased blood pressure [34]. Intracerebroventricular (i.c.v.) injection of APA specific inhibitor EC33 in rats, leads to a decrease in blood pressure and activates the degradation of brain AngII by other peptidases (angiotensin-converting enzyme 2, endopeptidases or others) leading to a formation of peptides, inactive to AT1 receptors [9]. On the other hand, APA infusion by i.c.v. results in a significant increase in blood pressure [32]. Those ex-

periments confirm the leading role of AngIII and the enzyme generating it (APA) in the regulation of central blood pressure [23]. According to the above studies, APA which generates AngIII may be considered a potential therapeutic target for the treatment of hypertension. The enzyme specific inhibitors are currently tested with a view to a possible clinical use [14].

On the other hand, APA-deficient mice are known to develop a mild hypertension [18]. It has been speculated that the total absence of APA during fetal and adult life may induce compensatory mechanisms of yet unknown nature for blood pressure regulation resulting in a slight hypertensive effect [14].

Role of APA in preeclampsia

The human fetus produces bioactive peptides such as oxytocin and vasopressin, as well as angiotensin II [21]. These peptides are highly uterotonic and vasoactive [17] and their secretion increases alongside with the fetus growth or under the action of stress factors. The peptides have low-molecular weight, so they can pass through the fetoplacental unit and affect the maternal organism [17]. It is believed that preeclampsia – a hypertensive disorder during pregnancy is caused by an overproduction of AngII in the fetus due to a failure in APA production and/or activation, since APA is the main enzyme responsible for degradation of AngII [16]. The high levels of AngII in fetus result in an increase in AngII concentration in maternal serum as well, and a subsequent raise in blood pressure. The experiments show that before and immediately after development of preeclampsia APA levels in maternal blood serum are substantially increased pointing out at a response to counter AngII increase [19]. The main goal of the treatment of preeclampsia is to decrease only maternal blood pressure without affecting the fetus. APA has a molecular weight of 109 kDa and does not cross the placental barrier [18]. In this respect, APA is an important candidate for the treatment of preeclampsia by its infusion in maternal organism.

APA and local renin-angiotensin systems

The renin-angiotensin systems are two types – systemic and local [11]. The systemic RAS regulates blood pressure, electrolyte and fluid homeostasis. The local RASs play autocrine, paracrine and intracrine physiological roles. These local RASs have been found in a lot of organ systems such as pancreas, heart, kidney, vasculature and adipose tissue, nervous, reproductive and digestive systems [11]. APA is a part of RAS. In the pancreas, the local RAS plays an important role in regulating local blood flow, control the secretion of digestive enzymes, glucose – stimulated insulin release, etc. [11]. Studies show that the pancreatic RAS components are responsive to various stimuli, including hypoxia, pancreatitis, hyperglycaemia, diabetes mellitus type 2, and pancreatic cancer [11]. The role of APA in those pathological conditions remains to be evaluated in the future.

Participation of aminopeptidase A in angiogenesis and tumorigenesis

Neovascularization consists of vasculogenesis and angiogenesis. Vasculogenesis is a process of formation of new capillaries from angioblasts and angiogenesis is a development of pre-existing vessels [24]. Angiogenesis is a result from a complex of interactions between vascular cells and cells from the surrounding environment [28]. Angio-

genic vasculature is a target for therapy in cancer [29]. Aminopeptidase A is expressed in blood vessels from several types of human tumors and is undetectable or barely detectable in normal vasculature [15]. Studies show that APA -- deficient mice have a decreased neovascularization. Treatment of mice who have tumors with APA – inhibitors shows reduction of tumor growth [15].

Aminopeptidase A is expressed in neoplastic lesions of the uterine cervix and its expression is upregulated as the lesion progresses from cervical intraepithelial neoplasm toward invasive squamous cell carcinomas [26]. Studies show that APA may play a promoting role in neoplastic transformation and disease progression in cervical neoplasm [6]. Immunohistochemical studies show that APA is strongly expressed at the invasive front of the tumor lesions [26]. These findings support the fact that tumor–stromal interaction is essential for the expression of aminopeptidases, including APA in these types of tumors. In other kinds of tumors like Angiotensin II-mediated cervical cancer, overexpression of APA reduces the invasive potential[26]. That is so because Angiotensin II is not only a vasoconstrictor but it is also a growth factor that stimulates cell migration and invasiveness of some kinds of tumors[20].

APA is normally expressed in the brush-border membrane of renal tubules where it takes part in the luminal hydrolisis of polypeptides. Recent studies show that APA is over-expressed in clear cell renal cell carcinoma patients. However, the enzyme activity measured biochemically, is lower in comparison to the normal renal tissue [29]. This discrepancy could be explained either by inhibition of catalytic activity throughout the action of yet unknown cellular factors or by point mutation in the zinc binding motif of the protein. Obviously, APAis involved in pathogenesis of renal cancer, although the mechanism of this involvement remains to be elucidated in future studies [29].

Aminopeptidase A is expressed in human malignant gliomas and metastatic carcinomas in the brain [15]. The enzyme is overexpressed in perivascular cells and it is enzymatically active. APA may play a role in several functions such as secretion of growth factors, modulation of the extracellular matrix and regulation of vascular permeability [15].

The expression of aminopeptidase A has been detected in other kinds of tumors. Studies show that benign prostatic stroma exhibit no APA expression, but stromal cells surrounding prostatic carcinoma cells demonstrate an increased APA expression [3].

Fibroblasts are heterogeneous group of structural cells whose function is to produce all the precursors for extracellular matrix [1]. They take part in maintaining and repairing the normal tissue. They also synthesize and respond to a lot of cytokines and

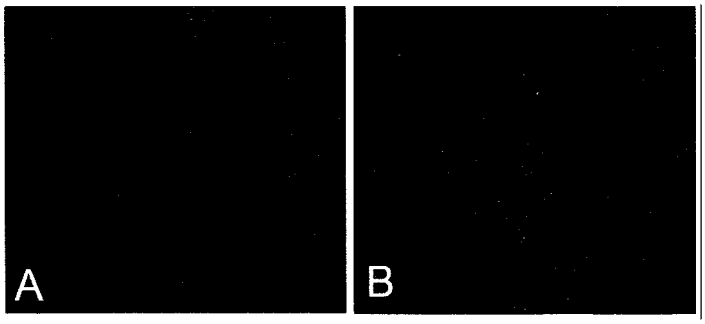

Fig. 1 Cytochemical demonstration of APA activity using a novel fluorescent method. Low enzyme activity in normal mouse fibroblasts (A); a substantially higher APA activity in mouse fibrosarcoma cells (B). 400x

mediators and are involved in the process of inflammation and healing [1]. Fibroblasts participate in tumorigenesis as they stimulate premalignant and malignant epithelial cells to proliferate and to form tumors in mice [10].

Recently, we examined the expression of APA in normal fibroblasts and in fibroblasts from mouse fibroblastoma using enzyme histochemistry. The results showed that in normal fibroblasts APA was weakly expressed but in fibroblasts from fibrosarcoma APA was visibly more active (Fig. 1).

In view of these preliminary results, it seems possible that the enzyme is involved in the regulation of malignant stromae fibroblasts. Since fibroblasts are the main cell type in all kinds of solid tumor stromae, it would be valuable to continue the above studies in order to elucidate the enzyme participation in the formation of tumor microenvironment.

In conclusion, APA activity is important for tumorigenesis. The enzyme role in different types of tumors deserves to be studied in order to establish its diagnostic and/or prognostic value.

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