ORIGINALNI RAD

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INTERAKCIJE PROKAINA SA KLJUČNIM PROTEINIMA U RAZVIĆU SRCA ZEBRICA: *IN SILICO* ANALIZA

Nikola Mitović[1], Milica Milošević[2], Maša Ristić[3], Ljubica Dimitrijević[4], Sanjin Kovačević[1], Jelena Nešović Ostojić[1], Marija S. Stanković[1]

[1] Institut za patološku fiziologiju, Medicinski fakultet Univerzitet u Beogradu, Beograd, Republika Srbija
[2] Institut za kardiovaskularne bolesti „Dedinje", Beograd, Republika Srbija
[3] Klinika za endokrinologiju, dijabetes I bolesti metabolizma, Univerzitetski klinički centar Srbije, Beograd, Republika Srbija
[4] Specijalna Bolnica „Sveti Sava", Beograd, Republika Srbija

[*] Korespondencija: Nikola Mitović, Institut za patološku fiziologiju, Medicinski fakultet Univerziteta u Beogradu, Beograd, Republika Srbija; e-mail: nikolamitovic@gmail.com

SAŽETAK

Uvod/Cilj: Prokain je lokalni anestetik iz aminoestarske grupe koji blokira natrijumove voltažno-zavisne kanale. S obzirom na to da može proći kroz placentu u jonizovanom obliku, postavlja se pitanje njegovog uticaja na embrionalni razvoj. Zebrice, koje imaju značajan stepen homologije sa ljudskim genomom, omogućavaju proučavanje razvoja kardiovaskularnog sistema sa pouzdanom ekstrapolacijom na čoveka. Ispitivanje uticaja prokaina na ključne proteine u razvoju srca zebrica pomoću molekularnog dokinga.
Metode: Prvo je izvršen skrining interakcije prokaina i celog ljudskog proteoma koristeći *FINDSITE*^{comb} softver. Na osnovu značajnih interakcija sa visokim stepenom preciznosti analize, selektovano je 113 proteina. Pomoću *ZFIN* baze je određen stepen homologije između selektovanih ljudskih proteina sa zebričinim, tkivna-specifičnost i vremena ekspresije. Devet proteina su ispunili sve kriterijume: *kcnh6a, kcnh7, kcnh5a, kcnh2a, psen2, rbfa* i *zfpl1* i dalje su ispitivani molekularnim dokingom u AutoDock Vina programu.
Rezultati: Većina proteina se eksprimira visokom stopom tokom blastule. Doking rezultati su pokazali da *scn1laa* protein i prokain imaju najnižu vrednost Gibsove slobodne energije (-6 kCal/mol), dok je za *zfp11* protein vrednost bila najviša (-4,4 kCal/mol). Vezivanje prokaina na ispitivane proteine pokazalo je slične aminokiselinske sekvence unutar iste familije proteina.
Zaključak: Prokain ostvaruje interakcije sa proteinima uključenim u razvoj srca zebrica u *in silico* uslovima. Dalje analize na živim embrionima su potrebne kako bi se dopunili ovi rezultati.

Ključne reči: prokain, razvoj srca, zebrice, molekularni doking.

Uvod

Prokain predstavlja kratkodelujući lokalni anestetik iz aminoestarske grupe koji se primenjuje u stomatologiji i veterini (1). Mehanizam dejstva ostvaruje putem blokade natrijumovih voltažno-zavisnih kanala. Nakon resorpcije, metaboliše se u plazmi pod dejstvom pseudoholinesteraze koja ga hidrolizuje u para-amino benzoičnu kiselinu koja se putem bubrega izlučuje urinom. Vreme poluživota iznosi svega 7,7 minuta (2). Iako je najizraženije dejstvo prokaina na blokadu natrijumovih kanala u manjoj meri deluje antagonistički i na N-metil-D-aspartat, nikotinske i serotoninske receptore (3).

Prokain u svom jonizovanom obliku može proći kroz placentu, pa se opravdano postavlja pitanje o njegovom eventualnom uticaju na razviće ploda (4). Podaci na ovu temu su deficitarni, pa tako današnje preporuke savetuju da se primenjuje u slučaju stomatoloških ili nekih drugih intervencija samo ukoliko benefiti preovlađuju potencijalne rizike. Zbog svog mehanizma dejstva, efekti prokaina bi pogotovu trebali biti ispitani na razvojne strukture u kojima su jonski kanali visoko eksprimirani, kao što su to npr. srčane ćelije.

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ORIGINAL ARTICLE

INTERACTION OF PROCAINE WITH KEY PROTEINS FOR HEART DEVELOPMENT IN ZEBRAFISH: *IN SILICO* ANALYSIS

Nikola Mitović[1], Milica Milošević[2], Maša Ristić[3], Ljubica Dimitrijević[4], Sanjin Kovačević[1], Jelena Nešović Ostojić[1], Marija S. Stanković[1]

[1] Institute for Pathological Physiology, Faculty of Medicine, University of Belgrade, Belgrade, Republic of Serbia
[2] Institute for Cardiovascular Diseases "Dedinje", Belgrade, Republic of Serbia
[3] Clinic for Endocrinology, Diabetes, and Metabolic Diseases, University Clinical Center of Serbia, Belgrade, Republic of Serbia
[4] Special Hospital "Saint Sava", Belgrade, Republic of Serbia

[*] Correspondence: Nikola Mitović, Institute for Pathological Physiology, Faculty of Medicine, University of Belgrade, Belgrade, Republic of Serbia; e-mail: nikolamitovic@gmail.com

SUMMARY

Introduction/Aim: Procaine is a local anesthetic from the amino ester group that blocks voltage-gated sodium channels. Since it can cross the placenta in its ionized form, its potential impact on embryonic development is of concern. Zebrafish, which have a significant degree of homology with the human genome, allow for the study of cardiovascular system development with reliable extrapolation to humans. Investigation of the effects of procaine on key proteins involved in zebrafish heart development using molecular docking.
Methods: First, a screening of the interaction between procaine and the entire human proteome was performed using FINDSITE^{comb} software. Based on significant interactions with a high degree of analysis precision, 113 proteins were selected. Using the ZFIN database, the degree of homology between the selected human proteins and zebrafish proteins, tissue specificity, and expression timing were determined. Nine proteins met all the criteria: kcnh6a, kcnh7, kcnh5a, kcnh2a, psen2, rbfa, and zfpl1, and were further investigated through molecular docking in the AutoDock Vina program.
Results: Most of the proteins were highly expressed during the blastula stage. Docking results showed that the scn1laa protein and procaine had the lowest Gibbs free energy value (-6 kCal/mol), while the zfp11 protein had the highest value (-4.4 kCal/mol). Procaine binding to the tested proteins revealed similar amino acid sequences within the same protein family.
Conclusion: Procaine interacts with proteins involved in zebrafish heart development under *in silico* conditions. Further analyses on live embryos are needed to complement these findings.

Keywords: procaine, heart development, zebrafish, molecular docking

Introduction

Procaine is a short-acting local anesthetic from the amino ester group that is used in dentistry and veterinary medicine (1). The mechanism of action is achieved by blocking voltage-gated sodium channels. After resorption, it is metabolized in the plasma by the enzyme pseudocholinesterase through hydrolysis into para-amino benzoic acid, which is then excreted by the kidneys via urine. The half-life is only 7.7 minutes (2). Although the most pronounced effect of procaine is on the blockade of sodium channels, it also acts, to a lesser extent, antagonistically on N-methyl-D-aspartate, nicotinic and serotonin receptors (3).

Procaine in its ionized form can pass through the placenta, so the question of its possible influence on the development of the fetus is justified (4). Data on this topic is lacking, and therefore, current recommendations advise that it should be applied in case of dental or some other interventions, only when benefits outweigh potential risks. Due to its mechanism of action, the effects of procaine on developmental structures, in which ion channels are highly expressed, such as heart cells, should be investigated.

Zebrice (lat. *Danio rerio*) su slatkovodne ribe čije potomstvo se pokazalo kao dobar model za ispitivanje razvića i razvojnih abnormalnosti. Zbog relativno kratkog perioda embriogeneze (4 dana) eksperimenti traju kratko, ekonomične su za održavanje, a transparentnost embriona omogućava lako praćenje velikog broja morfometrijskih i funkcionalnih parametara (5). Visok stepen homologije sa ljudskim genomom, promene u morfologiji i funkcionalnosti kardiovaskularnog sistema mogu biti proučavane na zebricama sa pouzdanom ekstrapolacijom na čoveka. Srce embriona je smešteno na prominentnoj poziciji, na ventralnoj strani, omogućavajući dobru vizuelizaciju razvića i analize srčane funkcije (6).

Proces razvića srca je regulisan brojnim mehanizmima u kojima su posrednici različiti proteini (7). Stoga, efekat štetnih noksi može interferirati sa navedenim regulatornim mehanizmima i dovoditi do različitih fenotipskih abnormalnosti. Interakcije noksi i proteina možemo ispitivati molekularnim dokingom kojim se utvrđuje orijentacija, afinitet i interakcija liganda na mestu vezivanja u proteinu (8).

Imajući u vidu sve navedeno, cilj našeg rada bio je ispitivanje interakcija prokaina sa ključnim proteinima u razviću srca zebrica uz pomoć bioinformatičke – doking analize.

Metode

Preliminarni skrining proteoma

Kako bi se utvrdile moguće interakcije između prokaina i proteina domaćina, izvršen je skrining kompletnog ljudskog proteoma korišćenjem *FINDSITE*^{comb} softvera. Ovaj softver analizira strukturu unetog molekula koristeći podatke iz različitih baza kao što su *PDB*, *chEMBL* i *DrugBank*, i poredi potencijalne strukturne sličnosti između unetog molekula i proteina iz ovih baza. Rezultati su prikazani u tabelarnom formatu i obuhvataju mTC vrednost (Tanimoto koeficijent – mera sličnosti između dva elementa), preciznost analize, naziv proteina iz *RefSeq* baze i opis proteina (9).

Za dalja istraživanja odabrani su proteini sa preciznošću analize većom od 70%, što je obuhvatilo ukupno 113 proteina (9). Ove analize su se odnosile na ljudske proteine. Zbog toga je uz pomoć *ZFIN* baze podataka utvrđen stepen homologije između ljudskih proteina i njihovih odgovarajućih kod zebrica, njihova tkivna specifičnost i ekspresije

Tabela 1. Podaci o ispitivanim proteinima preuzeti iz ZFIN baze podataka

Oznaka proteina	Naziv proteina	Lokalizacija	Funkcija
kcnh6a	Voltažno-zavisni kalijumov kanal, familija H, član 6a	Transmembranski protein	Srčana kontrakcija. Koristi se za proučavanje sindroma dugog QT i sindroma kratkog QT intervala. Eksprimiran i u probavnom traktu, mišićima, imunskim ćelijama.
kcnh7	Voltažno-zavisni kalijumov kanal, familija H, član 7	Transmembranski protein	Uloga u depolarizaciji i repolarizaciji ćelije. Konstitutivno eksprimiran u većini ćelija.
kcnh5a	Voltažno-zavisni kalijumov kanal, familija H, član 5a	Transmembranski protein	Učestvuje u transmembranskom transportu, održavanju membranskog potencijala
kcnh2a	Voltažno-zavisni kalijumov kanal, familija H, član 2a	Transmembranski protein	Učestvuje u patofiziologiji dugog QT sindroma i kratkog QT sindroma. Eksprimira se u srcu, mišićima, imunskom sistemu.
psen2	Presenilin 2	Endoplazmatski retikulum i Goldžijev aparat	Učestvuje u razvoju dilatativne kardiomiopatije.
rbfa	Ribozom vezujući faktor A	Mitohondrije	Učestvuje u obradi RNK molekula
zfpl1	Protein sličan cinkovim prstima 1	Goldžijev aparat i transmembranski protein	Učestvuje u vezivanju metalnih jona. Zadužen za razvoj srca, retroperiotoneuma i jetre.
scn8aa	Voltažno zavisni Na kanal, tip VIII	Transmembranski protein	Učestvuje u aksonskom transportu, eksprimira se u CNS-u, srcu, trigeminalnom ganglionu.
scnl1a	Voltažno zavisni Na kanal, tip I, alfa	Transmembranski protein	Učestvuje u aksonskom transportu, eksprimira se u CNS-u, srcu, nervnoj cevi.

Zebrafish (lat. *Danio rerio*) are freshwater fish whose offspring was shown to be a good model for the examination of growth and developmental abnormalities. Due to the relatively short period of embryogenesis (4 days), the experiments last a short time, they can be maintained at low cost, while the transparency of the embryo enables easy monitoring of a large number of morphometric and functional parameters (5). A high degree of homology with the human genome, changes in the morphology and functionality of the cardiovascular system can be studied in zebrafish with the reliable extrapolation to humans. The heart of the embryo is located in a prominent position, on the ventral side, thus allowing the good visualization of development and the analysis of cardiac function (6).

The process of heart development is regulated by numerous mechanisms in which various proteins are mediators (7). Therefore, the effect of noxious stimuli can interfere with the above mentioned regulatory mechanisms and lead to different phenotypic abnormalities. The interactions between noxious stimuli and proteins can be examined with the help of molecular docking, which determines the orientation, affinity and interaction of ligands at the binding site in the protein (8).

Having in mind all the above mentioned, the aim of our study was to examine the interaction between procaine and key proteins in the development of zebrafish with the help of bioinformatics – docking analysis.

Methods

Preliminary screening of the proteome

In order to determine possible interactions between procaine and host proteins, the complete human proteome was screened using FINDSITEcomb software. This software analyzes the structure of the input molecule using data from various databases such as PDB, chEMBL and DrugBank, and compares potential structural similarities between the input molecule and proteins from these databases. The results are presented in the form of tables and they include the mTC value (the Tanimoto coefficient – a measure of similarity between two elements), the precision of analysis, the name of the protein from RefSeq database and the description of the protein (9).

The proteins with the accuracy of analysis higher than 70% were selected for further research,

Table 1. Data on the examined proteins obtained from the ZFIN database

Protein label	Full name of the protein	Localization	Function
kcnh6a	Potassium Voltage-Gated Channel Subfamily H Member 6a	Transmembrane protein	Cardiac contraction. Used for studying long QT syndrome and short QT interval syndrome. Also expressed in the digestive tract, muscles, immune cells.
kcnh7	Potassium Voltage-Gated Channel Subfamily H Member 7)	Transmembrane protein	Role in cell depolarization and repolarization. Constitutively expressed in most cells.
kcnh5a	Potassium Voltage-Gated Channel Subfamily H Member 5a	Transmembrane protein	Participates in transmembrane transport and maintenance of membrane potential.
kcnh2a	Potassium Voltage-Gated Channel Subfamily H Member 2a	Transmembrane protein	Involved in the pathophysiology of long QT syndrome and short QT syndrome. Expressed in the heart, muscles, and immune system.
psen2	Presenilin 2	Endoplasmic reticulum and Golgi apparatus	Involved in the development of dilated cardiomyopathy.
rbfa	Ribosome Binding Factor A	Mitochondria	Participates in RNA molecule processing.
zfpl1	Zinc Finger Protein-Like 1	Golgi apparatus and transmembrane protein	Involved in binding metal ions. Responsible for the development of the heart, retroperitoneum, and liver.
scn8aa	Sodium Voltage-Gated Channel Alpha Subunit 8a	Transmembrane protein	Participates in axonal transport, expressed in the CNS, heart, and trigeminal ganglion.
scnl1a	Sodium Channel, Non-Voltage-Gated 1 Alpha Subunit	Transmembrane protein	Participates in axonal transport, expressed in the CNS, heart, and neural tube.

tokom embriogeneze (10). Na kraju, ustanovili smo da 9 proteina ispunjava sve kriterijume (homologi proteini koji su eksprimirani tokom embriogeneze u srcu zebrica), te su oni dalje analizirani. Nazivi, funkcije i lokalizacija ovih proteina preuzeti su iz *ZFIN* baze i prikazani su u Tabeli 1.

Priprema za molekularni doking

Strukture proteina (receptora) dobijene kristalografijom su preuzete sa *PDB* ili *AlphaFold* sajta. 3D struktura prokain-hidrohlorida (liganda) je preuzeta sa *PubChem*-a (11).

Molekularni doking

Molekularni doking je metoda koja se koristi za predviđanje toga kako će se ligand orijentisati i interagovati na mestu vezivanja unutar proteina (receptora). U ovom istraživanju primenili smo *AutoDock Vina* softver za predviđanje ovih interakcija, što je tehnika koja se široko koristi u procesu otkrivanja novih lekova (12).

Prilikom rada u ovom programu, prvo se unose strukture proteina u *PDB* formatu i liganda u *SDF* formatu, a zatim se, prema potrebi, vrše modifikacije. U našim analizama uklonili smo molekule vode iz proteina, jer one često nemaju značajnu ulogu u procesu vezivanja, čime smo olakšali računanje. Takođe, dodali smo polarne atome vodonika i naelektrisanja receptorima kako bismo povećali broj potencijalnih veznih mesta pre početka procesa dokinga. Proces je podešen tako da su sve veze liganda rotirajuće, dok su veze receptora fiksirane (12).

Nakon pokretanja dokinga, program simulira interakcije između različitih funkcionalnih grupa receptora i liganda. Na kraju, program prikazuje deset najrelevantnijih interakcija, uz vrednosti Gibsove slobodne energije i srednjeg kvadratnog odstupanja pozicija atoma (RMSD). Niže, negativne vrednosti Gibsove slobodne energije ukazuju na veću verovatnoću spontane reakcije, odnosno jače vezivanje između receptora i liganda, dok *RMSD* vrednosti omogućavaju procenu tačnosti dobijenih rezultata dokinga (12).

Rezultati

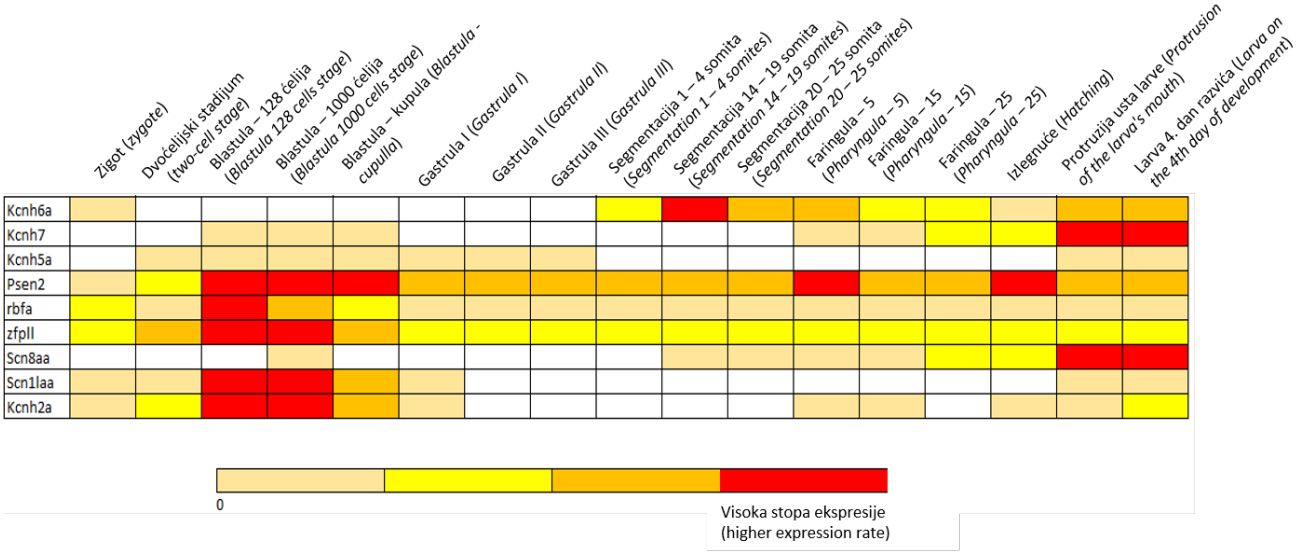
Stepen ekspresije ispitivanih proteina

Koristeći podatke preuzete sa *ZFIN* baze, kreirane su termalne mape koje prikazuju vreme i intenzitet ekspresije analiziranih proteina. Na X-osi mape nalazi se podela na 17 segmenata, pri čemu svaki segment predstavlja određenu fazu razvoja u kojoj se dešavaju specifične promene. Na Y-osi su prikazani ispitivani proteini. Ove mape omogućavaju praćenje dinamičkih promena u ekspresiji proteina tokom različitih faza embrionalnog razvoja, pružajući uvid u potencijalnu ulogu svakog proteina u tim ključnim periodima.

Prema dobijenim podacima, većina proteina (5 od 9) se eksprimira visokom stopom tokom perioda blastule (128 ćelija i 1000 ćelija) (Grafikon 1).

Molekularni doking

Molekularni doking je odrađen uz pomoć *AutoDock Vina* programa, gde su za poređenja uzete



Grafikon 1. Termalna mapa sa prikazom nivoa ekspresije ispitivanih proteina kroz različite periode embriogeneze

and 113 proteins were included (9). These analyses were related to human proteins. Therefore, the degree of homology between human proteins and the corresponding ones in zebrafish was determined with the help of the ZFIN database, as well as their tissue specificity and expression during embryogenesis (10). In the end, we found that 9 proteins met all the criteria (homologous proteins that are expressed during embryogenesis in the heart of zebrafish), and therefore, they were further analyzed. Names, functions and localization of these proteins were taken from the ZFIN database and are shown in Table 1.

Preparation for molecular docking

The structures of proteins (receptors), which were obtained by crystallography, were downloaded from PDB or AlphaFold website. 3D structure of procaine-hydrochloride (ligand) was downloaded from PubChem (11).

Molecular docking

Molecular docking is a method used to predict how a ligand will be oriented and how it will interact at the binding site in the protein (receptor). In this study, we applied the AutoDock Vina software for predicting these interactions, which is a technique that is widely used in the process of discovering new drugs (12).

While working in this program, the structures of proteins in PDB format and ligands in SDF format are entered first, and then, if necessary, modifications are made. In our analyses, we removed water molecules from proteins, because they often do not have a significant role in the binding process, thus simplifying the calculations. Also, we added polar hydrogen atoms and electric charges to receptors in order to increase the number of potential binding sites before the docking process starts. The process is set so that all ligand bonds are rotatable, while receptor bonds are fixed (12).

After docking has been started, the program simulates the interactions between different functional groups of the receptor and ligand. Finally, the program, displays the ten most relevant interactions, along with the values of Gibbs free energy and the root mean square deviation of atomic positions (RMSD). Lower, negative values of Gibbs free energy indicate a higher probability of spontaneous reaction, that is, a stronger binding between the receptor and ligand, while the RMSD values enable the assessment of the accuracy of obtained results of docking (12).

Results

The degree of expression of examined proteins

Using data downloaded from the ZFIN database, thermal maps, which show the time and intensity of expression of analyzed proteins, were created. There is a division into 17 segments on the X-axis of the map, where each segment represents a certain stage of development, in which specific changes occur. The examined proteins are shown

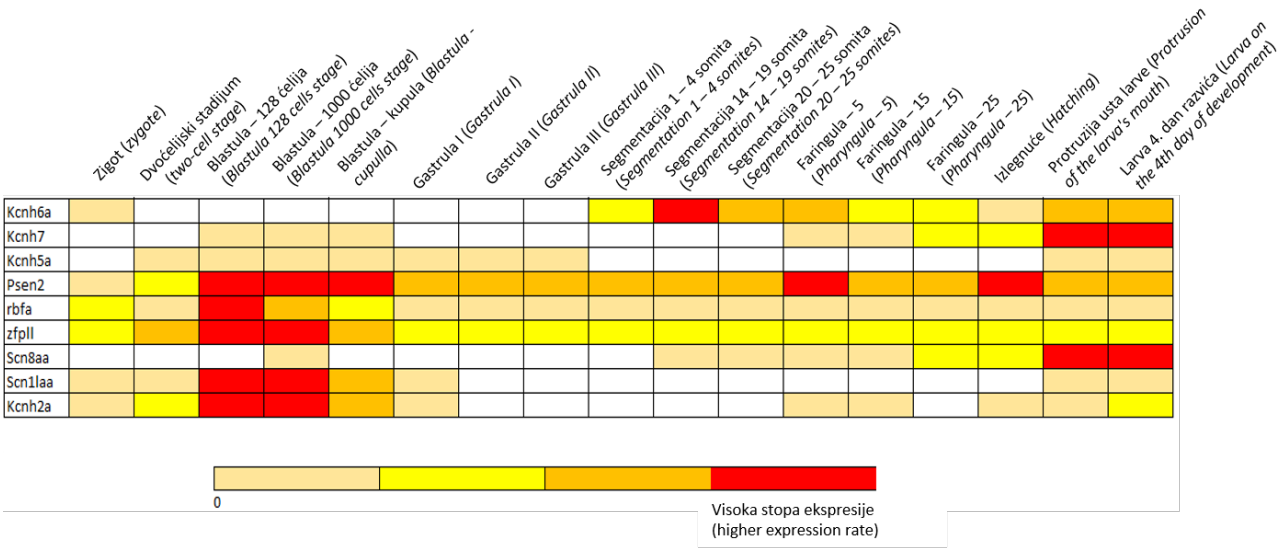


Figure 1. Heat map showing the expression levels of the examined proteins through different stages of embryogenesis

Grafikon 2. Vrednosti Gibsove slobodne energije u interakcijama izmedju prokaina i ispitivanih proteina. Prikazane vrednosti za svaki protein imale su RMSD niže od 3 (visoka preciznost dokinga), što je činilo po 2 interakcije po proteinu.

interakcije gde su vrednosti *RMSD*-a bile niže od 3 (za veće vrednosti se smatra da je preciznost analize mala).

Interakcija *scn1laa* proteina i prokaina je imala najnižu vrednost Gibsove slobodne energije i iznosila je - 6 kCal/mol. Za protein *zfp11* je vrednost Gibsove slobodne energije bila najmanja i iznosila je - 4,4 kCal/mol. Vrednosti energija ostalih interakcija su date u grafiku 2.

Pored vrednosti energija, ispitivali smo i vezna mesta prokaina na ispitivanim proteinima i pokazali da postoji određeni stepen sličnosti u vezivanju između proteina koji pripadaju familiji natrijumovih (*scn8aa*, *scn1la*) i kalijumovih (*kcnh6a*, *kcnh7*) voltažno-zavisnih kanala (Tabela 2). U slučaju natrijumovih kanala najčešće vezno mesto je sadržalo aminokiselinsku sekvencu koja ima u sebi leucin (LEU) i asparagin (ASN), dok u slučaju kalijumovi kanala je bila sekvenca koja sadrži histidin (HIS) i ileucin (ILE).

Diskusija

Prokain je kratkodelujući estarski lokalni anestetik koji se, uglavnom, danas primenjuje u stomatologiji i veterini (1). Prokain za sada nije ispitan u kontekstu embriotoksičnosti, ali je dozvoljen za primenu kod trudnica pre izvođenja dentalnih intervencija u posebnim indikacijama (2, 13). Efekti prokaina se ostvaruju blokadom voltažno zavisnih natrijumovih kanala, i posledič-

Tabela 2. Aminokiselinske sekvence i njihova redna mesta u ispitivanim proteinima sa kojima je prokain ostvario veze

Oznaka proteina	Aminokiselinska sekvenca za koju se prokain vezuje
kcnh6a	Ile523, Ile511, His1131
kcnh7	Ser36, His365, Leu364
kcnh5a	Tyr210, Trp292, Arg367
kcnh2a	Asn467, Phe468, Ile464
psen2	Trp208, Leu204, Phe184
rbfa	Arg294, Hist162, Asp267
zfpl1	Phe86, Trp82, Asn85
scn8aa	Asn904, Arg965, Lue1009
scnl1a	Phe1472, Asn885, Leu990

Skraćenice: Ile - ileucin, His – histidin, Ser – serin, Leu – leucin, Tyr – tirozin, Trp – triptofan, Arg – arginin, Asn – asparagin, Phe – fenil-alanin.

Figure 2. Gibbs free energy values in interactions between procaine and the examined proteins. The displayed values for each protein had RMSD below 3 (high docking precision), resulting in 2 interactions per protein.

on the Y-axis. These maps enable the monitoring of dynamic changes in the expression of proteins during different stages of embryonic development, providing insight into the potential role of each protein in those key periods.

According to the obtained data, most of the proteins (5 out of 9) are expressed at a high rate during the blastula period (128 cells and 1000 cells) (Figure 1).

Molecular Docking

Molecular docking was performed with the help of AutoDock Vina program, where comparisons were made using interactions, where RMSD values were lower than 3 (for higher values, the accuracy of the analysis was considered low).

The interaction between scn11aa protein and procaine had the lowest value of Gibbs free energy and it amounted to -6 kCal/mol. For the protein zfp11, the Gibbs free energy value was the lowest and it amounted to -4.4 kCal/mol. The energy values of other interactions are presented in Figure 2.

In addition to energy values, we also examined the binding sites of procaine on examined proteins and we showed that there was a certain degree of similarity in binding between proteins that belong to the family of sodium (scn8aa, scn1la) and potassium (kcnh6a, kcnh7) voltage-gated channels (Table 2). In the case of sodium channels, the most

Table 2. Amino acid sequences and their positions in the examined proteins with which procaine formed interactions

Protein label	Amino acid sequence to which procaine binds
kcnh6a	Ile523, Ile511, His1131
kcnh7	Ser36, His365, Leu364
kcnh5a	Tyr210, Trp292, Arg367
kcnh2a	Asn467, Phe468, Ile464
psen2	Trp208, Leu204, Phe184
rbfa	Arg294, Hist162, Asp267
zfpl1	Phe86, Trp82, Asn85
scn8aa	Asn904, Arg965, Lue1009
scnl1a	Phe1472, Asn885, Leu990

Abbreviations: Ile – Isoleucine, His – Histidine, Ser – Serine, Leu – Leucine, Tyr – Tyrosine, Trp – Tryptophan, Arg – Arginine, Asn – Asparagine, Phe – Phenylalanine.

no sprečavanjem prenosa signala (2). Poznavajući efekte postavlja se opravdano pitanje uticaja ovog anestetika na razviće struktura koji obiluju jonskim kanalima, kao što je srce. Pored klasičnih *in vivo* modela za ispitivanje toksičnosti danas se sve više koriste i kompjuterske analize i simulacije – *in silico* analize (14).

Razviće je dinamičan proces u kome dolazi do velikog broja promena koje su orkestrirane regulisanim stopama ekspresije različitih proteina (15). U našem radu smo pokazali da je većina proteina eksprimirana u visokoj stopi u periodu blastule, 2 h i 15 min nakon oplođenja, što nam može ukazati na kritičnu tačku u razviću gde bi prokain mogao ostvariti toksične efekte. Ekstrapolirajuću ove podatke na čoveka, ukazujemo na činjenicu da je ovo vrlo rani period kada obično žena ni ne sumnja da je trudna. Tarner i autori su sproveli studiju na 60.000 trudnica tokom 6 godina sa primenom prokaina tokom dentalnih intervencija i nisu pokazali da dolazi do značajnog porasta u komplikacijama na plodu (16). Međutim, u ovom radu su trudnice bile izlagane prokainu u odmakloj trudnoći i dobijeni rezultati mogu da se objasne ili gore navedenom kritičnom tačkom ili razlikom u metabolisanju prokaina kod čoveka i zebrica.

Na osnovu rezultata molekularnog dokinga utvrdili smo da prokain, u srcu zebrica, najviše ostvaruje efekte na kalijumove i natrijumove voltažno-zavisne kanale što je u skladu sa njegovim primarnim efektom (2). Pored ovih, ističu se još 3 proteina: presenilin 2, ribozom vezujući faktor A i protein sličan cinkovim prstima 1, koji su bitni za razviće srca ali ne posreduju u transportu navedenih jona. Njihove funkcije su vezane za različite procese, poput energetskog metabolizma, sinteze proteina, obrade primarnog tranksripta itd. (17–20). Uzimajući u obzir rezultate radova u kojima su animalnim modelima isključivani geni koji kodiraju ispitivane proteine može se očekivati ispad i u strukturi (nepravilno organizovana srčana mišićna vlakna i fibroza) i u funkciji srčanog mišića (bradikardija i produžen QT interval) (21,22).

Na kraju smo ispitivali vezna mesta prokaina na proteinima. Slične aminokiselinske sekvence su utvrđene među proteinima koji pripadaju istim familijama što ukazuje na blisko filogenetsko poreklo (23). Hov i autori su sekvencirali genom zebrica i pokazali homologiju u 70% gena u odnosu na čovekov genom (24). Filogenetski starije strukture poput jonskih kanala, pokazuju visok stepen homologije među vrstama pa je značaj naših dobijenih rezultata utoliko veći jer možemo pretpostaviti da će iste interakcije biti ostvarene i na nivou čovekovih (homologih) proteina (25).

Zaključak

Prokain u in silico uslovima ostvaruje značajne interakcije sa proteinima koji učestvuju u razvoju srca kod zebrica. Posebno je uočeno da je većina ovih proteina visoko eksprimirana u fazi blastule, što potencijalno označava ključnu tačku u razvoju u kojoj bi prokain mogao ispoljiti najtoksičnije efekte. Ekstrapolacija ovih rezultata na čoveka ukazuje da je ovo rani stadijum trudnoće, kada još uvek nisu prisutni znaci koji upućuju na graviditet što može biti značajan aspekt u kontekstu budućih primena prokaina. Dobijene rezultate ne treba koristiti samostalno već u sprezi sa rezultatima na živim embrionima koje treba dopuniti u nekim budućim istraživanjima.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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common binding site contained the amino acid sequence including leucine (LEU) and asparagine (ASN), while in the case of potassium channels, it was a sequence containing histidine (HIS) and isoleucine (ILE).

Discussion

Procaine is a short-acting local anesthetic, which is today mainly used in dentistry and veterinary medicine (1). Procaine has not been examined so far in the context of embryotoxicity, but it has been allowed for use in pregnant women before dental interventions in special indications (2,13). The effects of procaine are achieved by blocking voltage-gated sodium channels, and consequently by preventing transmission of signals (2). Knowing the effects, the legitimate question is raised about the impact of this anesthetic on the development of structures rich in ion channels, such as the heart. In addition to classic *in vivo* models for toxicity testing, today computer analyses and simulations – *in silico* analyses are increasingly used (14).

Development is a dynamic process, in which a large number of changes occur that are orchestrated by the regulated expression rates of various proteins (15). In our study, we have shown that most proteins are expressed at a high rate in the blastula period, 2h and 15 minutes after fertilization, which may indicate a critical point in development, where procaine could have toxic effects. By extrapolating these data to humans, we have pointed to the fact that this is the very early period when usually a woman does not even suspect that she is pregnant. Turner et al. conducted a study that included 60,000 pregnant women during 6 years, when procaine was administered during dental interventions and they did not show a significant increase in fetal complications (16). However, in this study, pregnant women were exposed to procaine in late pregnancy, while the obtained results can be explained either by the above mentioned critical point or by the difference in metabolizing procaine in humans and zebrafish.

Based on the results of molecular docking, we determined that procaine, in the heart of zebrafish, had most effects on potassium and sodium voltage-gated channels, which is in accordance with its primary effect (2). In addition to these, three more proteins stand out: presenilin2, ribosome-binding factor A and zinc finger-like proteins, which are important for the development of the heart but do not mediate in the transport of the mentioned ions. Their functions are connected with various processes, such as energy metabolism, synthesis of proteins, processing of primary transcript etc. (17-20). Taking into consideration the results of studies, in which protein-encoding genes were excluded in animal models, disorders related to the structure can be expected (irregularly arranged cardiac muscle fibers and fibrosis), as well as defects related to the function of the cardiac muscle (bradycardia and prolonged QT interval) (21,22).

Finally, we examined the binding sites of procaine on proteins. Similar amino acid sequences have been found among proteins belonging to the same families, which points to the close phylogenetic origin (23). Hov et al. sequenced the genome of zebrafish and showed homology in 70% of genes in comparison to the human genome (24). Phylogenetically older structures such as ion channels show a high degree of homology between species, so the importance of our obtained results is greater because we can assume that the same interactions will be realized at the level of human (homologous) proteins (25).

Conclusion

Procaine under *in silico* conditions achieves significant interactions with proteins that are involved in heart development in zebrafish. It was observed that most of these proteins were highly expressed at the blastula stage, which potentially marks the key point in development where procaine could exert its most toxic effects. The extrapolation of these results to humans indicates that this is the early stage of pregnancy, when signs of pregnancy are not present, which may be an important aspect in the context of future applications of procaine. The obtained results should not be used independently, but together with the results obtained on live embryos, which should be supplemented in some future research.

Competing interests

The authors declared no competing interests.

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