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MOLEKULSKE OSNOVE PATOGENOSTI NEKIH HERPESVIRUSA

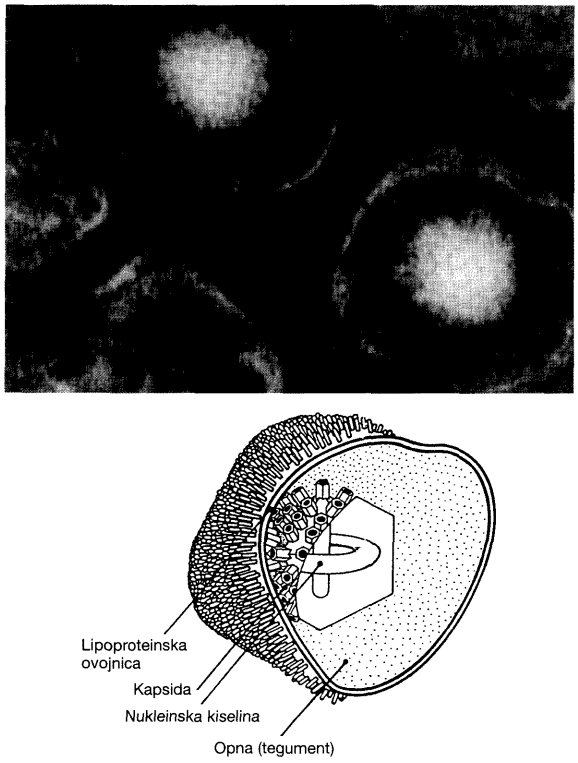
Uvod

Suvremene tehnike molekularne biologije omogućile su duboko pronicanje u srž molekulnih zbivanja na razini "virus-stanica" te uvelike pridonijele boljem poznavanju patogeneze virusnih infekcija. Smatra se da opis određenog virusa nije potpun ako u njegovu genomu nisu identificirani geni odnosno kodirajuće sekvencije za njegove glavne proteine te ako nije određena njihova uloga u ciklusu replikacije. Zahvaljujući životinjskim modelima, u novije doba razjašnjeni su mnogi temeljni događaji u interakciji herpesvirus-domaćin. U tom pogledu posebnu ulogu imaju glikoproteini virusne ovojnice i određeni proteini važni za neurotropizam i latenciju herpesvirusa. U ovom kratkom pregledu bit će riječi o novijim dostignućima u karakterizaciji herpesvirusnog genoma, glikoproteina i pojedinih enzima te njihovoj ulozi u nastanku infekcije, kao i o mogućnostima konstrukcije novih vakcinalnih sojeva genetičkim inženjeringom. Prvenstveno će se govoriti o virusu bolesti Aujeszkoga (BA), virusu zaraznog rinotraheitisa goveda (ZRG), a zatim o virusima varicella-zoster i herpes simplex tip 1 (HSV-1) čovjeka.

Herpesvirusi su vrlo rasprostranjeni u prirodi i čine veliku, po patogenosti vrlo raznoliku skupinu virusa. Obzirom na podrijetlo i antigenski sastav do sada je opisano 106 različitih serotipova izdvojenih iz preko 50 životinjskih vrsta. Spomenimo samo kako je u čovjeka opisano 7 antigenski različitih herpesvirusa, u drugih primata 27, u goveda 5, konja 5, svinje 2, psa i mačke 1, a u domaće peradi i ptica 15. Ostali su dokazani u mnogih vrsta glodavaca, gmazova, vodozemaca i riba [1,2,3].

Svrstani su u potporodice: *Alphaherpesvirinae* (rodovi *Simplexvirus* i *Varicellovirus*), *Betaherpesvirinae* (rodovi *Cytomegalovirus*, *Muromegalovirus* i *Roseolovirus*) i *Gammaherpesvirinae* (rodovi *Lymphocryptovirus* i *Rhadinovirus*). Po morfologiji su svi herpesvirusi međusobno slični (sl. 1). Razlikuju se prema svojim biološkim osobinama, patogenosti i građi virusnog genoma. Dakako da svi nemaju jednako medicinsko značenje ni za humanu, a ni za veterinarsku medicinu. Virusi o kojima ćemo govoriti pripadaju alfaherpesvirusima roda *Varicellovirus*, a svrstani su pod nazivima: ljudski (humani) herpesvirus tip 3 (HHV-3), goveđi herpesvirus tip 1 (GHV-1) i svinjski herpesvirus tip 1 (SHV-1) [3].

HHV-3 je virus varicella-zoster, a uzrokuje vodene kozice kod djece i rijetku bolest "zoster" u odraslih. GHV-1 uzročnik je zaraznog rinotraheitisa (ZRG),



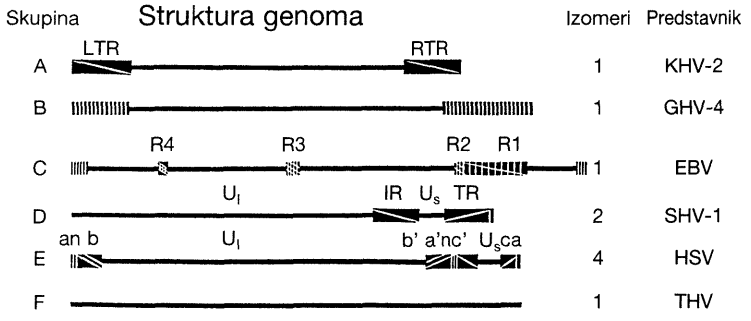
Sl. 1.

Morfologija herpesvirusa. (A) Elektronskomikroskopska snimka virusa herpes simplex. (B) Shematski prikaz herpesvirusne čestice (prema Horzinek, 1985[8]). Herpesvirusi su veliki od 120 do 200 nm. Imaju lipoproteinsku ovojnicu na kojoj se nalaze izdanci (peplomere) dugi do 8 nm. Svaka peplomera predstavlja određeni glikoprotein. Kapsida im je ikozaedarne simetrije sa 162 prizmatične kapsomere. U kapsidu je u obliku vretena upakirana DNA. *Bar*: 100 nm.

zaraznoga pustularnog vulvovaginitisa odnosno balanopostitisa, pobačaja, ooforitisa, encefalitisa i sistemnih infekcija goveda. SHV-1 uzročnik je bolesti Aujeszkoga (BA) ili lažne bjesnoće. Redovito je smrtonosan za prasad u dobi do 2 tjedna i druge domaće i slobodno živuće sisavce, koje napada. Odrasle svinje su skladište uzročnika. HSV-1 uzrokuje akutni herpetični gingivostomatitis, herpetični egzem, keratokonjunktivitis, encefalitis i herpes labialis u čovjeka [4,5,6].

Građa Genoma Herpesvirusa

Genom herpesvirusa građen je od dvolančane DNA, koja, ovisno o vrsti virusa, sadrži od 124000 do 235000 parova baza. Iz toga proizlazi da bi oni najveći mogli kodirati za preko 100 proteina. Većina herpesvirusa u svom genomu ima ponavljajuće sekvencije veće od 100 parova baza, smještene na različitim mjestima pa se na osnovi toga i veličine genoma mogu svrstati u 6 skupina (sl. 2). U genomu skupine A, u kojoj se, uz druge, nalaze ljudski herpesvirus 6 i konjski herpesvirus 2, slijed s jednog kraja genoma se izravno ponavlja na drugom kraju. U skupini B, s govedim herpesvirusom 4 i majmunskim herpesvirusom saimiri 2 kao predstavnicima, završni slijed se izravno ponavlja mnogo puta na oba kraja. U skupini C, čiji je predstavnik Epstein-Barrov virus (uzročnik infektivne mononukleoze čovjeka), broj završnih ponavljanja je malen, ali se na drugim mjestima u genomu mogu naći ponavljajuće sekvencije veće od 100 parova baza, koje dijele genom na nekoliko odsječaka. Skupina D, u koju su svrstani HHV-3, SHV-1 i GHV-1 ima genom sastavljen od jedinstvenoga dugog (unique long U_L) i jedinstvenoga kratkog područja (unique short U_S), koje je omeđeno završnim i unutarnjim ponavljajućim slijedom obratne orijentacije. Kod predstavnika skupine E, humanog virusa herpes simplex tip 1 i 2 (HSV-1 i HSV-2), oba područja, jedinstveno dugo i jedinstveno kratko, omeđena su ponavljajućim slijedovima obratne orijentacije. U genomu skupine F herpesvirusa, čiji je predstavnik majmunski tupaia herpesvirus, nema ponavljajućih slijedova [2,3].



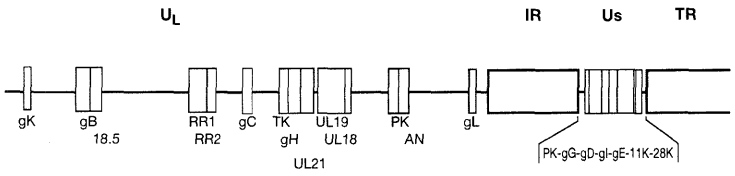
Sl. 2.

Shematski prikaz građe genoma herpesvirusa (prema Roizman i sur., 1995[3]). KHV-2 = konjski herpesvirus tip 2; GHV-4 = goveđi herpesvirus tip 4; EBV = Epstein-Barrov virus; SHV-1 = svinjski herpesvirus tip 1; HSV = herpes simplex virus; THV = tupaia herpesvirus.

Genom SHV-1, GHV-1 i HHV-3

Genom naslovljenih herpesvirusa čini pravocrtna, dvolančana DNA molekula. Ona kod HHV-3 sadrži 124884 parova baza i može kodirati za 67 proteina od kojih

je identificirano oko polovice [7,8]. Genom SHV-1 sadrži oko 150000 parova baza i može kodirati za više od 70 proteina [9], a GHV-1 136000 i može kodirati za najmanje 69 proteina [10]. Raspored gena u genomu ovih virusa i HSV-1 čovjeka uvelike je sličan. Kao primjer ovdje prikazujemo kartu genoma SHV-1 (virus BA), na kojoj se vidi položaj pojedinih gena za glikoproteine o kojima se raspravlja (sl. 3). Osim toga, slijed aminokiselina mnogih proteina ovih virusa je također homologan te je i njihova biološka zadaća slična ili identična [8,9,10,11,12].



Sl. 3.

Karta genoma SHV-1 (prema Mulder i sur., 1997[12]). Genom se sastoji od jedinstvenoga dugog (U_L) i jedinstvenog kratkog (U_S) područja. Oba područja međusobno su odijeljena obratnim, tzv. unutarnjim (IR) i završnim (TR) ponavljajućim sekvencijama. Izdvajamo gene koji kodiraju za glikoproteine B (gB), C (gC), D (gD), E (gE), G (gG), H (gH), I (gI), K (gK), i L (gL); podjedinice za ribonukleotid reduktaze RR1 i RR2, timidin kinazu (TK), alkalnu nukleazu (AN) i kodirajuća područja za proteinske kinaze (PK).

Virusni glikoproteini

Nositelji infektivnosti i virulencije herpesvirusa su njihovi glikoproteini, zatim neki enzimi važni za metabolizam DNA ili fosforilaciju te proteini koji sudjeluju u spajanju virusne DNA s kapsidom.

Ulazak herpesvirusa u stanicu domaćina zbiva se posredovanjem virusnih glikoproteina, koji poput izdanaka strše iz virusne lipoproteinske ovojnice (sl. 1). Za razliku od drugih virusa, herpesvirusi sadrže veći broj ovojničnih glikoproteina. Dosada ih je identificirano 11 u HSV-1, 10 u SHV-1, 10 u GHV-1 [10,12,13]. Kako su glikoproteini alfaherpesvirusa međusobno slični ili homologni, dogovoreno je da se označavaju velikim slovima abecede analogno HSV-1 (18th International Herpesvirus Workshop, Pittsburgh, Pennsylvania, 1993.). Tako sada nose oznake gB (prije gII za SHV-1 i gI za GHV-1), gC (prije gIII), gD (gp50), gE (gI za SHV-1 i gII za GHV-1), gG (gX), gH (gH), gI (gp63), gK (UL53), gL (UL1), gM i gN. Iako međusobno sličan, genom alfaherpesvirusa ipak nije podudaran. Tako je u HHV-3 poznato samo 6 glikoproteina. U njega nisu ustanovljeni gD i gG [8,14]. Također, postoje razlike u proteinskom sastavu npr. GHV-1 i HSV-1. Proteini UL0.5, UL3.5, *circ*, i US1.5 dokazani su u GHV-1, ali ne i u HSV-1. S druge strane drugih 12 proteina, među kojima je i glikoprotein gJ HSV-1 nije dokazano u GHV-1 [10]. Nadalje HSV-1 ima u US području 12, a HHV-3 samo 4 gena.

Virusni glikoproteini odgovorni su za adsorpciju virusa na stanične receptore te za penetraciju virusa u stanicu. Oni određuju virulenciju i tropizam virusa za

određeno tkivo, a prijeko su potrebni i za oslobađanje virusa iz stanice i njegov izravan prijenos sa stanice na stanicu. Neki glikoproteini služe kao receptori za Fc područje imunoglobulina G ili fragmenta C3b komplementa [15,16]. Njihova uloga u replikaciji i patogenezi razjašnjena je zahvaljujući upotrebi monoklonih protutijela, zasebnoj ekspresiji pojedinih njihovih gena u eukariontskim stanicama i proizvodnji delecijskih mutanata.

Od nabrojenih glikoproteina, četiri (gC, gE, gI i gG) nisu nužna za umnožavanje virusa u staničnim kulturama pa su nazvani neesencijalnima (tablica 1). Međutim, svi su oni ustanovljeni u terenskim punovirulentnim izolatima virusa BA [17,18,19] i virusa ZRG [20] pa se smatraju važnima za preživljavanje virusa u životinjskoj populaciji. Suprotno njima gB, gD, gH i gL su esencijalni za infektivnost virusa, jer virusna čestica kojoj nedostaje jedan od njih ne može prodrijeti u stanicu domaćina [12].

Uloga glavnih glikoproteina alfaherpesvirusa

Glavnim glikoproteinima alfaherpesvirusa smatraju se gB gD i gC. Glikoproteini gB i gD mogu se dokazati već za 2-3 sata nakon infekcije te se u procesu umnožavanja virusa javljaju kao rani (early) ili β proteini, dok je gC kasni ili γ protein [21,22]. Naime, proces umnožavanja herpevirusa odvija se u jezgri domaćina u nekoliko faza. Prvo se prepisuju jako rani (immediate-early) geni, koji kodiraju za jako rane ili α proteine. Ti prepoznaju samo specifične sekvencije na virusnom genomu, što dovodi do transkripcije nove skupine tzv. ranih (early) gena, koji kodiraju za β proteine važne za sintezu DNA. Posljednja se eksprimira skupina gena, koji pretežito kodiraju za strukturne (gliko)proteine virusne čestice ili kasne γ proteine. Nakon sinteze svih komponenti, nove virusne čestice nastaju na unutarnjoj membrani stanične jezgre, putuju kroz endoplazmatski retikulum i Golgijev aparat te se oslobađaju iz stanice. Kao primjer navodimo da se novoumnoženi SHV-1 može dokazati već za 5 sati nakon inokulacije stanične kulture. Njegov titar naglo raste te za sljedećih 7 sati dostiže oko $10^{7.5}$ $TCID_{50}$ na 10^6 inokuliranih stanica [11].

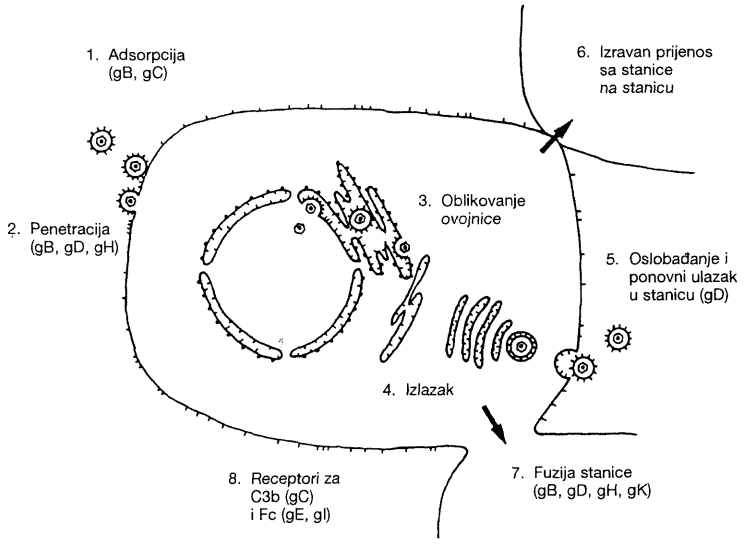
Glikoprotein gB je glavni protein virusne ovojnice alfaherpesvirusa i potiče tvorbu neutralizacijskih protutijela u inficiranom domaćinu. U svih homolognih virusa igra važnu ulogu u penetraciji slobodnog viriona u ciljnu stanicu, tj. u fuziji virusne ovojnice sa citoplazminom membranom stanice. Također je potreban za izravno širenje virusa s primarno inficirane stanice na susjednu neinficiranu stanicu (sl. 4; tablica 1.). Heterologni gB može zamijeniti homologni. Tako gB goveđeg herpesvirusa 1 može zamijeniti i preuzeti ulogu gB svinjskog herpesvirusa 1, a gB svinjskog herpesvirusa 1 može zamijeniti gB virusa herpes simplex 1 čovjeka [23,24]. To jasno potvrđuje funkcionalnu sličnost glikoproteina gB u različitih vrsta alfaherpesvirusa.

Glikoprotein gC sudjeluje u adsorpciji SHV-1, GHV-1 i HSV-1 na stanicu domaćina. Taj glikoprotein veže se zapravo na heparan sulfat proteoglikan na površini stanice [13,25,26,27,28]. Međutim, ta veza je samo inicijalna i dosta labava. Za virus BA i HSV-1 ustanovljeno je kako se stabilna veza između virusa i stanice uspostavlja pomoću glikoproteina gD, koji se veže na do sada nepoznati stanični receptor [9,12,29]. Prisutnost heparan sulfat proteoglikana na površini većine stanica

Tablica 1.

Uloga glikoproteina virusa bolesti Aujeskoga (BA) i zaraznog rinotraheitisa goveda (ZRG) u procesu umnožavanja (prema Mettenleiter, 1994.[9]; Baranowski i sur., 1996.[22]; Nauwynck, 1997.[11]).

Glikoprotein	Esencijalan		Adsorpcija		Penetracija		Širenje sa st. na stan.		Virulencija	
	BA	ZRG	BA	ZRG	BA	ZRG	BA	ZRG	BA	ZRG
gB	+	+	-	+	+	+	+	+	-	-
gC	-	-	+	+	-	-	-	-	+	+
gD	+	+	+	-	+	+	+	+	-	-
gE	-	-	-	-	-	-	+	+	+	+
gG	-	-	-	?	-	?	-	?	-	?
gH	+	+	-	-	+	+	+	(+)	-	-
gI	-	-	-	-	-	-	-	+	(+)	(+)
gK	-	-	?	?	?	?	+	?	?	?
gL	+	?	-	?	+	+	+	?	?	?
gM	-	-	?	?	(+)	?	-	?	?	(+)
gN	?		?		(+)		-		?	



Sl. 4.

Shematski prikaz uloge glikoproteina virusa herpes simplex tip 1 čovjeka (prema Manservigi i Cassai, 1991.[14]).

kralježnjaka koje imaju jezgru objašnjava veliku proširenost herpesvirusa u životinjskim svijetu. Proteoglikani se nalaze u rahlom vezivnom tkivu, bazalnoj membrani i citoplazminoj membrani stanice. Kolageni i proteoglikani povezuju površinske komponente stanice s citoskeletnim elementima, a vjerojatno i jezgrom. Stoga oni djeluju kao vrlo osjetljivi radarski sustav koji stanici daje sve informacije o tome što se zbiva u njezinom mikrookolišu. Heparan sulfat proteoglikan je ustanovljen u stanicama plućnoga, jetrenog i drugih tkiva. Jetrena stanica ima čak 4x108 tih receptorskih molekula [30]. Iznenađujuće je to da gC nije esencijalan za virusno umnožavanje, što pretpostavlja da i drugi virusni glikoproteini prepoznaju heparan sulfat proteoglikan kao receptor. U odsutnosti homolognog gC, HSV-1 veže se na heparan pomoću gB [14,31,32]. Glikoprotein gC važan je i kao imunogen, jer potiče tvorbu humoralne i stanične imunosti. Čini se da je i tip citopatskog učinka u različitim staničnim kulturama uvjetovan količinom gC na površini inficirane stanice. Povećanjem njegove količine preteže zaokruživanje stanica, a smanjenjem ili njegovom odsutnošću preteže tvorba sincicija [33].

Glikoprotein gD je također esencijalan za virusnu infektivnost. On je odgovoran za penetraciju virusa u ciljnu stanicu. Za razliku od HSV-1 i GHV-1, on nije potreban za izravan prijenos SHV-1 iz stanice u stanicu te se virusna čestica bez njega može u domaćinu širiti samo izravno sa stanice na stanicu. Virioni koji se oslobode iz stanice, a ne posjeduju gD ne mogu više inficirati novu stanicu [34]. On je ujedno i najvažiji imunogen alfaherpesvirusa. Životinje cijepljene pročišćenim gD virusa BA bile su zaštićene od izazivačke infekcije virulentnim virusom [9].

Uloga drugih (manjih) glikoproteina alfaherpesvirusa

Glikoprotein gE tvori kompleks s **glikoproteinom gI**, koji je važan za intersinaptičko širenje virusa u središnjem živčanom sustavu [35,36]. On je nositelj virulencije i neurotropizma alfaherpesvirusa. Posreduje u prenošenju virusa sa stanice na stanicu i oslobađanju virusa iz stanice [9,12,14,22,37]. Iako ne u svim sojevima jednako, nedostatak gE bitno smanjuje virulenciju SHV-1 i GHV-1 [38,39]. Zbog toga je on od velikog značenja za konstrukciju delecijskih mutanti lišenih gena za gE, koje mogu poslužiti kao vakcinalni sojevi virusa BA i virusa ZRG [18,40,41]. Glikoprotein gE pojačava stupanj latencije alfaherpesvirusa [37]. Na površini stanica inficiranih HSV-1 ustanovljeni su receptori za Fc područje imunoglobulina. Ta aktivnost pridaje se prvenstveno glikoproteinu gE, ali kompleks s gI pojačava afinitet prema spomenutim receptorima [42]. Fc-receptorska aktivnost dosada nije ustanovljena u SHV-1 i GHV-1 [22]. Iako kompleks gE/gI čini funkcionalnu jedinicu alfaherpesvirusa, za GHV-1 je dokazano da nedostatak glikoproteina gI ipak učinkovitije smanjuje virulenciju virusa nego samo nedostatak gE [43].

Glikoprotein gG nije esencijalan za umnažanje virusa i nema učinka na virulenciju HSV-1 i SHV-1 [9,44]. Suprotno tome, glikoprotein gG-negativna mutanta GHV-1 ipak ima oslabljenu virulenciju [43].

Glikoprotein gH je strukturni protein. Važan je za penetraciju virusa u stanicu i njegovo širenje od stanice do stanice, ali za razliku od gB i gD ne sudjeluje u adsorpciji. Nekovalentnom vezom je vezan u kompleks gH/gL pa je uloga glikoproteina

gH i gL podudarna [9,22]. Glikoprotein gH sudjeluje u staničnoj fuziji HSV-1 [14]. Stvaranje kompleksa gH/gL bitno je za intracelularni transport HSV-1 [45].

Glikoprotein gK virusa herpes simplex 1 je odgovoran za fuziju inficiranih stanica, ali ne izravno. On ne dospijeva na površinu inficirane stanice, već se nakuplja u endoplazmatskom retikulumu i jezgrinoj membrani [46]. Bez njega je onemogućen izlazak virusnih čestica iz inficirane stanice [47]. Biološke osobine gK zasad su nepoznate u SHV-1 i GHV-1.

Glikoprotein gM nije esencijalan za replikaciju HSV-1 u staničnoj kulturi. Mutanta lišena toga glikoproteina slabije se širi i umnaža u stanicama živčanog sustava, ali u njima ipak ostaje u latentnom obliku [48]. Nedostatak gM uvelike pridonosi oslabljivanju patogenosti SHV-1 [49], ali njegova uloga ipak ostaje nedorečenom budući da se u vakcinalnom soju Bartha lišenom gena za gM, virulencija može ponovno uspostaviti vraćanjem kompleksa gE/gI i gC [50].

Važniji virusni enzimi

Spomenut ćemo dva virusna enzima važna za replikaciju virusne DNA. Jedan je timidin kinaza (TK), a drugi ribonukleotid reduktaza (RR). Timidin kinaza je potrebna za fosforilaciju deoksitimidina do monofosfata, a ribonukleotid reduktaza reducira ribonukleotid-difosfate do deoksi-proizvoda, koji se koriste za sintezu virusne DNA. Kako se oba nalaze u stanicama koje se mogu dijeliti, ali ne i u stanicama koje se ne mogu dijeliti, nije teško zaključiti da su prijeko potrebni za umnožavanje virusa u stanicama koje se ne dijele, kao što su živčane i periferne mononuklearne stanice [11]. Virusne mutante bez TK i RR imaju smanjenu virulenciju i slabo su patogene za domaćina. U pokusnoj infekciji te mutante uzrokovale su blagu kliničku sliku BA u svinja. Mutanta lišena RR se 100 do 1000 puta slabije umnožava u nosnoj i orofaringealnoj sluznici od mutante bez TK. To znači da je RR potrebnija za replikaciju virusa od TK [12].

Alkalna nukleaza (UL12) nije esencijalna za umnožavanje virusa u staničnim kulturama. Dokazano je da je odgovorna za virulenciju virusa BA u miša. U HSV-1 igra važnu ulogu prilikom dozrijevanja virusa odnosno upakiravanja DNA u kapsidu. dUTPaza nositelj je neurovirulencije HSV-1 za miša. Njezin homolog nije dokazan u SHV-1 i GHV-1 [12].

Proteinske kinaze (PK) reguliraju fosforilaciju proteina, a poznato je da one imaju glavnu ulogu u prenošenju signala, regulaciji rasta i diferencijaciji stanica. Jedna kodirajuća sekvencija za virusnu PK nalazi se u U_L13, a druga u U_S3 području genoma alfaherpesvirusa. Ukloni li se ona u području U_L13, SHV-1 se i nadalje može umnožavati u staničnoj kulturi i ostaje patogen za miša, dok mutanta lišena gena za PK u području U_S3 ima smanjenu virulenciju i sporije se replicira u staničnim kulturama. Ustanovljeno je da ne može normalno pupati na jezgrinoj membrani epitelnih stanica nosne sluznice i stanične kulture SK-6. Stoga se virusne U_S3-mutante nakupljaju perinuklearno [12].

Neurotropizam i virulencija

Kao što je spomenuto, herpevirusi o kojima raspravljamo uzrokuju infekcije središnjega živčanog sustava (SŽS), što govori da su manje ili više neurotropni. Naročito izražen neurotropizam ima SHV-1. Taj virus u domaćina prodire kroz nazofaringealno područje. Infekcija započinje njegovim umnažanjem u stanicama sluznice nosa i ždrijela. Nakon infekcije perifernih živčanih stanica dospijeva intraaksonalnim prijenosom do regionalnih živčanih ganglija. On se može replicirati u živčanim i glija stanicama i dospjeti u središnji živčani sustav, gdje uzrokuje encefalitis smrtonosan za prasad na sisi [35,51,52]. Za GHV-1 nije još potpuno razjašnjeno dospijeva li u središnji živčani sustav aksonalnim putem ili je posljedica viremije, što pretpostavlja prodiranje kroz hematoencefalnu prepreku. Prodiranje kroz hematoencefalnu prepreku moglo bi objasniti zagonetku zašto GHV-1 ipak samo sporadično uzrokuje encefalitis. Za razliku od njega SHV-1 se u preživača redovito prenosi živčanim putem i redovito uzrokuje encefalitis [53].

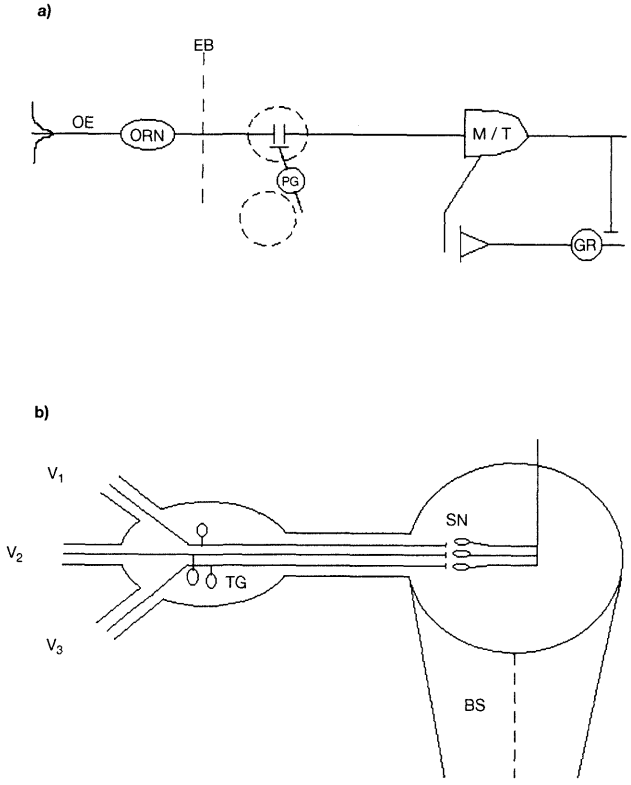
Na štakorskom modelu proučavan je način prodiranja herpes simplex virusa u SŽS. Ustanovljeno je da se virus prenosi preko sinaptički povezanih neurona pa su herpesvirusi poslužili i kao transneuronski markeri za proučavanje neuroanatomske organizacije [54,55].

Važnu ulogu u transneuronskom širenju SHV-1 ima neesencijalan gE, koji je, kako smo vidjeli, nositelj virulencije virusa. On ima određenu regulacijsku ulogu u širenju virusa sa stanice na stanicu. Prijeko je potreban za transneuronski (preko sinapsi) prijenos virusa. Virulentan virus u kojem je eksprimiran gE dospijeva u mozak prasadi za 4 do 7 dana, dok mutante u kojima nije eksprimiran gE tj. glikoprotein gE-negativne mutante teško dospijevaju u središnji živčani sustav. Naime, gE-negativna mutanta SHV-1 se, doduše, umnožava u perifernom živčanom tkivu i inficira neurone prvog reda te se širi prema središnjem živčanom sustavu olfaktornim i trigeminalnim putem, ali se, za razliku od virulentnog virusa, ne prenosi ili se u znatno manjoj mjeri prenosi na živčane stanice drugoga i trećeg reda (sl. 5) [35,56,57]. To znači da je gE važniji za transneuronski prijenos nego za penetraciju i umnožavanje SHV-1 u živčanim stanicama prvog reda. Osim toga, nužan je za širenje virusa u simpatikusu i parasimpatikusu [57]. Glikoproteini gE i gI omogućuju infekciju neurona prvoga reda retino-recipijentnog područja i odatle prijenos virusa do središnjeg živčanog sustava, ali određenu ulogu u širenju ima i koncentracija inokuliranog virusa [58,59]. Prema tome, kompleks gE/gI odgovoran je za prijenos virusa u živčevlju, jer omogućuje oslobađanje virusa iz živčanih stanica prvog reda i fuziju neurona na sinapsama [35,59]. Ipak, neki noviji radovi govore o mnoštvenoj ulozi gE te više ističu njegovo posredništvo u virulenciji i neurotropizmu, nego njegov izravni učinak u sklopu same virusne čestice [60].

Glikoprotein gE je nositelj neuroinvazivnosti i u GHV-1 [10] i HSV-1 [61,62], a pretpostavlja se i u HHV-3 [8].

Latencija

Herpesvirusi uzrokuju latentne infekcije. Tijekom latentne faze ne mogu se u inficiranom domaćinu dokazati infektivne virusne čestice ni virusni antigeni, ali se



Sl. 5.

Shematski prikaz transneuronskog širenja SHV-1 (a) olfaktornim putom i (b) trigeminalnim putom (prema Mulder i sur., 1994[35]). (a) Olfaktorni receptorski neuroni (ORN) u olafktornom epitelu (OE) pružaju aksone kroz etmoidnu kost (EB) u olfaktorni bulbus (OB), gdje završavaju u neuropilima, nazvanima glomeruli. Ovdje se aksoni sinapsama vežu na drugi red neurona, na periglobularne (PG) i mitralne (M/T). Konačno granulski neuroni (GR) (treći red neurona) stvara recipročne sinapse s mitralnima. (b) Neuroni u trigeminalnom gangliju (TG) primaju aferentne aksone iz oftalmičkog (V1), maksilarnog (V2) i mandibularnog (V3) živca i pružaju eferentne aksone trigeminalnim živcem, koji završavaju u moždanom deblu (BS). Tu se sinapsama vežu na drugi red neurona u senzornom nukleusu (SN) trigeminalnog živca.

osjetljivim suvremenim metodama, npr. pomoću lančane reakcije polimerazom ili hibridizacijom, može dokazati virusna DNA na mjestu latencije [53,63]. Pojava stre-

sa dovodi do reaktivacije virusa i njegovog ponovnog izlučivanja iz inficirane jedinke. Latentna DNA SHV-1 i GHV-1 pretežito se nalazi u trigeminalnim ganglijima, ali i u olfaktornom bulbusu, nosnoj sluznici, tonzilama i perifernim limfocitima [63,64,65]. Idealno mjesto ugnježđenja virusne DNA su, dakle, visoko diferencirane i dugo živuće stanice koje se ne dijele, kao što su živčane i limfoidne stanice. Latentno inficirane stanice ne smiju biti razorene, već zaštićene od apoptoze i destrukcije pomoću imunoloških mehanizama. Stoga se za vrijeme latentne faze virusni proteini ili uopće ne proizvode ili su podnošljivi za imunološki sustav [53].

Latencija SHV-1 uvelike ovisi o genotipu vakcinalnog soja i načinu njegove aplikacije. U intramuskularno vakciniranih svinja dokazana je manja količina latentnoga virulentnog virusa rabljenog za izazivačku infekciju nego u intranazalno vakciniranih [66].

Molekulni mehanizmi koji sudjeluju u uspostavljanju i održanju latencije nisu još poznati. Zna se da je u genomu HSV-1, SHV-1 i GHV-1 dokazana aktivnost tzv. LAT (latency associated transcripts) transkripcijske jedinice [9,10]. U genomu SHV-1 ona iznosi 12,6 kb, a pokriva cijeli regulacijski gen IE180 (immediate early 180) i većim dijelom regulacijski gen EP0 (early protein 0) [67]. Njezina uloga je ipak još uvijek spekulativna.

Za razliku od drugih alfaherpesvirusa, latencija HHV-3 nije povezana s prisutnošću LAT-a. Također nije poznati ni mehanizam reaktivacije toga virusa, premda se obično povezuje s imunosupresijom ili imunosnom disfunkcijom. Na štakorskom modelu ustanovljena je ekspresija nekoliko "otvorenih okvira čitanja" (open reading frames ORF) ORF 4, ORF 29, ORF 62, ORF 63 toga virusa [8].

Pretpostavlja se da reaktivaciju herpesvirusa vjerojatno potiče neuropeptid induciran stresom, što bi dovelo do ekspresije virusnoga ili staničnog transaktivatorskog proteina i posljedične replikacije virusa. Od jako ranih proteina (IE) nekoliko ih ima regulacijsku ulogu. Npr. ICP0 (infected cell protein) može aktivirati široki raspon virusnih gena. Indukcija ekspresije gena za ICP0 može biti dovoljna da započne reaktivacija latentnog herpesvirusa. Metabolizam dugo živućih, nereplicirajućih stanica u kojima virus ostaje u latentnoj fazi, vjerojatno nije dovoljno aktivan za uspješnu virusnu replikaciju. Stoga mora doći i do ekspresije virusnih gena koji kodiraju za virusne enzime. Tako je poznato da TK-negativne mutante herpesvirusa imaju slabu moć reaktivacije [53].

Delecijske mutante

Poznavanje funkcije pojedinih (gliko)proteina alfaherpesvirusa našlo je svoju primjenu u proizvodnji novih vakcina protiv herpesvirusnih zaraza životinja.

Poznate su prirodne delecijske mutante SHV-1 i GHV-1 s krnjim genomom za gE [18,40,68]. One se mogu proizvesti i genetskim inženjerstvom ukloni li se iz genoma herpesvirusa gen ili geni, koji kodiraju za (gliko)proteine odgovorne za njihovu virulenciju. Molekularna istraživanja vakcinalnog soja Bartha (prirodna mutanta) virusa BA pokazala su da mu je genom u odnosu na punovirulentne terenske izolate kraći za 3.6 kb u U_S području i da ne može kodirati za gE i gI [9,69]. Nedostatak gE bitno smanjuje virulenciju virusa BA [38]. U svinja kojima je intranazalno apliciran gE-negativan soj ne javljaju se više živčani poremećaji,

znakovi respiratorne bolesti, groznica ni anoreksija. Sojevi s istodobnom delecijom za gE, TK i gG ne prenose se s vakciniranih na nevakcinirane svinje [12]. U veterinarskoj medicini gE i TK/gG negativni sojevi upotrebljavaju se za proivodnju tzv. "obilje-ženih ili markiranih" vakcina protiv herpesvirusnih zaraza. Uporabom tih vakcina mogu se razlikovati inficirane od vakciniranih životinja. To je važno za iskorjenjivanje životinjskih herpesvirusnih zaraza [18,39,40,70,71].

Vakcinalni soj Zagreb (Za) goveđeg herpesvirusa 1

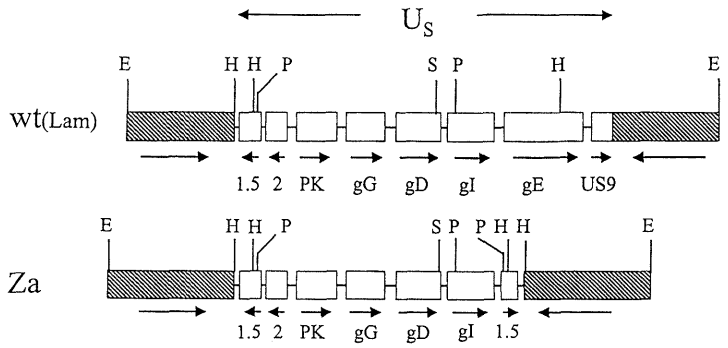
Budući da je vakcinalni soj Zagreb prva opisana prirodna gE-negativna mutanta GHV-1, slično kao i vrlo poznati soj Bartha SHV-1, osvrnut ćemo se ukratko na njegovo podrijetlo. Soj S1/85 GHV-1 bio je ugrađen u monovalentnu vakcinu protiv ZRG-a i bivalentnu protiv ZRG-a i parainfluence 3 goveda [72]. U virološkom laboratoriju Zavoda za mikrobiologiju i zarazne bolesti s klinikom Veterinarskog fakulteta Sveučilišta u Zagrebu desetak puta je bio propasiran u kontinuiranoj staničnoj kulturi goveđega embrijskog bubrega (bovine embryonic kidney, BEK) i liofiliziran. Dalje je bio obrađivan u Centraal Diergeneeskundig Instituut, Lelystad, u Nizozemskoj, gdje je s tim sojem napravljen čitav niz eksperimenata od 1990. do najnovijeg doba. Liofilizat je bio resupendiran u destiliranoj vodi, dva puta propasiran u staničnoj liniji goveđe embrijske traheje (embryonic bovine trachea, EBTr) i nazvan Za1. Dalje je bio tri puta uzgajan u stanicama EBTr i pročišćen tako da je u svakoj pasaži nakon pojave citopatskog učinka odabran mali "plaque", jer se populacija virusa sastojala od mješavine malih i velikih plakova. Tada se već dugo znalo da gE delecijska mutanta SHV-1 tvori male plakove, što je kasnije opisano i za gE delecijsku mutantu GHV-1[73]. Nakon trokratnog pročišćavanja dobiven je soj nazvan Zagreb 4, kasnije preimenovan samo u soj "Zagreb". Analiza njegova genoma restrikcijskim enzimom *Hind*III pokazala je kako mu u području U_S nedostaje fragment od 8.2 kb, ali se pojavio novi od 7.4 kb, što je upućivalo na deleciju u području U_S. U daljnjoj obradbi *Eco*RI (C) fragment, koji obuhvaća cijelo U_S područje bio je kloniran u pACYC 184 i nazvan p775. Analizom toga fragmenta restrikcijskim enzimima dokazano je kako soj Zagreb (Za) ima deleciju od 2.7 kb u U_S području, koja obuhvaća čitav "otvoreni okvir čitanja" za gen gE i gen US9 (sl. 6). To je ujedno i prvi dokaz da je gen US9 u GHV-1 neesencijalan [74].

Od soja Zagreb pripravljene su vrlo dobre "markirane" vakcine protiv ZRG-a [40,75].

Inače se u U_S području genoma GHV-1 nalazi barem šest neesencijalnih (US1.5, US2, US3 (PK), US4 (gG), US7 (gI) US8 (gE) i jedan esencijalan gen US6 (gD)(76). Sada je dokazan i gen US9(74).

Vektorske i plazmidne vakcine

Genom virusa BA i ZRG može poslužiti i kao vektor stranih gena. Tako je gen koji kodira za protein E1 virusa svinjske kuge ugrađen u gE-negativni soj virusa BA. Svinje cijepljene tom rekombinantom bile su istodobno zaštićene od BA i



Sl. 6.

Shematski prikaz građe U_S područja punovirulentnog divljeg soja Lam GHV-1 i rekombinante Zagreb (Za). Soj Zagreb ima kraće područje U_S zbog delecije od 2.7 kb, koja obuhvaća gene gE i US9 (prema Rijsewijk i sur., 1999[74]).

svinjske kuge [77]. Goveda pak cijepljena gE-negativnom mutantom virusa ZRG u čiji je genom umjesto gena za gE ugrađen gen za protein G respiratornoga sincicijskog virusa bila su istodobno zaštićena od ZRG-a i infekcije govedim respiratornim sincicijskim virusom [78].

Premda još uvijek u eksperimentalnoj fazi, najznatniji napredak na području vakcinologije u novije doba postignut je proizvodnjom tzv. "DNA ili plazmidnih" vakcina. U tim vakcinama plazmidi su vektori gena koji kodiraju za imunogene glikoproteine gC i gD virusa BA [79]. DNA vakcina pripravljena od mješavine plazmida koji kodiraju za gB, gC, gD i gE virusa BA bila je djelotvornija od inaktivirane vakcine [80].

Zaključak

Molekularna virologija znatno je napredovala posljednjih dvadesetak godina. Omogućila je identifikaciju i određivanje funkcije pojedinih virusnih gena mnogih virusa. Određivanje sekvencije čitavog genoma alfaherpesvirusa olakšalo je razvitak vrlo osjetljivih i specifičnih dijagnostičkih postupaka i konstrukciju rekombinantnih virusa s krnjim genomom ili zamjenom pojedinih gena. Konačni cilj je do kraja odrediti ulogu pojedinih virusnih komponenata važnih u patogenezi herpesvirusnih zaraza kako bi se moglo smišljeno omesti virusnu replikaciju i prekinuti bilo akutnu litičku, bilo latentnu infekciju. Identifikacija virusnih komponenata i proučavanje molekulnih mehanizama replikacije virusa od velike su koristi za dijagnostiku, preventivu i antivirusnu terapiju.

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SAŽETAK

Molekulne osnove patogenosti nekih herpesvirusa

U članku se ukratko raspravlja o nekim aspektima interakcije svinjskog herpesvirusa 1, goveđeg herpesvirusa 1 te virusa herpes simplex i varicella-zoster čovjeka s prijemljivim stanicama domaćina. Prikazane su nove spoznaje o organizaciji herpesvirusnih genoma, fizička karta genoma svinjskog herpesvirusa 1 i smještaj gena za virusne glikoproteine. Govori se o ulozi pojedinih ovojničnih glikoproteina i drugih proteina važnih za infekciju, (neuro)virulenciju, latenciju i prijenos herpesvirusa. Spominju se delecijske mutante i utjecaj suvremenih spoznaja na pripravu novih vakcina. U tom kontekstu prikazane su osnovne značajke genoma vakcinalnog soja Zagreb goveđeg herpesvirusa 1.

SUMMARY

Molecular Level of the Pathogenicity of some Herpesviruses

In this brief review, several aspects of the interaction of suid herpesvirus 1, bovine herpesvirus 1, herpes simplex virus and varicella-zoster virus with host susceptible cells are presented. They include herpesviral genomes organization, physical map of the suid herpesvirus 1 and location of glycoprotein genes, functional analysis of viral envelope glycoproteins and some other proteins with relation to infection, (neuro)virulence, latency and transmission. Furthermore, deletion mutants and the impact of new advances on the construction of novel vaccines are presented. In this context, the genome characteristics of Zagreb vaccine strain of bovine herpesvirus 1 are given.

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DAS GEFÄHRLICHE ETHMOID

Es ist bekannt dass bei der Tieflage der Lamina cribrosa der mediale Teil des Daches des ethmoidalen Labyrinthes durch eine dünne, verschieden breite, manchmal dehiszente Knochenplatte gebildet ist. An unserer Schädelsammlung haben wir Fälle gefunden wo das Dach des ethmoidalen Labyrinthes überhaupt nicht vom Stirnbein sondern ausschliesslich vom Ethmoid gebildet wurde so dass die Ethmoidalzellen, von der Dura bedeckt, seitlich von der Lamina cribrosa den Boden der vorderen Schädelgrube bildeten.

Metarial und Methoden

Um die Beteiligung der ethmoidalen Zellen an der Bildung des Bodens der vorderen Schädelgrube zu bestimmen haben wir 72 Präparate von mazerierten zerlegten Schädelknochen vom 3. bis 35. Lebensjahr u.zw. 22 Präparate vom 3.-8. Lebensjahr, 20 Präparate vom 9-19 Lebensjahr und 30 Präparate vom 20-35 Lebensjahr untersucht. Uin die Verhältnisse der ethmoidalen Zellen zu der vorderen Schädelgrube zu ilustrieren haben wir auch CT Aufnamen dieser Region angefertigt (Somatom, DR Siemens, Schichten in der Frontalebene, Dicke der Schnitte 2 mm, hohe Resolution.

Literatur

In der Literatur finden wir Angaben dass die oberen und medialen Ethmoidalzellen in der Regel vom Stirnbein bedeckt sind [2,7,12,13,15]

Der mediale Teil des Daches des ethmoidalen Labyrinthes wird bei der Tieflage der Lamina cribrosa durch eine dünne gelegentlich dehiszente Knochenplatte, die dem Ethmoid gehört, gebildet [4,5,6].

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Resultate

Das ethmoidale Labyrinth entwicklet sich als erste von den Nasennebenhöhlen und erreicht bei der Geburt schon beträcliche Grösse. Im zweiten Lebensjahr erstreckt sich der Pneumatisationsvorgang in der Richtung der künftigen Stirnhöhle, die am Anfang des 3. Lebensjahrs schon die ersten Zellen zeigt. Das Ethmoid schiebt sich in die Incisura ethmoidea des Stirnbeins ein. Bis zum 9. Lebensjahr ist die Incisura ethmoidea relativ eng. Ihre Breite beträgt 8-11 mm was etwa der Breite der Lamina cribrosa und den seitlich von derselben liegenden dünnen Streifen von Knochensubstanz enspricht. Von dem 9. Lebensjahr an entwickelt sich das ethmoidale Labyrinth mächtig. Die sich entwickelnden ethmoidalen Zellen werden nach den Angaben in der Literatur, vom Stirnbein bedeckt sodass das Dach des ethmoidalen Labyrinthes vom Stirnbein gebildet wird [1,7,8,9,10,11,12,14]. Wir haben aber an unserem Material, in der Lebensperiode vom 9-35 Lebensjahr, in der Mehrzahl der Fälle Präparate gefunden wo die ethmoidalen Zellen in der ganzen Ausdehnung oder im vorderen Teil des Ethmoids vom Stirnbein unbedeckt blieben und den Boden der vorderen Schädelgrube bildeten. Dies geschah in der Fällen in denen die Pneumatisation des Stirnbeins nicht in derselben Intensität wie diejenige des Ethmoids erfolgte, sondern auf relativ kleine Buchten beschränkt blieb. Durch die intensive Pneumatisation schob das immer breiter werdende Ethmoid die Ränder der Incisura ethmoidea auseinander. Die Breite der Incisur betrug besonders im hinteren Teil sogar bis 35 mm. Die Incisur war entweder im ganzen Umfang gleich breit (Abb. 1a, 1b) und liess die Ethmoidalzellen komplett unbedeckt oder sie war nur im hinteren Teil breit sodass die vorderen oder teilweise auch die mittleren Zellen von Stirnbein unbedeckt blieben (Abb. 2a, 2b). Die vom Stiernbein nicht abgedeckten Ethmoidalzellen bildeten den Boden der vorderen Schädelgrube. In diesen Fällen war die Reichrinne kaum vorhanden. Die Breite des stark pneumatisierten Ethmoids betrug bis 35 mm. Die Ränder der Incisur waren in solchen Fällen durch eine Sutur mit dem Oberrand der Lamina papyracea verbunden (Abb. 3a, 3b). An unserem Material haben wir im Alter vom 9 bis zum 35 Lebensjahr (50 Präparate) 19 Fälle gefunden wo die Incisura ethmoidea extrem breit war und die Ethmoidalzellen vom Stirnbein komplett oder im hinteren 3/4 unbedeckt den Boden der vorderen Schädelgrube bildeten. In einigen Fällen (13 Präparate) war die Kontur der Incisura ethmoidea unregelmässig so dass nur einige, in der Regel, hintere Ethmoidalzellen vom Stirnbein unbedeckt blieben und den Boden der vorderen Schädelgrube bildeten. Fälle wo das Stirnbein in üblicher Weise die ethmoidalen Zellen bedeckt und den Boden der vorderen Schädelgrube bildet haben wir in der erwähnten Lebensperiode nur an 18 Präparaten gefunden.

Diskussion

Im Alter von 9-35 Lebenjahr (50 Präparate) war in 19 Fällen das Dach des ethmoidalen Labyrinthes nicht vom Stirnbein abgedeckt sondern es wurde in den Boden der vorderen Schädelgrube inkorporiert. Nur der vordeste Teil des Ethmoids seitlich von der Crista galli war teilweise oder komplett vom Stirnbein bedeckt. Die Incisura ethmoidea war besonders im hinteren Teil sehr breit. An 13 Präparaten

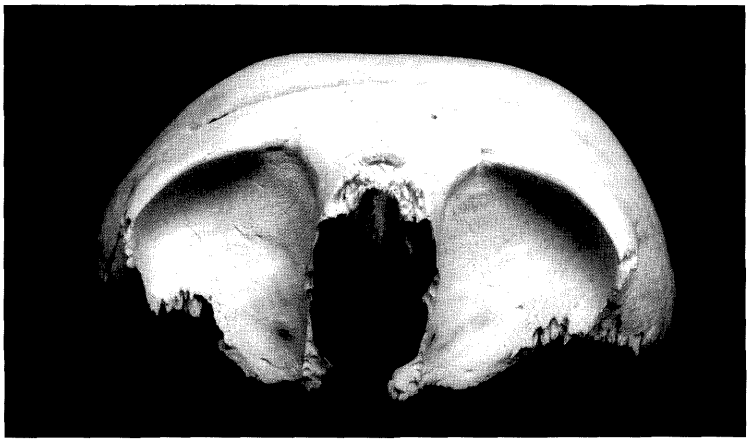

Abb. 1a:

Stirnbein eines 11 jährigen Kindes. Incisura ethmoidea ist extrem breit.

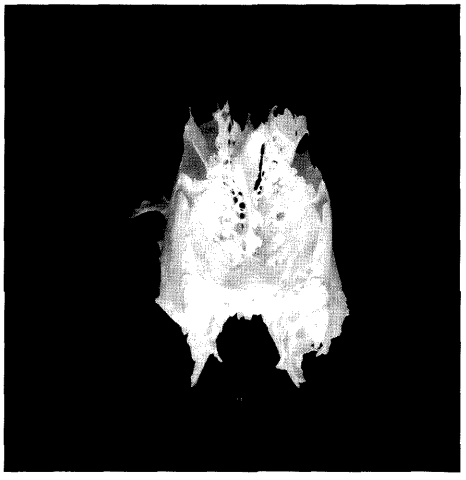

Abb. 1b:

Das dazugehörige Ethmoid. Die Ethmoidalzellen lateral von der Lamina cribrosa sind nicht vom Stirnbein bedeckt.

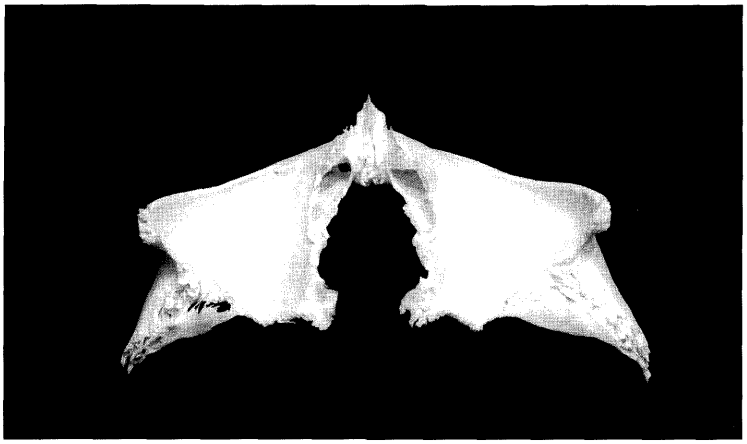

Abb. 2a:

Stirnbein eines 21 jährigen Mannes. Incisura ethmoidea ist besonder im hinteren Teil 35 mm breit.

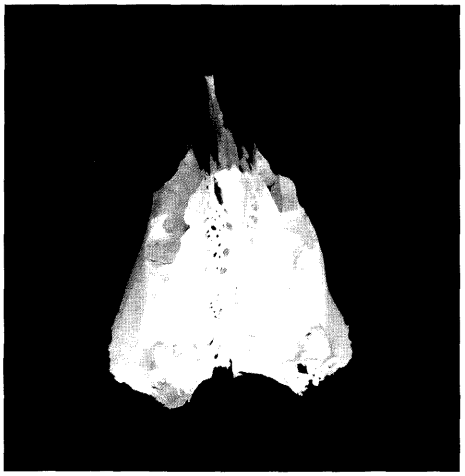

Abb. 2b:

Das dazugehörige Ethmoid. Die mittleren und hinteren Ethmoidalzellen sind vom Stirnbein unbedeckt.

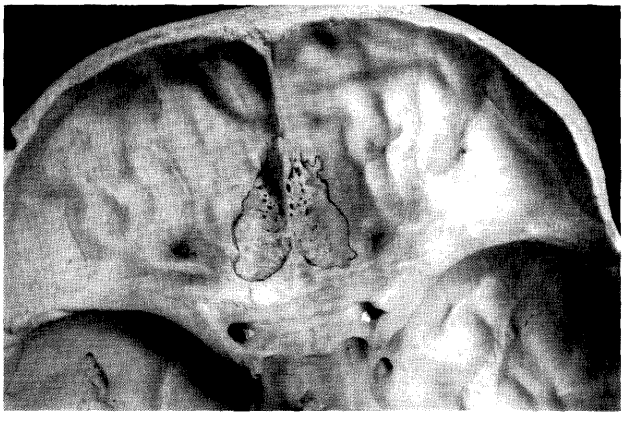

Abb. 3a:

Schädelbasis eines 35 jährigen Individums. Die lateral von der Lamina cribrosa liegenden Ethmoidalzellen bilden den Boden der vorderen Schädelgrube.

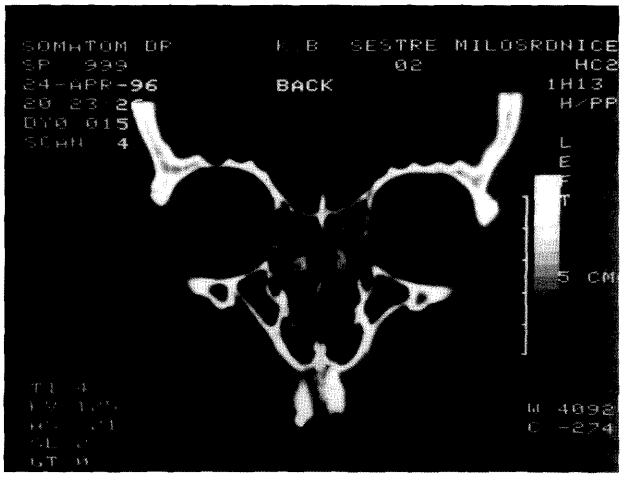

Abb. 3b:

Tomogram desselben Präparates. Man sieht lateral von der Lamina cribrosa die dünnen Stellen der vorderen Schädelgrube von den Ethmoidalzellen gebildet.

waren die Konturen der Incisura ethmoidea unregelmässig mit teilweisen Erweiterungen bis zu 30 mm. An diesen Stellen wurde der Boden der vorderen Schädelgrube von einzelnen Ethmoidalzellen gebildet. Die breitesten Incisuren haben wir am Ende des zweiten und am Anfang des dritten Dezeniums gefunden. Nur an 18 Präparaten haben wir eine relativ enge Incisura ethmoidea und eine Abdeckung des Ethmoidallabyrinthes durch das Stirnbein gefunden.

Auf den ersten Blick scheinen die Angaben aus der Literatur den unseren Befunden zu widersprechen. Ein Erklärung liegt in der Tatsache dass in der diesbezüglichen Literatur das Alter der uritersuchten Individuen nicht angegeben ist. Es handelt sich scheinbar um Präparate von älteren Individuen wo die Pneumatisation der Nasennebenhöhlen abgeschlossen war [2, 3]. An unserem Material handelt es sich um ausgesprochen jüngere Individuen wo die Stirnhöhle nicht ihren definitiven Pneumatisationsgrad erreicht hat. Die Riechgrube war in dieser Lebensperiode noch nicht gesenkt. Erst durch eine intesivere Entwicklung der Stirnhöhle wölbt sich der Boden der vorderen Schädelgrube gegen das Endocranium vor was eine tiefere Lage der Lamina cribrosa besonders in ihrem Vorderteil und Bildung einer tieferen Riechgrube zu Folge hat. Bei der Tieflage der Lamina cribrosa werden die von ihr seitlich gelegenen dünnen Knochenplatten breiter und bilden den medialen Teil des Daches des ethmoidalen Labyrinthes [4,5,6]. Die Pneumatisation der Stirnhöhle kann sich nach einigen Autoren sogar nach dem 40 Lebensjahr fortsetzen [2,3].

Da sich die mittlere Nasenmuschel mit ihrem vorderen Anteil an den Rand der Lamina cribrosa anhäftet kann man bei der Ethmoidectomie, auch wenn man mit dem Instrument lateral von der Insertion der mittleren Muschel bleibt doch in das Endocranium eindringen [4,5,6].

Unsere Untersuchungen haben gezeigt dass in einer gewissen Lebensperiode noch eine weitere Gefahr bei der Ethmoidektomie besteht u.zw. in den Fällen wo das ganze Dach des ethmoidalen Labyrinthes oder der grösste Teil desselben vom Stirnbein unbedeckt bleibt und den Boden der vorderen Schädelgrube bildet. Unserer Meinung nach sind solche Verhälnisse der Incisura ethmoidalis zu dem Ethmoidallabyrinth Folge eines intensiven Wachstums und excessiver Pneumatisation des Ethmoids in einer bestimten Lebensperiode. Eine geeignete CT Aufnahme wird uns preoperativ eine Orientation geben können (Abb. 3b).

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ZUSAMMENFASSUNG

Das gefährliche Ethmoid

Vom 9. Lebensjahr entwicklet sich das ethmoidale Labyrinth mächtig. Die sich entwickelnden ethmoidalen Zellen werden, wie es in der Literatur beschrieben und in den Atlanten abgebildet ist, vom Stirnbein bedeckt, so dass das Stirnbein das Dach des ethmoidalen Labyrinthes bildet. Die Pneumatisation des Stirnbeins erfolgt aber nicht in derselben Intesität wie diejenige des Ethmoids. Falls die Pneumatisation des Stirnbeins nur auf relativ kleine Buchten beschränkt bleibt während die Pneumatisation des Ethmoids intensiv weiter schreitet drängt das in die Breite wachsende Ethmoid die Ränder der Incisura ethmoidea auseinander so dass die Breite der Incisur besonders im hinteren Teil bis zu 35 mm betragen kann. In solchen Fällen deckt das Stirnbein wenn überhaupt nur die vordersten Ethmoidalzellen während die mittleren und hinteren Zellen, vom Stirnbein nicht abgedeckt, den Boden der vorderen Schädelgrube bilden. An unserem Material (72 Präparate) haben wir, im Alter vom 9-35 Lebensjahr (50 Präparate), 19 solche Fälle gefunden. In weiteren 13 Fällen war die Kontur der Incisura ethmoidea unregemässig sodass nur einige von den Ethmoidalzellen unbedeckt blieben und den Boden der vorderen Schädelgrube bildeten. Diese Ethmoidalzellen waren in mehreren Fällen dehiszent. Das als Regel beschriebene Verhältniss des Stirbeins zu den Ethmoidalzellen fanden wir in dieser Lebensperiode in der Minderheit (18 Präparate) der Fälle. Diese Befunde sind äusserst wichtig bei der Ethmoidectomie da man durch die von Stirnbein unbedeckten Ethmoidalzellen in die vordere Schädelgrube eindringen kann.

Schlüsselwörter: Ethmoid, vordere Schädelgrube, Stirhöhle

SUMMARY

The dangerous ethmoidal bone

The ethmoidal labyrinth undergoes with nine year of age intensive growth. The developping ethmoidal cells are, as cited in the literature and represented in atlases, by that time covered by frontal bone which represents the roof of the ethmoidal labyrinth. The pneumatisation of the frontal bone was in a certain period of life less intensive than the one of the ethmoid. The consequence of an intensive pneumatisation of the ethmoid was the enlargement of the gap of incisura ethmoidea which reached in its posterior part even 35 mm in width. In such cases the frontal bone did not cover the ethmoidal cells at all or it covered only the anterior cells. The ethmoidal cells were consequently incorporated in the floor of the anterior cranial fossa. On our material (72 specimens) we found in the life period from 9 to 35 (50 specimens) years of age 19 such cases. In 13 further specimens the shape of incisura ethmoidea was irregular so that only some of the ethmoidal cells remained uncovered by the frontal bone and formed the floor of the anterior cerebral fossa. These ethmoidal cells displayed in many cases rarefications. The relation of the frontal bone to the ethmoidal labyrinth described in the literature as normal appeared in our specimens in this life period in minority of cases (18 specimens). These findings are very important in ethmoidectomy because the anterior cranial fossa can easily be injured througth the thin roof of the ethmoidal cells incorporated in the floor of the fossa.

Key words: ethmoid, anterior cranial fossa, frontal sinus

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INTERFERON TREATMENT OF UTERINE CERVICAL CARCINOMA

THE RESULTS OF STUDIES CONDUCTED A LONG TIME AGO

Introduction

Interferon can be considered an important therapy for many gynecological diseases. It has been shown to be effective in the treatment of genital herpes [1,2,3,4]; condylomata acuminata [5,6]; uterine cervical precancerosis, interaepithelial neoplasia [7,8,9]. A treatment of the uterine carvical cancer was published by Lippman at al. [10,11]. According to Lippman at al. [10], the response rate is 58% [11 of 19] in patients with stage IIB or higher disease level and 66% [10 of 15] in patients with bulky disease (at least one dimension >10cm). Patients were treated with 13-cis Retinol Acid (cRA) lmg/kg orally per day and r.IF'N alpha $6x10^6$ IU sc per day for at least 2 months. The local adjuvant treatment of the cervical uterine carcinoma was published by Ikić, Krušić and coll [12,13 14,15]. After the application of human natural leucocytic interferon (HNLI) as local adjuvant treatment before surgery in patients with the uterine cervical as carcinoma the response was as follows: exellent in 6 patients, very good in 5, moderate in l, poor in 2 and no response in one [14]. The aim of our study is the follow-up of the uterine cervical carcinoma patients both HNLI treated and non treated up to 20 years ago, and to analyse the difference between these two groups. The IFN clinical studies are slow and it requires long assessment time. Therefore, it is valuable to analyse studies taken a long time ago which may lead to the procedure of using IFN as a part of the uterine cervical carcinoma treatment.

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Patients and methods

Patients. Fifty seven patients out of 72 volunteering patients with biopsy proven cervical uterine carcinoma took part in the final study. Twenty seven randomized patients were treated with HNLI and followed up, 30 randomized patients were included and followed up in the control group without being treated with HNLI. The staging of disease conformed with the 1987 Intemational Federation of Gynecology and Obstetrics (FIGO) staging system [16]. Surgical material removed from HNLI both treated and control patients was also examined. None of the patients had received any prior therapy. On completion of HNLI therapy the patients treated with HNLI, as well as patients in the control group, were treated with surgery. Table 1 shows the patients clinical stage, according to FIGO classification.

TABLE 1

PATIENTS CLINICAL STAGE. FIGO CLASSIFICATION

	IA	IB	IIA, IIB	TOTAL
HNLI TREATED	8	9	10	27
CONTROL GROUP	8	10	12	30

Interferon. HNLI was applied locally at $2x10^6$ units daily in pessaries. HM,I was given for 21 days before surgery. HNLI is semipurified concentrated (10^4 per 1 mg) protein,. human natural lekocytic interferon. The follow-up of some patients, both HNLI treated and not HNLI treated in the control group, has been organized for up to 20 years.

Statistics. The data were analyzed by using Kaplan-Meier method for survival analysis [17] and Cox's F-test for the comparison of the groups. The analysis was made by means of the statistical programme, Statistica, Stat Soft inc. Tulsa OK 741 10, USA.

Results

In the group of interferon treated patients 1 A, all the patients survived as well as patients in the control lA group. There were no differences between these two groups. There were 8 patients in the interferon treated group and 8 patients in the control group. The patients were followed up from 197 to 240 months in the HNLI treated group and 195-240 months in the control group.

Table 2 shows survivals of the 1B patients. In the group of interferon treated 1B patients 2 of 9 died, and in the control group 5 of 10 patients died. Regression lines for interferon treated and control 1B patients are shown in FIG 1. Survival analysis according to Cox's F-test shows that the difference in survival between the interferon treated 1B patients and the control group is significant on the level 9% $P<0,09$ which suggests there would have been an even greater difference, if we have had more patients.

TABLE 2

CARCINOMA, CERVICIS UTERI 1B

PATIENTS	SURVIVAL IN MONTHS	PATIENTS	SURVIVAL IN MONTHS
1	191	1	205
2	202	2	194
3	204	3	216
4	216	4	209
5	202	5	204
6	196	6	+60
7	216	7	+48
8	+60	8	+48
9	+18	9	+4
		10	+22
LIVING/DEAD = 7/2		LIVING/DEAD = 5/5	

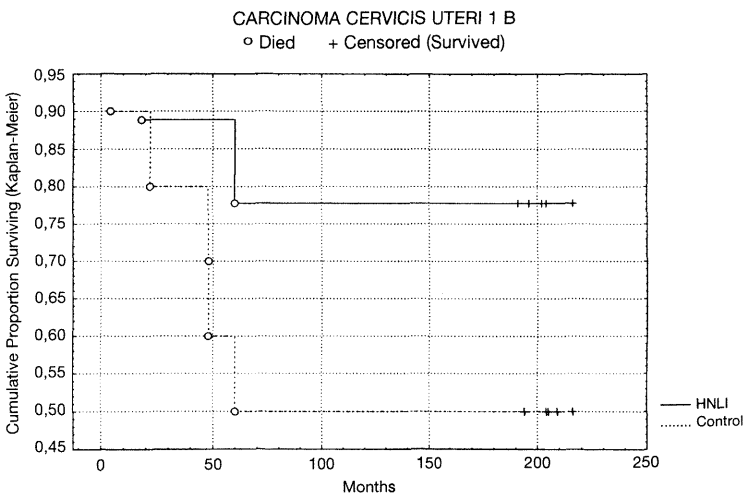

FIG 1

Table 3 shows survivals of the 2A and 2B patients. In the group of interferon treated 2A and B patients 5 of 10 died and in the control group 11 of I2 patients died. Regression lines for interferon treated and control 2A and B patients are shown in FIG 2. Survival analysis according to Cox's F-test shows that the difference in survival between the interferon treated 2A and B patients and control group is significant on the level 2% P<0, 02.

TABLE 3

CARCINOMA, CERVICIS UTERI 2A, 2B

HNLI		CONTROL	
PATIENTS	**SURVIVAL IN MONTHS**	**PATIENTS**	**SURVIVAL IN MONTHS**
1	216	1	214
2	192	2	+84
3	197	3	+48
4	214	4	+13
5	210	5	+13
6	+48	6	+13
7	+24	7	+24
8	+17	8	+32
9	+13	9	+36
10	+40	10	+36
		11	+30
		12	+34
LIVING/DEAD = 5/5		LIVING/DEAD = 1/11	

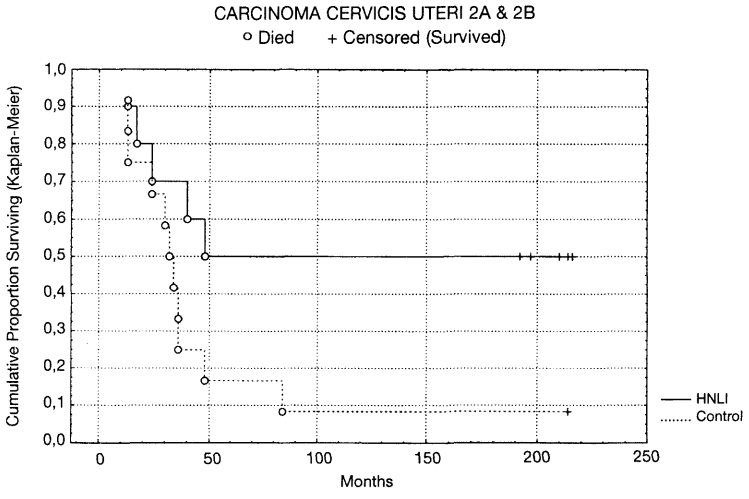

FIG 2

Discussion

The results of our long term follow-up show that it is possible to achieve the cure of the uterine cervical carcinoma patients 2A and 2B, treated locally with HNLI before surgery and that the cure persist. The difference in survival between the HNLI treated 2A and 2B patients and the control group is significant on the level of 2% P<0,02. The difference between groups 1B and control group was significant on the level 9% P<0,09. In our study, patients with squamous cell carcinoma of the cervix, were treated with HNLI $2x10^6$ units locally, daily for 21 days before surgery. In Lippman et al. study, r.IFN alpha 2a at the rate of $6x10^6$ units daily was applied subcutaneously for two months and 13 cRA 1 mg/kg daily, orally, after surgery [10,11]. It seems, then, that retinoids are potent regulators of epithelial differentiation within many neoplastic cell system [19]. In any case, in Lippman et al. study, the dosing of r.IFN alpha 2a after surgery was several times higher, the route of application different and the treatment duration longer than in our study where HNLI was applied locally before surgery [10,11]. Are there any signs that HNLI, which is mixture of many subtypes and r. IFN alpha 2a differ in clinical efficacy? That is an important question but we still lack data for clear-cut comparisons. The effects produced by HNLI are also comparable with those produced by with very pure interferon, although we have an impression that HNLI administered locally requires smaller doses than r.IFN alpha 2a [20,21]. Local interferon (IFN) application into tumor before surgery was introduced by Ikić et al. [12,14]. The local production of IFN has an important physiological role in the immune system. After the local application of HNLI to the tumor site, before surgery, the tumor cells were blocked and their dispersal during surgery was thus prevented, reducing the number of micrometastasis and increasing the chance of survival [18]. Therefore we advise for to be IFN administer locally in pessaries at least $2x10^6$ units daily before surgery for 3-4 weeks for the treatment of the patients IB, IIA, IIB or of higher grade and then to give them IFN after surgery, similar to Lippman et al. procedure.

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SAŽETAK

Liječenje cervikalnog karcinoma interferonom

Liječenje interferonom cervikalnog karcinoma uterusa pokazalo je pozitivne rezultate kod 50-60% pacijentica s 2A i 2B ili višeg stadija. Visoki mortalitet žena oboljelih od cervikalnog karcinoma uterusa pokazuje na potrebu potvrđivanja ovih rezultata. Zbog toga korisno je analizirati rezultate istraživanja izvršenih mnogo godina ranije koja mogu pridonijeti liječenju interferonom pacijentica oboljelih od cervikalnog karcinoma uterusa.

Dvadesetsedam randomiziranih pacijentica liječeno je s prirodnim humanim leukocitnim interferonom (HNLI) prije operativnog zahvata. HNLI primijenjen je lokalno, dnevno, $2x10^6$ jedinica kao prašak u pesaru u toku dvadesetjednog dana prije operacije. Trideset randomiziranih pacijentica u kontrolnoj skupini nisu liječene s HNLI. FIGO klasifikacija interferonom liječenih pacijentica bila je IA:8; IB:9; i B:10, ukupno 27. FIGO klasifikacija pacijentica u kontrolnoj skupini koje nisu liječene s HNLI bila je IA:9; IB:10; IIA, B:12; ukupno 30.

Rezultati promatranja pacijentica analizirani su nakon 20 godina pomoću Kaplan-Meirove metode i Cox-ovog F testa. U grupi IA sve su pacijentice preživjele, pa nije bilo razlike između HNLI liječenih pacijentica i onih u kontrolnoj skupini. U grupi IB umrle su 2 od 9 pacijentica liječenih s HNLI, u kontrolnoj skupini 5 od 10. Analiza prema Cox-ovom testu pokazala je da je razlika značajna na razini 9% P< 0,09. U grupi HNLI liječenih 2A i B pacijentica umrlo je 5 od 10, a u kontrolnoj skupini 11 od 12. Analiza prema Cox-ovom F testu pokazala je kako je razlika značajna na razini 2% P=<0,02.

Lokalna produkcija interferona igra važnu ulogu u našem imunološkom sustavu. Glavni razlog za primjenu HNLI prije kirurškog zahvata je induciranje reaktivnosti tumorske strome, regionalnih limfnih čvorova, te sprječavanje mikrometastaza prilikom kirurškog zahvata. Mišljenja smo da je potrebno prije operacije primijeniti interferon lokalno pomoću pesara u količini od najmanje $2x10^6$ jedinica u toku 3-4 tjedna za liječenje cervikalnog karcinoma uterusa kod pacijentica s 1B, 2A, 2B ili višim stupnjem, a zatim nastaviti poslije operacije 1-2 mjeseca.

SUMMARY

Interferon treatment of uterine cervical carcinoma

Background. The Interferon treatment of uterine cervical carcinoma has shown promising results, i. e. the response rate of 50%-60% in patients with stage IIA and IIIB or of higher grade. High mortality rate in women due to uterine cervical carcinoma indicates the need for the confirmation of these results. Therefore, it is valuable to analyse the results of studies conducted a long time ago which may contribute to establishing the procedure of using interferon as a part of the uterine cervical carcinoma treatment.

Methods. Twenty seven randomised patients were treated with human natural leucocytic interferon (HNLI) and followed up, while 30 randomized patients were followed up in the control group and were not treated with with HNLI. FIGO classification of IFN treated patients was IA:8, IB:9, IIA;IIB:lO; total 27. FIGO classification of control nontreated patients was IA:9, IB: 10 IIA and IIB:l2; total 30 patients. HNLI was applied locally at $2x10^6$ units daily in pessaries for 21 days before surgery. The data were analysed by means of Kaplan-Meier methods for survival analysis and by means of Cox's F-test for comparison between groups. We followed up the HNLI treated and nontreated patients up to 20 years.

Findings. In the group IA all the patients survived. There was not any difference between the IFN treated and the control group. In the group of IB patients 2 of 9 died and in the control group 5 of 10 patients died. Survival analysis according to Cox's F-test shows that the difference is significant at the level 9% $P<0.09$. In the group of IFN treated 2A and 2B patients 5 of 10 died, and in the control group 11 of 12 patients died Survival analysis according to Cox's F-test shows that the difference is significant at the level 2% $P<0.02$.

Interpretation. Local production of IFN has an important physiological role within the immune system. The main purpose of IFN local administration before surgery is to induce the reactivity of tumor stroma and regional lymph nodes and to inhibit micrometastasis. In our opinion it is important to apply IFN locally in pessaries at least $2x10^6$ units daily before surgery for 3-4 weaks for the treatment of uterine cervical carcinoma patients IB, IIA, IIB or of higher grade and then to give IFN after surgery.

Contributors: Drago Ikić was responsible for writing up the paper. Josip Krušić MD, ScD, departed from the life during preparation of the manuscript, was responsible for the design, execution and clinical work. Davor Ivanković was responsible for statistical analysis of data.

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PROPHYLACTIC DIGITALIZATION BEFORE HIP ARTHROPLASTY IN SO CALLED ELDERLY AND MIDDLE-AGED PATIENTS

Introduction

The cardiovascular system of patients who undergo anesthesia and orthopedic hip surgery is a factor of multiple stress resulting from respiratory depression and myocardial contractility. The change of body temperature, arterial blood pressure, ventricular filling pressure, blood volume and activity of the autonomous nervous system, are factors contributing to the stress. Complications during the anesthesia and operation such as large blood loss, infection, lung embolism, myocardial infarction are the additional strain on the cardiovascular system. Even when cardiovascular diseases had been compensated before surgery, the patient's organism may be unable to sustain increased perioperative complications without heart arrest, myocardial ischemia or both. [1-6].

Besides already known direct or indirect effects of anesthetic agents on the heart, mediating primarily through the autonomous nervous system, the additional factors such as uncontrolled ventilation or unadequatly controlled hypoxia, acidosis, hypercarbia, may further depress the myocardial contractility and decrease the arrhythmia threshold. The combination of mentioned variables may lead to changes in the arterial and central venous pressure, minute volume, heart frequency and rhythm.

A high frequency of perioperative life-threatening cardiac complications and mortality of middle-aged and elderly patients undergoing total hip endoprothesis has been established by a retrograde analysis of an electrocaradiogram and on X-ray film of the heart and lungs. Therefore, we have undertaken a prospective study to establish the effect of preoperative treatment with digoxin in patients in whom this treatment was necessary. The main aim of this research was to estimate the dimension of surgical risk and preoperative prophylactyc treatment with digitalis glycosides.

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Patients and methods

One hundred and forty-one patients with anticipated latent systolic heart disfunction who underwent hip arthroplasty at the Orthopedic Clinic, School of Medicine, University of Zagreb, have been included into the analysis of preoperative preparations and perioperative complications. The patients varied in age from 40 to 90 years (mean age, 63 years). They were divided into three groups based on preoperative basic cardiac analysis, electrocardiogram examination and the determination of heart volume from a chest roentgenogram. The research results of preoperative and postoperative parameters were compared with data of 120 patients in the average age of 67 years (40-89) from the control group, which earlier underwent the same operation, but were neither preoperative prepared nor selected according to the cardiovascular status. Their data were taken from medical records.

Electrocardiogram was made in all patients before and 24 hours after operation. Following parameters were analyzed: heart frequency, arrhytmias and conduction disturbances. The following ECG signs of ischemic cardiac disease and digitalis saturation were selected: a) S-T segment, b) corrected Q-T interval calculated by the formula of BAZETT [7], i.e. according to the nomogram of KISSIN et al. [8, 9], derived from this formula. The corrected Q-T interval is considered to reflect the digitalis effect if it measures 0.37 seconds or less [9]. The measured Q-T interval in seconds was corrected for heart frequency by means of the following expression:

$$\text{corrected Q-T interval (s)} = \frac{\text{corrected Q -T interval (s)}}{\text{R-R interval (s)}}$$

The upper limit of corrected normal Q-T interval values for woman measures 0.432 seconds and for men 0.422 seconds. In all patients the serum potassium level was analyzed.

The calculation of heart volume, (anterior and left lateral views) expressed in millilitres per square meter of the body surface area was done by a formula derived by JEFFERSON [10].

$$V = \frac{L \times B \times D \times 0.43}{A}$$

in which L is the long diameter from the superior vena cava and right atrial junction to the cardiac appex; B, the broad diameter from the diaphragm/right atrial junction to the pulmonary trunk/left atrial appendix junction; D, the depth diameter representing the greatest horizontal depth of the heart; and 0.43-MxK, in which M is the magnification factor (Q68); K, the ellipsoid constant (0.63); and A is the body surface area in square meters derived from the height and weight tables.

In 49/141 patients because of painful hip mobility, arm ergometer test with maximal load was performed using an apparatus of the firm "MONARK".

Estimation of the patients was done according to the functional classification of the New York Heart Association (NYHA) [11]: class I: dyspnea under greater load, under usual circumstances without discomfort, class II: smaller activity limitations - dyspnea under usual load, class III: significant body activity reduction - dyspnea under smaller load, no discomfort at rest, class IV: dyspnea at rest - heart failure.

The decrease of functional aerobic capacity more than 25% [16] directs attention to the fact that examinees are cardiac patients or they are limitedly capable of load toleration [12,13].

These are patients with anticipated latent cardiac failure systolic disfunction without symptoms at rest, a history of dyspnea under lighter and heavier load, arterial hypertension, left ventricular hypertrophy, arrhythmias in ECG, and roentgenographic findings of increased heart volume. In patients with cardiac enlargement and decreased cardiac reserve during the load test of class II and III (NYHA classification) [11], the prophylactic therapy with low medigoxin doses (Lanitop "Pliva") was carried out 3-5 days before hip operation and 5 days after operation or longer, depending upon the indications [14,15]. For medigoxin administration, estimation of serum digoxin levels was done by radioimmunoassay by commercial kits supplied by Abbot. The digoxin (medigoxin) concentration was measured on a sampl drawn 5 hours after oral dose of digoxin in order to find out the concentration in a state of dynamic balance. Blood for this analysis was taken from the vein in the quantity of 2 ccm and put in the test-tube with heparin with the patient in supine position. In the analysis of serum digoxin concentration a level of 0.7 to 2.5 mmol/L was considered as therapy one. In all patients the possible presence of infection, anemia, infusion, hypervolemia, renal insufficiency, arterial hypertension and tachycardia because of pain and myocardial infarction were analyzed.

Statistical analysis was performed by the x^2 test. The significance of distribution results before and after operation was analyzed by the Stuart's test [16] which serves for examining the homogeneity of marginal distribution in data classification before and after operation.

Results

Group 1 consisted of 49/141 patients with suspected latent cardiac failure aged 63 years (range, 40-76). In group 2, there were 48/141 patients in the age of 69 years (range, 45-90) with chronic heart failure. Patients of both groups were receiving cardiac glycosides. Group 3, comprised 44/141 patients who had no heart disease and who preoperatively did not receive cardiac glycosides. Group 4 consisted of 120 patients aged 67 years (range, 40-90) who were not preoperatively randomized and did not receive cardiac glycosides. Distribution according to groups, age and sex is shown in Table 1. The mean value of heart volume of Group 1 with latent cardiac failure was 641.89 ml-m^2; range, 362-917 ml-m^2. The increase in heart volume was found in 36/49 patients with arterial hypertension.

The results of load tests showed that 19/49 (38.7%) patients had lowered cardiorespiratory capacity, 28/49 (57.14%) patients belonged to the second class according to the NYHA classification and 21/49 (42.85%) patients who received prophylactic digitalis preoperatively to the third class. The relation between cardiac enlargement and reduced functional aerobic ability in the first group of our patients is presented in Table 2.

In 46/49 (93.87%) of the patients, the systolic blood-pressure - heart frequency product was greater than 12000 (18.19%) and 20/49 (40.81%) patients could hardly bear the load lesser than 3.6 METs. Before and after surgical procedure patients of

Table 1.

HEART CONDITION ACCORDING TO AGE AND SEX

Group	Number (N) of patients/% N/%	Age < 65 y N/%		Age > 65 y N/%		♀ N/%		♂ N/%		TOTAL N
Latent heart failure	I - 49	27	55%	22	45%	23	17%	26	53%	49
Congestive heart failure	II - 48	17	35%	31	65%	37	77%	11	23%	48
Normal heart condition	III - 44	23	52%	21	48%	41	93%	3	7%	44
Control group	IV - 120	28	23%	92	77%	76	63%	44	36%	120
TOTAL		95	36%	166	64%	177	68%	84	32%	261
		x^2 = 21.06,d.t.=3, P<0.01						x^2=25.75,d.t.=3, P<0.01		
										MED: ≈ 65

Table 2.

CARDIOMEGALY WITH REGARD TO THE FUNCTIONAL AEROBIC ABILITY IN 49/141 OF OUR PATIENTS

Decrease of functional aerobic ability		Heart volume ml/m² N/% < 550		551-700		701-900		Number and percentage > 900		TOTAL	
26-40%	moderate	4	8%	12	25%	3	6%	1	2%	20	41%
41-50%	severe	3	6%	12	25%	6	12%	2	4%	23	47%
> 51%	very severe	-		5	10%	1	2%	-		6	12%
TOTAL		7	14%	29	60%	10	20%	3	6%	49	100%

Group 1 and 2 with latent and chronic heart failure received cardiac glycosides. Plasma digoxin concentration measured before and after operation was 0.5 - 1.0 nmol/L, and 0.6 -1.45 nmol/L, respectively. An analysis of heart frequency data of all four groups of patients from pre- and 24 h postoperative ECGs using the Stuart's test disclosed that there was a statistically significant differences between postoperative heart frequencies in patients of Groups 3 and 4 who received no cardiac glycosides. In Groups 3 and 4 frequency of postoperative tachycardia ($P < 0.05$) was also statistically significant compared to preoperative findings. In patients of Groups 1 and 2 who received digitalis glycosides there was no postoperative tachycardia ($P > 0.05$) as compared to the findings before the operation. The potassium values and renal function on which the therapy with digitalis depends was within the normal range. In all four groups of patients, there was a statistically significant postoperative depression of ST segment < 1 mm as compared to the preoperative findings and a significantly longer Q-T interval (Table 3).

By an analysis of disturbances in formation and conduction in an electrocardiogram of digitalized patients with latent heart failure before and after orthopedic operation it was established, that there was no significant difference ($P > 0.05$) between these parameters. The frequency of intraoperative and postoperative complications classified according to the Groups of our patients, is shown in Table 4.

Table 3.

Statistical significance of the difference (Stuart's test) of the heart frequency, S-T segment depression and corrected Q-T interval before and 24 hours after surgical procedure in our patients according tofunctional groups

Group	Heart frequency > 100/min	S-T segment depression > 1,5 mm	Corrected Q-T interval ♀ > 0,432" ♂ > 0,422"
I Latent heart failure	P > 0.05	P > 0.05	Q = 11.77 P > 0.05
II Congestive heart failure	P > 0.05	P > 0.05	Q = 4.47 P > 0.05
III Healthy	P > 0.05	P > 0.05	Q = 10.27 P > 0.05
IV Control group	P > 0.05	P > 0.05	Q = 9.48 P > 0.05

Table 4.

INTRAOPERATIVE AND POSTOPERATIVE COMPLICATIONS IN OUR PATIENTS

Complications Number (N) and percentage (%)	I Group 49	II Group 48	III Group 44	IV Group 120
Sinus tachycardia > 100/min	11 22%	12 28%	23 52%	34 28%
Sinus bradycardia < 50/min	-	1 8,0%	-	1 0,80%
Arterial hypertension	-	2 4,0%	2 4,5%	- (No date)
Arterial hypotension	-	1 2,0%	7 16%	6 5,0%
Stenocardia	3 6%	2 4%	-	- (No date)
Myocardial infarction	1 2%	-	-	-
Congestive heart failure - Myocardiac decompensation	-	1 2%	-	22 18%
Pneumonia	-	2 4%	-	6 5%
Fatal pulmonary embolism	-	-		3 2%
Oliguria	-	-	-	5 4%
prolonged postoperative treatment	-	-	-	24 20%
Cerebrovascular insult/stroke	-	-	-	3 2,5%
Cardiac arrest during introduction into anesthesia	-	-	-	5 4%
Postoperative cardiac death	-	-	-	7 6%

Discussion

A decreased rate of morbidity and mortality in perioperative period compared to the controls is a result of preoperative estimation, selection and prophylactic treatment with digitalis of the patients with myocardial insufficiency about to undergo major surgery. The degree of momentary functional myocardial capacity during surgery and classification of cardiac load according to the NYHA nomenclature shows a significant correlation between the NYHA classification, morbidity and mortality in patients who underwent major surgery [17,18].

A good indicator of functional ability of cardiovascular system (obtained by the exercise tolerance test) is considered to be maximal aerobic capacity, respectively that load expressed in Watts, in which the heart is not capable any more to transport oxygen quantity which meets requirements of surgical load. The concept of oxygen transport and ventilation refers to the minute volume as the main determinant of maximal oxygen utilization, i.e. maximal aerobic capacity [12,13,19].

Of the patients who were included in the first group (49/141) and were without signs of heart failure at rest, with a history of dyspnoea in strain, with arterial hypertension (36/49, 73.46%) and cardiac enlaragement (42/49, 85.71%), 22/49 (44.83%) were capable to tolerate only a low degree of load. All patients had reduced functional aerobic capacity and reduced cardiac reserve.

The rate-pressure product is considered to be a significant hemodynamic parameter in myocardial oxygen consumption [19,20,21]. According to the Loeb's study [19], this product is a better indicator of heart failure than the systolic pressure itself. It has been established that tachycardia as a result of stress more often leads to ischemia and heart failure than stress caused by hypertension. Objective data of exercise tolerance test could be applied in the operating-room in everyday work [19]. Efforts have been made to keep lower during the induction into anesthesia the rate-pressure product at which during ergometry ischemic pain appears, in order to prevent myocardial ischemia and its complications. The critical level of arterial systolic pressure (ASP) and heart frequency product for stenocardia is fixed - 23000 [19] in the majority of cardiac patients. Reports suggest that when surgical procedure is prolonged, ST-segment depression becomes more pronounced and if one does not intervene with lowering of arterial pressure, the myocardial pump function could be compressed and by reducing the minute volume it could lead to heart arrest. ST-segment depression in an electrocardiogram 24 hours after operation is a sign of elevated pressure of the left ventricular filling, ischemia and reduced relaxing of the myocardium.

In Groups 1 and 2 without tachycardia, ST-segment depression may be considered as the effect of digitalis glycosides and in Groups 3 and 4 with tachycardia who were not administered digitalis glycosides, as a result of ischemic heart disease, which is explained by a possible disturbed relationship between oxygen supply and demand during the operation. The ischemic ST-segment response is generally defined as a depression of the ST-segment 1 mm or more below the base line. It often occurs in patients with arterial hypertension and with an increase of diastolic arterial pressure, and is normalized by reduction of blood pressure. Arterial hypertension occurring 30-60 minutes after a large operation is often the consequence of hypoxia, hypercarbia, pain, strain with liquids and is treated causally [20,21]. Postoperative significantly longer Q-T interval ($P < 0.05$) in our patients draws attention to the possible functional changes of the myocard, caused by acidosis, hypoxia and possible transitory anemia.

It is well known that the prolonged Q-T interval in an electrocardiogram is connected with a lethal outcome because of a malignant change in ventricular rhythm. In our study the corrected Q-T was prolonged in all groups except in Group 2 receiving cardiac glycosides for longer period of time [22-25]. When the Q-T interval in an electrocardiogram indicating electrical ventricular systole is prolonged, the recovery of excitable myocardial threads is unequal. Therefore, an impulse may meet unequally recovered ventricular muscle parts and by the "reentry" mecha-

nism lead to paroxysmal ventricular tachycardia and finally to ventricular fibrillation [26-29]. A malignant change of the heart rhythm may also appear without the prolonged Q-T interval [30]. The correlation between myocardial infarction, preoperative systolic arterial blood pressure, intraoperative hypotensive episodes, and arrhythmias, without typical pain is significant and the lethality is 50% [31,32]. Therefore, electrocardiogram recording in an early postoperative phase is recommended, when the possibility of poor outcome is the greatest. Postoperative lung oedema, myocardial infarction and ventricular tachycardia have frequency greater than 25% in the incidence of cardiac death. Patients with severe heart disease do not tolerate the use of vasodilatators and it is often necessary, to apply inotropic drugs intraoperatively. Our study shows that by a selection of patients and digitalization it is possible to reduce the incidence of cardiac complications in the postoperative course. Exercise tolerance testing is an effective way to identify persons at high risk of cardiac complications. It enables an insight into the development of arrhytmias under the load and thus a choice of perioperative medical treatment. Digitalis glycosides may prevent or at least control postoperative heart frequency, i.e. tachycardia which belongs to relevant clinical symptoms of systolic cardiac failure [33]. Systolic cardiac failure may complicate postoperative treatment even in patients who do not have anamnestic data on cardiac arrhythmias [34,35].

Conclusion

Middle-aged and elderly patients with dyspnea during an effort, cardiomegaly and decreased functional aerobic capacity should be prophylactically digitalized by a dilatory saturation dose 3 to 5 days prior to major elective orthopedic surgery in order to increase inotropic heart reserves and to prevent postoperative myocardial ischemia and congestive heart failure. Preoperative treatment of patients with latent heart failure with digitalis glycosides does not only increase cardiac reserve but also reduces the negative inotropic effect of anesthetic agents and thus decreases the possibility of supraventricular and ventricular arrhythmias, i.e. heart failure.

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SAŽETAK

Profilaktička digitalizacija pred artroplastiku kuka u bolesnika tzv. starije i srednje dobi

U cilju smanjenja perioperacijskih komplikacija sa strane srca, skupina od 141 bolesnika srednje i tzv. starije dobi pred artroplastiku kuka podijeljena je u 3 podskupine. Prvu skupinu sačinjavali su bolesnici sa suspektnim latentnim zatajivanjem crpne funkcije srca, u drugoj skupini bili su bolesnici sa znacima zatajivanja crpne funkcije srca, dok su treću skupinu činili bolesnici koji su imali očuvanu funkciju srca.

Svi su klinički i laboratorijski parametri analizirani prije i nakon kirurškog zahvata i uspoređeni su s podacima 120 bolesnika kojima je učinjena artroplastika kuka, a nisu prethodno kardiološki izabrani kao što im nije proveden postupak preoperativne primjene medigoksina.

U bolesnika s otežanim disanjem u naporu i s velikom sjenom srca radiološkim pregledom, učinjen je pokus opterećenjem ručnim ergometrom. Kod bolesnika sa smanjenim funkcijskim aerobnim kapacitetom i smanjenom rezervom srca, primijenjene su male doze medigoksina prije operacije. Osobita je pozornost usmjerena ka korigiranom Q-T intervalu u elektrokardiogramu kao znaku učinka digitalisa, kao i prema depresiji S-T segmenta u smislu znaka ishemijske bolesti srca kao i učinka digitalisa.

Frekvencija srca u digitaliziranih bolesnika s latentnim zatajivanjem crpne funkcije srca nije bila značajno promijenjena prije i nakon operacije, dok je u nedi-gitaliziranih bolesnika postojala značajna razlika. Glikozidi digitalisa mogu spriječiti i kontrolirati poslijeoperacijsku frekvenciju srca koja može biti znakom kom-plikacija. Učestalost perioperacijskih kardijalnih komplikacija u sve tri skupine bolesnika značajno je niža u odnosu prema poredbenoj skupini. Rezultati poredbenih mjerenja smetnji stvaranja i provođenja podražaja prije operacije, tijekom ergometrije, poslije liječenja digoksinom i nakon operacije pokazuju da su smetnje stvaranja podražaja povećane tijekom ergometrije, tijekom operacije i ranog poslijeoperacijskog perioda u nedigitaliziranih, dok nema razlike u digitaliziranih bolesnika s latentnim znacima zatajivanja crpne funkcije srca.

Bolesnike biološki (ne nužno i kronološki) starije životne dobi sa zaduhom pri naporu, kardiomegalijom i smanjenim funkcijskim aerobnim kapacitetom prije velikog kirurškog zahvata potrebno je 3-5 dana liječiti malim dozama medigoksina sa svrhom poboljšanja inotropne sposobnosti srca i sprječavanja perioperacijskih komplikacija.

SUMMARY

Prophylactic digitalization before hip arthroplasty in so called elderly and middle-aged patients

In order to reduce perioperative cardiac complications in 141 hospitalized orthopedic middle- and older aged patients sheduled for hip arthroplasty, we have distinguished three groups of patients:

Group I **with suspected latent heart failure,**
Group II **with chronic myocardial insufficiency,**
Group III **with normal heart condition.**

All observed parameters established before and after operation as well as analyzed complications, have been compared with the same parameters of 120 patients with already performed hip arthroplasty who were not cardiologically selected and medically treated with digoxin. Preoperatively, the arm exercise testing was applied in patients with dyspnoea at strain and with cardiac enlargement. Patients with impaired functional aerobic capacity and reduced cardiac reserve, before the operation received small doses of digoxin. Special attention has been paid to the values of the corrected Q-T interval in an electrocardiogram, to which the specificities of changes conditioned by the digoxin are attributed, as well as to S-T segment depression as a sign of ischemic heart disease in non-digitalized patients and digitalis effect (non toxicity)($P<0.05$). By the preoperative use of digitalis glycosides in patients with bordering compensation in latent cardiac failure, efforts were made to increase inotropic cardiac reserve and to reduce negative inotropic effect of anesthetic agents to the myocardium and to reduce the cardiac morbidity and mortality in orthopedic patients, caused by the early ischemic cardiac disease and congestive heart failure.

Key words: perioperative complications, arm ergometry, latent heart failure, prophylactic digitalization, hip arthroplasty.

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FUNCTIONAL ABILITY IN PATIENTS WITH ARTERIAL HYPERTENSION – A FOLLOW-UP

ABSTRACT

A population sample consisting 4.214 subjects of both sexes aged 35-54 years was examined in 1969, selected at random from the Municipal register from six regions of Croatia: two urban, two rural and two semiurban. A sample consisting of 180 subjects of both sexes from urban registry from the city of Zagreb Centre, who were grouped into persistently hypertensive, periodically hypertensive and control subjects, were followed up over a period of 15 years. In addition to clinical examination and blood pressure measurement a resting electrocardiogram and repeated post-exercise electrocardiograms were taken for all subjects. The aim of this study was to examine the effect of elevated blood pressure on ECG findings at rest and after exercise. In hypertensive male subjects the most frequent changes in resting electocardiogram were ST segment depressions. No such changes were detected in women. From the observed ST segment changes it was not possible to establish how long it would take them to manifest themselves at a higher rate than in persons having normal blood pressure. In the 15-year period, the rate of arrhythmias at exercise in males appeared to be increasing with age. It was not possible, however, to ascertain whether this may have been due to increased blood pressure only.

Introduction

Among major risk factors for cardiovascular diseases, high blood pressure[1] may be singled out, as almost 20 per cent of the adult population is reported to have

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arterial hypertensions[2]. Epidemiological studies conducted in non-selected populations in highly developed industrialized countries show that 50 per cent of hypertensive subjects have heart disease with cardiac enlargement and/or electrocardiographic signs of left ventricular hypertrophy[1]: an electrocardiogram however plays an important diagnostic role, especially during and after exercise[3,4].

Arterial hypertension induces direct and indirect effects on cardiovascular and other systems[3-6]. The presence in the population of mild forms of hypertension are also bound to induce cardiac changes which can be registered by means of resting or exercise electrocardiogram. Since population studies which could provide an answer to this question call for a great deal of effort and money[7-9], more questions invariably follow: what results may we expect to achieve on a smaller population sample, how constant are the changes registered and how long does it take for some electrocardiographic changes, that cannot be defined as accidental but occur as a results of elevated blood pressure, to become apparent. With more severe forms of arterial hypertension, left ventricular hypertrophy is an abnormality which is relatively frequently registered on the electrocardiogram[10]. With milder forms of hypertension, however, no such relatively drastic changes are expected, but only those that are connected with a lower degree of impairment according to the Minnesota code[11]. The present study aims to show whether, in a comparatively small number of subjects, over a period of years, ECG changes at rest and after exercise can be single out, which could be related to high blood pressure.

Sample and methods

A population sample from Croatia consisting of 4.214 subjects of both sexes aged between 35 and 54 was examined in 1969, selected at random from the Municipal register of the population in six regions of Croatia: two urban, two rural two semiurban in the interior and of the coast. Apart from a physical examination and laboratory tests, the examination included blood pressure measurement according to WHO criteria and an electrocardiogram at rest and at and after exercise using a bicycle ergometer. The exercise was of the same grade and lasted five minutes, with a pedalling speed of 50 r.p.m. The load for men was 150 W and for women 100 W. Blood pressure measurements were taken for the same subjects again in 1972.

On the basis of blood pressure values determined in 1969 and 1972, in 1980 we selected a group of 90 men and 90 women among subjects living in the Zagreb Centre community using a random sampling method and classified them in three groups (Table 1). The first group consisted of the subjects who had elevated diastolic pressure (95 mmHg or higher) in both 1969 and 1972. The second group included the subjects with elevated diastolic pressure in one of the two measurements. The third, control group, consisted of the subjects whose blood pressure was normal at both examinations. In the 1980 study the subjects were tested by means of the maximal exercise treadmill test according to the Bruce protocol[5]. The level of maximal work was chosen according to the subject's age and sex with respect to the pulse rate. The test was stopped if the established indications for termination of exercise were present[6] or if blood pressure reached a value of 240/130 mmHg. In

Table 1.

Data of examinee

	Years of examination	Persistently hypertensive	Periodically hypertensive	Normotensive	TOTAL
MEN	**1980**	**30**	**30**	**30**	**90**
	1984	**29**	**28**	**28**	**85**
		1 died in 1982 (malig.lymphoma)	*1 died in 1982* (reason unknown) *1 died in 1983* (myocardial infarction)	*1 died in 1982* (brain tumor) *1 died in 1982* (myocardial infarction)	**5**
WOMEN	**1980**	**30**	**30**	**30**	**90**
	1984	**28**	**28**	**27**	**83**
		1 living (stroke)	*2 living* (veneous thrombosis in the legs)	*1 living* (moved away)	**7**
		2 living (veneous thrombosis in the legs)		*1 died* (haematemasis) *1 living* (myocardial infarction)	**7**

1984 all the 1980 subjects tested were again summoned for examination, of whom 168, 85 men and 83 women, were tested under indentical conditions. The remaining twelve subjects either died in the preceding four-year period or were spared treadmill testing because of locomotive complaints. The ECG tracings were coded according to the Minnesota Codell using uniform criteria i.e. all ECGs were interpreted by the same doctors, except in 1984, when ECGs were read by two doctors independently whose interpretations showed good agreement as confirmed by subsequent control. The ECG responses were then grouped, so that, for example all ST segment changes coded 4-1 to 4-4 were placed in the same group. Thus, a greater frequency of individual finds was achieved. All data were statistically analysed along these lines.

It is possible that the responses have been modified to some extent by drugs taken by some of our subjects. Persistently hypertensive subjects who were taking drugs in non-therapeutic doses or at irregular intervals were taken in 1980 to be without therapy, the same as the control grup. This applied to a man with a daily dose of 20 mg propranolol and a woman who occasionally took 40-80 mg oxprenolol. The remaining subjects were without therapy in 1980. In 1984 the two above mentioned patients were examined after three weeks of abstinence. Among 29 persistently hypertensive male patients who were taking any drug whatsoever in 1984, three received a combination of reserpine, clopamide and dihydroergochristine, one methyldopa, one guanethidine and four beta adrenergic blocking agents. Of 28 periodically hypertensive male patients three were taking reserpine, of whom one was taking beta adrenergic blockers, one was on a combination of

reserpine and hydrochlorothiazide and one digitalis. Among 28 women with persistent hypertension four were taking, a combination of reserpine and hydrochlorothiazide of whom one also took 80 mg propanolol, two hydrochlorothiazide, two methyldopa and four beta adrenergic blocking agents. Od 28 periodically hypertensive women four were receiving a combination of resorpine, clopamide and dihydroergochristine, of whom one had taken medigoxin, and two were treated with beta adrenergic blocking agents. The control groups of both sexes, as in 1980, did not receive any therapy. The data obtained were processed using on electronic computer. Statistical analysis was done with the chi-square testl[2].

Results

Table 2. shows resting and after exercise ST-segment changes in the 15-year period. While in 1969 five out of 30 men with persistent hypertension had ST changes at rest, fifteen years later there were eight out of 30. None of control male subjects had ST-segment changes. The presence of ST-segment changes was hardly detectable among persistently hypertensive women at any examination. A small number of ST changes were identified in women with periodically high blood pressure and in the control grup.

Table 2.

ST-segment changes in the electrocardiogram at rest (and after exercise) over a 15-year period

SEX	YEARS	1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		TOTAL
		Normal values	Pathologic. values	Normal values	Pathologic. values	Normal values	Pathologic. values	Normal+Pathological values
Men	1969	24 (21)	5 (8)	26 (22)	2 (6)	27 (22)	1 (6)	(85) n.s. (ns)
	1980	23 (19)	6 (10)	28 (19)	0 (9)	28 (22)	0 (6)	(85) p<0.01 (ns)
	1984	21 (15)	8 (14)	26 (19)	2 (9)	28 (21)	0 (7)	(85) p<0.01 (ns)
							p=0.9954	(p=9.949)
Women	1969	27 (22)	1 (6)	24 (21)	4 (7)	23 (20)	4 (7)	(83) n.s. (ns)
	1980	28 (22)	0 (6)	23 (18)	5 (10)	21 (17)	6 (10)	(83) p<0.05 (ns)
	1984	28 (21)	0 (7)	23 (18)	5 (10)	21 (16)	6 (11)	(83) p<0.05 (ns)
							p=0.9997	(p=9.903)

Finding post-exercise are put in parenthesis. In males, a rise in frequency with age is noted among the persistently hypertensive subjects, it is smaller among those who were periodically hypertensive, while no major change is observed in the control group. In women ST-segment changes are less common among those with hypertension and they occur more frequently in the control group.

T-wave changes at rest and after exercise over a period of 15 years are shown in, Table 3. They appear at rest to be more common in persistently hypertensive men than in the controls. The subjects with periodically elevated blood pressure tend to be somewhere in between. The finding for women is entirely different. The changes are less prevalent among persistently hypertensive subjects.

Table 3.

T-wave changes in the electrocardiogram at rest (and after exercise) over a 15-year period

SEX	YEARS	1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		TOTAL
		Normal values	Pathologic. values	Normal values	Pathologic. values	Normal values	Pathologic. values	Normal+Pathological values
Men	1969	22 (22)	7 (7)	25 (20)	3 (8)	26 (22)	2 (6)	85 n.s. (ns)
	1980	25 (26)	4 (3)	27 (20)	1 (8)	27 (25)	1 (3)	85 n.s. (ns)
	1984	22 (25)	7 (4)	22 (22)	6 (6)	27 (24)	1 (4)	85 n.s. (ns)
							p=0.9954	(p=9.9970)
Women	1969	26 (22)	2 (6)	24 (21)	4 (7)	23 (22)	4 (5)	83 n.s. (ns)
	1980	27 (25)	1 (3)	24 (18)	4 (10)	24 (21)	3 (6)	83 n.s. (ns)
	1984	27 (24)	1 (4)	22 (15)	6 (13)	20 (19)	7 (8)	83 n.s. (ns)
							p=0.9890	(p=9.9830)

Post-exercise T-wave changes over the follow-up period are put in parenthesis. No rule seems to be applicable to the freqency of this finding in both sexes.

Table 4. shows the frequency of arrthythmias in the 15-year period as recorded by resting ECG and after exercise. Persistently hypertensive and control male subjects do not greatly differ with respect to this finding at rest. The difference, however, becomes much more pronounced over the 15-year period, although not significantly. The results for women are similar. There are no major differences between the groups with respect to blood pressure values, while over a period of 15 years these differences tend to become noticeable. The prevalence of arrhythmias over a period of 15 years is similar in persistently hypertensive and control subjects of both sexes.

The prevalence of arrhytmias after exercise are put in parenthesis. While in 1969 one in 29 hypertensive subjects had heart-rate imparirment after exercise testing, fifteeen years later eight men with persistent hypertension had this abnormality. Of 28 control subjects who were exercise tested in 1969 one had arrhythmia, while fifteen years later there were five. Most changes took place over the last four years. The differences are statistically significant. The findings for women are similar, but the differences are not statistically significant.

Table 4.

Arrhythmias in the electrocardiogram at rest (and after exercise) over a 15-year period

SEX	YEARS	1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		1969 and 1972 Elevated blood pressure		TOTAL
		Normal values	Pathologic. values	Normal values	Pathologic. values	Normal values	Pathologic. values	Normal+Pathological values
Men	1969	28 (28)	1 (1)	27 (25)	1 (3)	28 (27)	2 (6)	85 n.s. (ns)
	1980	23 (29)	6 (0)	23 (28)	5 (0)	24 (27)	1 (3)	85 n.s. (ns)
	1984	23 (21)	6 (8)	27 (21)	1 (7)	24 (23)	1 (4)	85 n.s. (ns)
							p=0.4131	(p=0.0324)
Women	1969	28 (27)	0 (1)	27 (28)	1 (0)	23 (27)	4 (5)	83 n.s. (ns)
	1980	24 (27)	4 (1)	23 (27)	5 (1)	24 (25)	3 (6)	83 n.s. (ns)
	1984	24 (23)	4 (5)	27 (25)	1 (3)	20 (23)	7 (8)	83 n.s. (ns)
							p=0.4341	(p=0.7355)

Discussion

Haemodynamic stresses and biological changes over one's lifetime may cause many effects[15]. How they will affect the cardiovascular system, when and to what extent, will depend on many factors including the much referred to risk factors. It appears, however, that in interpreting certain findings the effect of risk factors may be rather difficult to assess and define strictly, even when objective measuring methods are applied[14].

For determining the effect of high blood pressure on the heart in our sample, we used the resting and exercise ECG and applied it repeatedly. The methodology was essentially the same over the 15-years. Analysis of resting ECG changes in three groups of subjects separately for sexes shows that no major significant differences between hypertensive and control subjects were present at the first examination in 1969. Thus, it took 11 to 15 years for the differences between hypertensive and control subjects to become noticeable in S-T segment depression. T-wave changes, ventricular conduction impairment, arrhythmias and other resting ECG abnormalitis did not differ within groups over the 15-year period. ST-segment depression is the most frequent ECG response which can be singled out over such a long period of study. In the sample of 4.214 persons from which ours was selected at a later stage the ECG abnormalities detected (left axis deviation, and tall R wave) were associated with high blood pressure[15]. The question of whether ECG changes, as realistic indicators, manifest themselves in hypertensive persons differently to the other subjects in the follow-up period remains to be answered. The finding for women is especially confusing as those in the 1984 control group and those who were periodically hypertensive had significantly more changes in ST-segment depressions

than the women who were classified as persistently hypertensive. Can we use here the results of other authors[16] who do not take ST-segment depression, to a certain degree, in women to be pathological or at least not in the same measure as in men to explain our own results? However, whether ST changes are the result of high blood presure or of some other cause, the fact remains that in this sample, of all resting ECG changes examined over the 15-year period, ST-segment depression in men, not in women is found to be the most common one, while all other ECG changes are less so.

Although the frequencies of ST segment depression after exercise are higher than at rest, statistical differences within groups are not significant. ST changes found to be more common in 1980 and 1984 than in 1969, are not significant within individual groups. Such findings, which are not greatly different from the ones recorded earlier, are similar to the results of analysis of T-changes but different to those pertaining to arrhythmia following exercise (mainly frequent ventricular premature beats). In males it has been possible to establish a relationship between arrhythmias and high blood pressure in the 15-year period, with the main differences occurring in the last four years. In women only tendencies could be noted as far as the finding of arrhythmias is concerned. It is pointed out that there are no statistically significant differences between hypertensive and control subjects. Thus, a key factor here appears to be the time factor of 15 and 4 years respectively, while the effect of high blood pressure on the prevalence of arrhythmias after exercise could not be separated. This finding can be compared with the results of Kramer et. a1[14]. who only established a relationship between progression of atherosclerosis and the time constant, but were unable to single out specific effects of hypertension and other risk factors. Our data indicate that the finding of arrthythmias in the examined sample is pathological. Namely, in 1984 eight (28%) out of 29 men with persistent hypertension had arrhythmias in contrast to five (18%) control subjects. Also, of 28 women with persistent hypertension five (18%) had arrhythmias in contrast to four (15%) from the control grup, which, to some extent, is similar to the prevalence of arrhythmias in patients with coronary heart disease[17,18]. Evidently, the effect of blood pressure on post-exercise ECG changes in the sense of arrhythmias has a centrain meaning and the etiopathogenesis of heart function impairment[19] has to do with arterial hypertension. However, a number of other factors are likely to be involed[20]. It should be noted that the physical exercise test in 1969 was carried out on a bicycle ergometer in contrast to later tests.

Apart from high blood pressure, it is not possible to exclude the effects of other risk factors on the obtained results.

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