Univerza v Mariboru

UNIVERZA V MARIBORU
FAKULTETA ZA NARAVOSLOVJE IN MATEMATIKO

DOKTORSKA DISERTACIJA

MATEMATIČNO MODELIRANJE VPLIVA NESTEROIDNIH ANTIREVMATIKOV NA ASPIRINSKO INTOLERANCO ASTME

Andrej Dobovišek

Mentor: red. prof. dr. Milan Brumen

Somentor: doc. dr. Aleš Fajmut

Junij 2012

Mentorjema,
red. prof. dr. Milanu Brumnu in doc. dr. Alešu Fajmutu,
se zahvaljujem za številne koristne diskusije in nasvete
pri raziskovanju in izdelavi doktorskega dela.
Vesel sem, da sem se lahko učil od vaju.

Fakulteti za naravoslovje in matematiko Univerze v Mariboru
se zahvaljujem za finančno pomoč pri sofinanciranju doktorskega študija.

Staršema in bratu ter njegovi družini
se zahvaljujem, da so verjeli vame,
mi v času študija stali ob strani in me
med študijem vsestransko podpirali ter razumeli.

Maji in njeni družini se zahvaljujem za podporo ter
razumevanje pri uresničevanju mojih ciljev.

Zahvaljujem se tudi prijateljem, kolegom in znancem, ki so
mi med študijem kakorkoli pomagali.

Povzetek

Pri približno 10−20 % astmatičnih bolnikov se po zaužitju aspirina, ibuprofena ali katerega drugega nesteroidnega antirevmatika (NSAR) razvije bronhokonstrikcija, vnetje sluznic nosu, žrela in vnetje oči ter izpuščaji na koži. Ta pojav imenujemo aspirinska intoleranca. Raziskave kažejo, da so pri aspirinsko-intolerantnih astmatikih v primerjavi z aspirinsko-tolerantnimi astmatiki in neastmatiki bistveno spremenjene genske ekspresije encimov prostaglandin H sintaz 1 in 2 (PGHS1 in PGHS2) ter levkotrien C_4 sintaze (LTC_4S) pri presnovi arahidonske kisline (AA) v belih krvnih celicah. Ključnega pomena pri razvoju bronhokonstrikcije in drugih simptomov aspirinske intolerance sta produkta presnove AA: vnetni mediator levkotrien C_4 (LTC_4) in protivnetni prostaglandin E_2 (PGE_2). NSAR inhibirajo PGHS1 in PGHS2, kar zniža koncentracijo PGE_2 in poviša koncentracijo LTC_4 v celicah. V doktorskem delu izdelamo matematični model, ki omogoča študij vpliva NSAR na pojav aspirinske intolerance. Osredotočimo se na modeliranje ciklooksigenazne in lipoksigenazne poti presnove AA, pri čemer upoštevamo tudi inhibitorni učinek NSAR na encima PGHS1 in PGHS2. Časovni potek koncentracije NSAR v krvni plazmi po zaužitju zdravila pa opišemo s standardnim farmakokinetičnim modelom, v katerem upoštevamo fazo absorpcije zdravila v krvno plazmo in eliminacije iz krvne plazme. Razmerje med koncentracijama PGE_2 in LTC_4 uporabimo kot osrednji kriterij za napoved bronhokonstrikcije, pri čemer je tveganje za pojav bronhokonstrikcije večje, kadar je razmerje med koncentracijama PGE_2 in LTC_4 manjše od ena. Z modelom preučujemo pojav aspirinske intolerance na treh različnih ravneh; najprej na ravni ekspresij encimov PGHS1, PGHS2 in LTC_4S, nato na ravni produkcije ključnih metabolitov PGE_2 in LTC_4 v celici in nato še na ravni organov, kjer preučujemo, kako je pojav bronhokonstrikcije odvisen od doze različnih NSAR. Izvedemo simulacije, v katerih napovemo časovne poteke koncentracij PGE_2 in LTC_4 v odvisnosti od različnih ekspresij encimov PGHS1, PGHS2 in LTC_4S pri neastmatikih, aspirinsko-tolerantnih astmatikih in treh različnih populacijah aspirinsko-intolerantnih astmatikov. Simulacije izvedemo brez ali ob prisotnosti NSAR. Pokažemo, da je pojav bronhokonstrikcije odvisen od doze uporabljenega NSAR. Za aspirin, ibuprofen in celecoxib ocenimo mejne doze, pri katerih je povišano tveganje za pojav bronhokonstrikcije. Napovemo, koliko časa po zaužitju NSAR se pojavi bronhokonstrikcija in koliko časa traja. Študiramo tudi strategijo, ki bi aspirinsko-intolerantnim astmatikom omogočila varno doziranje NSAR, brez tveganja bronhokonstrikcije. Predlagamo strategijo, pri kateri bi v kombinaciji z NSAR dozirali učinkovini, ki delujeta kot inhibitorja encima 5-lipoksigenaze (5-LOX): sintetični analog

PGE_2 − nocloprost in učinkovina ABT-761. Rezultati kažejo, da bi spremenjeni ekspresiji PGHS1 in LTC_4S utegnili biti osrednja vzroka, ki po zaužitju NSAR vodita do bronhokonstrikcije, znižana ekspresija PGHS2 pa najverjetneje vodi do drugih simptomov aspirinske intolerance. Napovedane mejne doze za aspirin in celecoxib so primerljive z eksperimentalno določenimi, o katerih poročajo v literaturi, za ibuprofen pa so nekoliko nižje. Z modelom napovedana časa od zaužitja NSAR do pojava bronhokonstrikcije in časovni interval trajanja bronhokonstrikcije sta enakega velikostnega reda, kot poročajo v literaturi. Podrobna analiza strategije doziranja NSAR z inhibitorjema 5-LOX kaže, da je nocloprost v kombinaciji z aspirinom potrebno dozirati le enkrat, v kombinaciji z ibuprofenom pa večkrat zaporedoma v različnih dozah, pri čemer so čas doziranja, doza in število doz nocloprosta odvisni od doze ibuprofena. Strategija se bistveno poenostavi, kadar namesto nocloprosta uporabimo ABT-761, ki je močan inhibitor 5-LOX s počasno fazo eliminacije iz krvne plazme.

UDK: 577:539.19(043.3)

543.635.353:616.2(043.3)

Ključne besede: matematični model, aspirinska intoleranca, nesteroidni antirevmatik, arahidonska kislina, levkotrieni, prostaglandini.

Abstract

In around 10-20 % of asthmatic patients ingestion of aspirin, ibuprofen and other non-steroidal anti-inflammatory drugs (NSAIDs) induces bronchoconstriction, inflammation of upper airways and skin rash. This phenomenon is called aspirin intolerance. Clinical data show that altered expressions of enzymes prostaglandin H synthases 1 and 2 (PGHS1 and PGHS2) and leukotriene C_4 synthase (LTC_4S) in arachidonic acid (AA) metabolism could be of central importance for occurrence of aspirin intolerance. The main role is attributed to AA metabolites: inflammatory mediator leukotriene C_4 (LTC_4) and anti-inflammatory prostaglandin E_2 (PGE_2). NSAIDs inhibit the enzymes PGHS1 and PGHS2 thus increase LTC_4 production and decrease PGE_2 production. In this work, a mathematical model is elaborated that enables the study of the impact of different NSAIDs on the occurrence of aspirin intolerance. Mathematical model of lipoxygenase and cyclooxygenase pathway in AA metabolism is developed in which inhibitory effect of NSAID on the enzymes PGHS1 and PGHS2 is taken into account. The time course of NSAID plasma concentration is described by the standard pharmacokinetic model with absorption and elimination phases. The ratio between PGE_2 and LTC_4 concentrations is used as the central criterion in predictions of bronchoconstriction. The risk of bronchoconstriction is increased when the ratio between PGE_2 and LTC_4 concentrations is lower than 1. The occurrence of aspirin intolerance is studied on three different levels: on the level of enzymes PGHS1, PGHS2 and LTC_4S expressions, on the level of PGE_2 and LTC_4 production in the cell and on the tissue or organ level, where the occurrence of bronchoconstriction is studied in dependence on different NSAIDs and their doses. We show that the risk of bronchoconstriction depends on the type of NSAID as well as on its dose. The limiting doses of aspirin, ibuprofen and celecoxib that may induce bronchoconstriction are calculated for different populations of aspirin-intolerant asthmatics. Further, we theoretically estimate the time between NSAID dosing and bronchoconstriction as well as duration of bronchoconstriction. We propose the strategy, which could enable safe managing of NSAIDs to aspirin-intolerant patients. The strategy is proposed in which different 5-LOX inhibitors, such as synthetic PGE_2 analogue – nocloprost or ABT-761, are used in combination with NSAIDs in order to avoid bronchoconstriction. Our results identify altered expression of PGHS1 and LTC_4S as the key elements of aspirin intolerance that lead to bronchoconstriction. Decreased expression of PGHS2 may lead to other symptoms of aspirin intolerance. Predicted limiting doses for aspirin and celecoxib are in the same range as measured threshold doses reported in literature. For ibuprofen, limiting

doses are lower than measured threshold doses. Theoretically estimated time between NSAID dosing and bronchoconstriction, as well as the estimated duration of bronchoconstriction for different populations of aspirin-intolerant patients, are in the same order of magnitude as those observed in clinical studies. When nocloprost is used in combination with aspirin only one dose of nocloprost is needed to avoid bronconstriction. In case of ibuprofen, several consecutive doses of nocloprost should be applied. This strategy is remarkably simplified when instead of nocloprost, ABT-761 is used in combination with NSAID.

UDK: 577:539.19(043.3)

543.635.353:616.2(043.3)

Key words: mathematical model, aspirin – intolerance, non steroidal anti-inflammatory drug, arachidonic acid, leukotrienes, prostagladins.

Seznam kratic in simbolov, uporabljenih v doktorskem delu

AA	arahidonska kislina
aiPGs	zaščitni oz. protivnetni prostagladini
PGE_2	zaščitni prostaglandin E_2
piPGs	vnetni prostaglandini
LTA_4	levkotrien A_4
LTB_4	levkotrien B_4
LTC_4	levkotrien C_4
5-HPETE	5-hidroperoksieikozatetraenoična kislina
PGHS1	encim prostaglandin H sintaza 1 oz. ciklooksigenaza 1
PGHS2	encim prostaglandin H sintaza 2 oz. ciklooksigenaza 2
5-LOX	encim 5-lipoksigenaza
LTA_4H	encim levkotrien A_4 hidrolaza
LTC_4S	encim levkotrien C_4 sintaza
MLCK	encim kinaza lahkih verig miozina
MLCP	encim fosfataza lahkih verig miozina
NSAR	kratica za zdravilo − nesteroidni antirevmatik
NA	modelno stanje, s katerim opišemo neastmatike
ATA	modelno stanje, s katerim opišemo aspirinsko-tolerantne astmatike
$AIA^{(1)}$	modelno stanje, s katerim opišemo aspirinsko-intolerantne astmatike z znižano ekspresijo encima PGHS1
$AIA^{(2)}$	modelno stanje, s katerim opišemo aspirinsko-intolerantne astmatike s povišano ekspresijo encima LTC_4S
$AIA^{(3)}$	modelno stanje, s katerim opišemo aspirinsko-intolerantne astmatike z znižano ekspresijo encima PGHS2
$[x]$	koncentracija snovi x
IC_{50}	koncentracija inhibitorja, ki za 50 % zmanjša hitrost encimske reakcije
Rf	razmerje $[aiPGs]/[LTC_4]$
v	hitrost encimske reakcije oz. aktivnost encima

v_{maks}	maksimalna hitrost encimske reakcije oz. maksimalna aktivnost encima
k	hitrostna konstanta
K	ravnotežna ali Michaelis-Mentenina konstanta
D	doza zdravila
F	razmerje med količino doziranega in v krvno plazmo absorbiranega zdravila
V	navidezni volumen porazdelitve zdravila
t_B	čas od doziranja NSAR do pojava bronhokonstrikcije
$t_{Rf=1}$	čas, ko je vrednost razmerja *Rf* enaka ena
$\Delta t_{Rf\leq 1}$	časovni interval, znotraj katerega je vrednost *Rf* manjša ali enaka ena

Kazalo

1 Uvod

Astma je kronična pljučna bolezen, ki v razvitem svetu prizadene približno 10 % ljudi [3, 11, 60]. Oboleli za astmo doživlja občasne ali pogostejše in dalj časa trajajoče astmatične napade, ki se kažejo kot težko in plitko dihanje, sopenje, tiščanje v prsnem košu ter kašljanje, h kateremu je pridruženo tudi izločanje sluzi [60]. Vzrok so zožene dihalne poti zaradi skrčenih gladkih mišičnih celic dihalnih poti, vnetih in odebeljenih sten dihalnih poti ter sluzi v dihalnih poteh [2, 60]. Astmatični napad lahko sprožijo različni alergeni, virusne okužbe dihal, telesni in čustveni napor ter nekatera zdravila [2, 50, 54, 74]. Ti vplivi v organizmu sprožijo produkcijo vnetnih mediatorjev, tj. snovi, ki so v organizmu vpleteni v vnetne procese. Do sedaj so odkrili, da je v patofiziološki mehanizem astme vključenih prek 50 vnetnih mediatorjev, ki se sproščajo iz belih krvnih celic eosinofilcev, bazofilcev, neutrofilcev, makrofagov in mastocitov ter celic endotelnega in epitelnega tkiva [2, 60]. Kot bronhokonstriktorji − snovi, ki sprožijo krčenje gladkih mišičnih celic dihalnih poti − so osrednjega pomena cisteinil levkotrieni, ki jih poleg prostaglandinov prištevamo med eikozanoide, tvorijo pa se kot produkti arahidonske kisline (AA), predvsem v eosinofilcih [2, 6, 9, 74].

Statistični podatki kažejo, da je približno od 10 % do 20 % astmatičnih bolnikov občutljivih na aspirin, ibuprofen in druga podobna zdravila, ki jih uporabljamo za zdravljenje bolečine in vnetja ter jih s skupnim imenom imenujemo nesteroidni antirevmatiki (NSAR). Občutljivost na NSAR se lahko pojavi pri astmatičnih bolnikih, lahko pa se razvije tudi pri povsem zdravih ljudeh [73]. Pri ljudeh v 30. letih se najprej pojavi vnetje nosne sluznice, ki sčasoma postane kronično. V nekaj mesecih se pojavijo še kronične težave z zamašenim nosom in izcedkom iz nosu, izguba vonja ter nosni polipi. Po približno dveh letih se nato pojavijo simptomi astme in občutljivost na aspirin [8, 73, 74]. Značilni simptomi, ki se po zaužitju aspirina ali drugega NSAR razvijejo pri teh bolnikih, so vnetje sluznice zgornjih in spodnjih dihal, oženje dihalnih poti ali bronhokonstrikcija, koprivni izpuščaj oz. urtikarija in otekanje tkiva okoli oči in ustnic ali angioedem [2, 74]. Skupek teh simptomov se v klinični praksi imenuje aspirinska astma ali aspirinska intoleranca [2, 8, 73, 74]. Simptomi aspirinske intolerance nastopijo v povprečju približno od 30 do 90 min po zaužitju NSAR [8, 61, 73, 74], bronhokonstrikcija pa lahko traja tudi do devet ur [69]. Približno 90 % bolnikov kaže simptome, ki so vezani na zgornje in spodnje dihalne poti, približno 10 % pa simptome, ki so

vezani na dihalne poti in kožo [61]. Zelo redki so bolniki, pri katerih se po zaužitju NSAR razvijejo le simptomi vnetja zgornjih dihalnih poti [8].

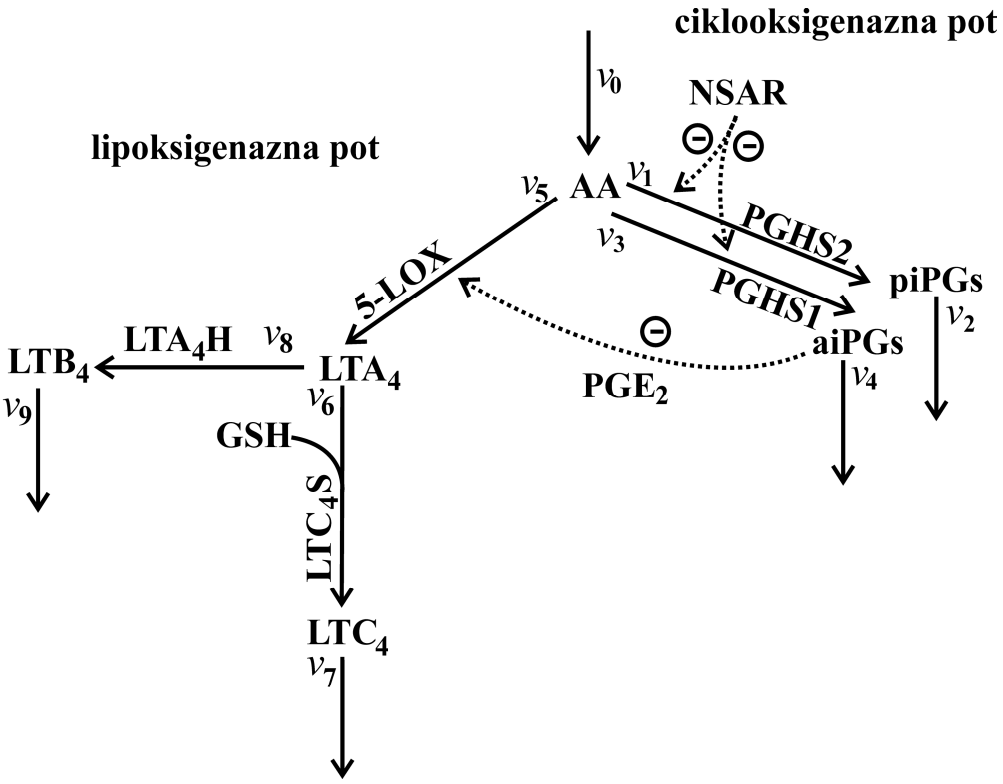
Zdravnik lahko na podlagi pogovora z bolnikom postavi sum na aspirinsko intoleranco, vendar pa se natančna diagnoza in potrditev aspirinske intolerance lahko opravi le na osnovi provokacijskih testov, ki so zaenkrat edina zanesljiva metoda, s katero potrdijo aspirinsko intoleranco [8]. Pri teh testih bolnikom v telo postopoma vnašajo manjše količine aspirina v izbranih časovnih presledkih, nato pa opazujejo, pri kateri dozi NSAR se pojavijo različni simptomi, ali pa s spirometričnimi meritvami merijo pljučno funkcijo bolnika in tako potrdijo bronhokonstrikcijo. Glede na mesto vnosa aspirina v telo ločimo štiri tipe provokacijskih testov: nosni, oralni, bronhialni ali inhalacijski in intravenozni provokacijski test [8, 74]. Postopki izvajanja testov so različni v različnih bolnišnicah. Razlikujejo se po odmerkih, časovnih presledkih v posameznih odmerkih in po kriterijih za pozitivnost testa, zato so testi, ki jih opravijo v različnih bolnišnicah, med seboj težko primerljivi [75].

Aspirin oz. acetilsalicilno kislino je leta 1897 odkril nemški kemik Felix Hoffmann [8]. Kmalu potem, ko so aspirin uvedli v klinično prakso, je leta 1902 Hirschberg pri nekaterih bolnikih prvič opazil občutljivost na aspirin [64]. Leta 1922 so Widal in sodelavci (1922) [80] objavili članek, v katerem so občutljivost na aspirin povezali z astmo in nosnimi polipi [10, 73, 74]. V 60. letih prejšnjega stoletja so raziskovalci spoznali, da patofiziološki mehanizem aspirinske intolerance poteka preko presnove arahidonske kisline (AA) [10]. K razumevanju asprinske intolerance je veliko prispevalo odkritje Johna R. Vanea, ki je v 70. letih prejšnjega stoletja odkril, da aspirin učinkuje na presnovo AA, kjer z inhibicijo encimov prostaglandin H sintaz 1 in 2 (PGHS1 in PGHS2) zavira produkcijo prostaglandinov [8]. Za to odkritje je John R. Vane leta 1982 dobil Nobelovo nagrado za medicino in fiziologijo. Nekaj let po tem odkritju so Szczeklik in sodelavci pokazali, da so bolniki, ki so intolerantni na aspirin, intolerantni tudi na druga zdravila s podobnim učinkovanjem [8].

Novejša spoznanja kažejo, da imata pri razvoju aspirinske intolerance osrednjo vlogo produkta presnove AA, cisteinil levkotrien C_4 (LTC_4) in prostaglandin E_2 (PGE_2) [2, 5, 6, 8, 70, 73, 74]. LTC_4 je agresiven vnetni mediator, ki z vezavo na receptorje na membrani gladkih mišičnih celic dihalnih poti sproži krčenje teh celic [5, 70]. Obstajata dva tipa receptorjev za cisteinil levkotriene, cisteinil levkotrien receptorja 1 in 2 (Cys-LT_1 in Cys-LT_2 receptorja) [47]. V študiji [47] so pokazali, da je za razvoj sile v gladkih mišičnih celicah bistvenega pomena stimulacija celice preko receptorja Cys-LT_1, medtem ko stimulacija celice preko receptorja Cys-LT_2 silo v gladki mišični celici le delno regulira. Aspirinsko-intolerantni astmatiki imajo v primerjavi z aspirinsko-tolerantnimi astmatiki in neastmatiki povišano

število receptorjev Cys-LT_1 in Cys-LT_2 [72, 73, 74] na membrani gladkih mišičnih celic dihalnih poti. PGE_2 je zaščitni oz. protivnetni prostaglandin, ki posredno zavira produkcijo LTC_4 [2, 32, 74] in relaksira gladke mišice dihalnih poti [26, 33, 45, 68].

Tarča učinkovanja NSAR je presnova AA v eosinofilcih in epitelnih ter endotelnih celicah [2, 8, 60, 65, 74]. Presnova AA poteka po dveh poteh − ciklooksigenazni in lipoksigenazni poti [2, 8, 9, 74]. Kinetična shema presnove AA, ki jo predlagamo v naših raziskavah [18, 19, 25], je prikazana na sliki 1.



Slika 1. Kinetična shema presnove AA, ki jo predlagamo Dobovišek in sodelavci [18].

V ciklooksigenazni poti se AA veže na encima prostaglandin H sintazi 1 in 2 (PGHS1 in PGHS2), kot produkti pa nastajajo protivnetni (aiPGs) in vnetni (piPGs) prostaglandini. Encim PGHS1 je stalno prisoten v celicah, PGHS2 pa je inducibilen encim, ki se aktivira ob vnetnih procesih [49]. V lipoksigenazni poti se AA najprej veže na encim 5-lipoksigenazo (5-LOX). V tej reakciji se iz AA tvori najprej 5-hidroperoksieikozatetraenoična kislina (5-HPETE), iz nje pa se v naslednjem koraku tvori levkotrien A_4 (LTA_4) [9]. Obe reakciji katalizira encim 5-LOX. 5-HPETE je zelo nestabilen produkt [38], zato smo v naših raziskavah [18, 19, 25] (glej priloge 1, 2 in 3) upoštevali, da se AA pretvori kar direktno v LTA_4 (glej sliko 1). LTA_4 se nato veže ali na encim levkotrien A_4 hidrolazo (LTA_4H), pri

čemer nastane levkotrien B_4 (LTB_4), ali pa na encim levkotrien C_4 sintazo (LTC_4S), pri čemer se ob prisotnosti glutationa (GSH) tvori LTC_4 [29]. Le-ta se izloči iz celic in se veže naprej na Cys-LT_1 in Cys-LT_2 receptorja na membrani gladkih mišičnih celic dihalnih poti. To sproži nadaljnje procese, ki vodijo do krčenja teh celic. Signalna pot, ki vodi do krčenja gladkih mišičnih celic dihalnih poti, ob njihovi stimulaciji z LTC_4 še ni povsem natančno raziskana.

Pomemben produkt ciklooksigenazne poti presnove AA je zaščitni prostaglandin PGE_2. Le-ta se izloča iz celic, v katerih poteka presnova AA, nato pa z vezavo na receptorje teh istih celic posredno inhibira encim 5-LOX, s čimer posredno zavira produkcijo LTC_4 [2, 32, 74]. Do sedaj so potrdili obstoj štirih vrst receptorjev za PGE_2, prostaglandin E receptorje 1, 2, 3 in 4 (EP_1, EP_2, EP_3 in EP_4 receptorji) [36]. Na membrani gladkih mišičnih celic aspirinsko-intolerantnih astmatikov je v primerjavi z aspirinsko-tolerantnimi astmatiki in neastmatiki število EP receptorjev zmanjšano [86]. Povsem natančno mehanizem, ki vodi do inhibicije encima 5-LOX še ni znan. Po poročanju raziskovalcev [32] je inhibicija 5-LOX reverzibilna in poteka preko deaktivacije t. i. FLAP proteina, ki aktivira 5-LOX. Posrednik v inhibiciji 5-LOX je interleukin 10 (IL-10).

Splošno je znano, da večina NSAR inhibira oba encima PGHS1 in PGHS2 [13, 49, 52, 71, 77], s čimer se zmanjša totalna produkcija prostaglandinov in s tem posledično tudi PGE_2. Ob znižani produkciji PGE_2 oslabi inhibicija encima 5-LOX, presnova AA se preusmeri po lipoksigenazni poti, produkcija vnetnega mediatorja LTC_4 pa se s tem poviša. To lahko posledično vodi do razvoja različnih kliničnih znakov aspirinske intolerance. Ker je opisan mehanizem učinkovanja NSAR enak pri vseh ljudeh, se pojavi vprašanje, zakaj se aspirinska intoleranca ne pojavi pri vseh ljudeh ali pa vsaj ne pri vseh astmatikih [74].

Eksperimentalne študije [15, 57, 58, 74] kažejo, da je genska ekspresija encimov PGHS1, LTC_4S in PGHS2 pri aspirinsko-intolerantnih astmatikih bistveno drugačna kot pri aspirinsko-tolerantnih astmatikih in neastmatikih. V študiji [58] so ugotovili, da je ekspresija encima PGHS1 pri aspirinsko-intolerantnih astmatikih 3-krat nižja kot pri aspirinsko-tolerantnih, med aspirinsko-tolerantnimi astmatiki in neastmatiki pa niso opazili statistično pomembnih razlik. Študiji [15, 74] kažeta, da je ekspresija LTC_4S pri aspirinsko-intolerantnih astmatikih 5-krat višja kot pri aspirinsko-tolerantnih astmatikih in kar 20-krat višja kot pri neastmatikih. Avtorji v študiji [57] pa so pokazali, da je ekspresija PGHS2 pri aspirinsko-intolerantnih astmatikih 6-krat nižja kot pri aspirinsko-tolerantnih astmatikih. Te eksperimentalne raziskave kažejo, da so ekspresije encimov PGHS1, LTC_4S in PGHS2 osrednjega pomena pri razvoju aspirinske intolerance in da bi utegnile obstajati različne populacije aspirinsko-intolerantnih astmatikov.

Schäfer in sodelavci [65] so objavili eksperimentalno študijo, v kateri so merili koncentracijo LTC_4 in PGE_2 v krvni plazmi aspirinsko-intolerantnih in aspirinsko-tolerantnih astmatikov ter neastmatikov pred in po inhalatornem provokacijskem testu z aspirinom [65]. Namen te raziskave je bil izdelati preprost krvni test za diagnozo aspirinske intolerance [65]. Rezultati iz te študije kažejo, da je koncentracija LTC_4 v krvni plazmi aspirinsko-intolerantnih astmatikov že pred samim doziranjem aspirina višja kot pri neastmatikih in aspirinsko-tolerantnih astmatikih, po doziranju aspirina pa se še dodatno poviša. Nasprotno so ugotovili za koncentracijo PGE_2, ki je v krvni plazmi aspirinsko-intolerantnih astmatikov nižja v primerjavi z aspirinsko-toleranimi astmatiki in neastmatiki, po doziranju aspirina pa se še dodatno zniža. Avtorji so z izmerjenimi podatki analizirali tudi razmerje med koncentracijama PGE_2 in LTC_4 v krvni plazmi. Podrobnejša analiza je pokazala, da je to razmerje v krvni plazmi aspirinsko-intolerantnih astmatikov vedno − pred in po doziranju aspirina − manjše od ena, pri aspirinsko-tolerantnih astmatikih in neastmatikih pa vedno večje od ena. Pri vseh aspirinsko-intolerantnih astmatikih, za katere so v študiji z biokemijskimi meritvami določili, da je vrednost zgoraj omenjenega razmerja manjša od ena, so s spirometričnimi meritvami potrdili tudi bronhokonstrikcijo po provokacijskem testu z aspirinom, ekspresij encimov PGHS1, LTC_4S in PGHS2 pa ti avtorji niso podrobneje analizirali. Na podlagi teh eksperimentalno pridobljenih podatkov predlagajo razmerje med koncentracijama PGE_2 in LTC_4 v krvni plazmi kot ustrezen kvantitativni kriterij, na osnovi katerega bi bilo možno ločiti med aspirinsko-intolerantnimi astmatiki in aspirinsko-tolerantnimi astmatiki ter neastmatiki, hkrati pa bi bilo iz vrednosti tega razmerja možno sklepati na povišano tveganje bronhokonstrikcije.

V drugih raziskavah [69, 73, 74, 78] poročajo tudi o mejnih dozah za aspirin in ibuprofen. Mejne doze so tiste, pri katerih so v kliničnih raziskavah pri aspirinsko-intolerantnih astmatikih opazili različne simptome aspirinske intolerance. Te doze predstavljajo zgornjo mejo tolerantnosti aspirinsko-intolerantnih astmatikov na NSAR. Največ študij poroča o mejnih dozah za aspirin. V študiji [69] poročajo, da so mejne doze za aspirin med 325 in 650 mg, v študiji [74] pa, da so mejne doze za aspirin med 30 mg in 150 mg. V študiji [78] poročajo, da so mejne doze za ibuprofen manjše od 400 mg.

Iz zgoraj opisanih eksperimentalnih študij vidimo, da raziskave aspirinske intolerance potekajo na treh različnih ravneh. Prva raven poteka na molekularnem nivoju, kjer raziskovalci [15, 57, 58, 74] merijo genske ekspresije encimov PGHS1, LTC_4S in PGHS2 pri neastmatikih, aspirinsko-tolerantnih astmatikih in aspirinsko-intolerantnih astmatikih. Druga raven raziskav poteka na celičnem nivoju, kjer raziskovalci merijo koncentracijo LTC_4 in

PGE_2 v krvni plazmi neastmatikov ter aspirinsko tolerantnih in aspirinsko-intolerantnih astmatikov ob prisotnosti in odsotnosti NSAR. Ena izmed ključnih raziskav je raziskava Schäferja in sodelavcev [65], v kateri so z biokemijskimi meritvami potrdili, da je za pojav bronhokonstrikcije pri aspirinsko-intolerantnih astmatikih značilno, da je vrednost razmerja med koncentracijama PGE_2 in LTC_4 manjša od ena. Tretjo raven raziskav predstavljajo različne klinične raziskave, v katerih so z opazovanjem kliničnih znakov aspirinske intolerance na ravni tkiv in organov ali s spirometričnimi meritvami določali mejne doze za aspirin in ibuprofen [63, 69, 73, 74, 78].

V naših raziskavah [18, 19, 25] (priloge 1, 2 in 3) predlagamo in razvijemo izviren matematični model, s katerim raziskujemo vpliv NSAR na pojav aspirinske intolerance. Z modelom je mogoče povezati številne eksperimentalne ugotovitve in pojasniti nekatere bistvene vzročne povezave med vsemi tremi zgoraj opisanimi nivoji raziskav. V našem modelu ekspresije encimov PGHS1, LTC_4S in PGHS2 nastopajo kot parametri. Tako eksperimentalno pridobljene podatke o ekspresijah encimov PGHS1, LTC_4S in PGHS2 iz študij [15, 57, 58, 74] uporabimo kot vstopne podatke, na osnovi katerih vpeljemo pet različnih modelnih stanj, s katerimi razlikujemo med neastmatiki, aspirinsko-tolerantnimi astmatiki in tremi različnimi populacijami aspirinsko-intolerantnih astmatikov. Pri tem privzamemo, da je ekspresija encima linearno povezana s totalno koncentracijo encima v celici in direktno vpliva na maksimalno aktivnost encima. Model omogoča, da v različnih modelnih stanjih (tj. za različne vrednosti ekspresij encimov PGHS1, PGHS2 in LTC_4S) preučimo časovne poteke koncentracij LTC_4 in PGE_2 ($[LTC_4]$ in $[PGE_2]$) v odvisnosti od časovno spremenljive koncentracije NSAR, ki jo lahko simuliramo za različne doze. Na ta način lahko pojasnimo, kako ekspresije encimov PGHS1, PGHS2 in LTC_4S vplivajo na časovne poteke $[LTC_4]$ in $[PGE_2]$ v krvni plazmi ob prisotnosti ali odsotnosti NSAR. Z uporabo razmerja $[PGE_2]/[LTC_4]$ kot osrednjega kvantitativnega kriterija za napoved bronhokonstrikcije pri aspirinsko-intolerantnih astmatikih pa lahko napovemo, pri kateri dozi NSAR bo tveganje za pojav bronhokonstrikcije povišano, in ocenimo, kako dolgo bo trajala. Model uporabimo tudi v namene predlogov in raziskav različnih strategij, s katerimi bi aspirinsko-intolerantnim astmatikom lahko omogočili varno doziranje NSAR, brez tveganja bronhokonstrikcije.

V naslednjem poglavju najprej podrobneje predstavimo matematični model, ki smo ga objavili v dveh izvirnih znanstvenih članki [18, 19] in poglavju v knjigi [25]. Predstavimo in opišemo osnovne modelne enačbe ter enačbe za hitrosti posameznih encimskih reakcij v presnovi AA, enačbo za opis časovnega poteka koncentracije zdravila v krvni plazmi in

modelne parametre. Nato sledi poglavje Rezultati in diskusija, ki je razdeljeno na dve podpoglavji. V prvem podpoglavju definiramo modelna stanja, s katerimi opišemo neastmatike, aspirinsko-tolerantne astmatike in tri različne populacije aspirinsko-intolerantnih astmatikov. Temu sledi verifikacija modela. Z le-to preučimo, ali so modelna stanja smiselno definirana. V ta namen analiziramo časovne poteke napovedi modelnih spremenljivk [LTC_4], [PGE_2] in razmerja [PGE_2]/[LTC_4] ob odsotnosti in prisotnosti NSAR ter jih primerjamo z ustreznimi eksperimentalnimi podatki iz literature [65]. Nato izvedemo senzitivnostno analizo, s katero preučimo občutljivost stacionarnih vrednosti razmerja [PGE_2]/[LTC_4] na majhne spremembe v vrednostih ekspresij encimov PGHS1, LTC_4S in PGHS2 v modelnih stanjih, ki ustrezajo opisu aspirinsko-intolerantnih astmatikov. Tudi senzitivnostno analizo izvajamo ob odsotnosti in prisotnosti zdravila v sistemu. V zadnjem podpoglavju podrobneje preučimo odvisnost razmerja [PGE_2]/[LTC_4] od doze NSAR. Najprej pokažemo, da je pojav bronhokonstrikcije odvisen od doze NSAR. Nato napovemo mejne doze izbranih NSAR, njihove vrednosti pa primerjamo z vrednostmi, ki so bile določene v kliničnih raziskavah [69, 78]. Na osnovi modelnih napovedi mejnih doz nakažemo možnosti teoretičnega presojanja tolerantnosti oz. intolerantnosti astmatičnih bolnikov na NSAR. Na koncu tega poglavja podrobneje predstavimo tudi različne strategije, ki bi lahko omogočile varno doziranje NSAR aspirinsko-intolerantnim astmatikom [18, 19, 25]. Osredotočimo se predvsem na strategijo, pri kateri bi poleg NSAR dozirali dva različna inhibitorja encima 5-LOX, sintetični analog PGE_2 – nocloprost ali ABT-761. Bolj podrobno pa z modelom preučimo tudi mejne doze selektivnega inhibitorja encimov PGHS1 in PGHS2 celecoxiba. Temu sledi poglavje s povzetkom bistvenih ugotovitev in zaključkov.

V zadnjem poglavju razpravljamo o nadaljnjih raziskovalnih izzivih in možnostih raziskovanja aspirinske intolerance in drugih boleznih, povezanih s produkti presnove AA. Sledi še literatura, dodatka A in B ter priloge 1, 2 in 3. V dodatku A so predstavljeni osnovni koncepti encimske kinetike, ki jih uporabimo pri zasnovi matematičnega modela, in izpeljava Cheng-Prussoeve enačbe. Slednjo smo v raziskavah uporabljali za izračun inhibitornih konstant zdravil za encime PGHS1, PGHS2 in 5-LOX iz poznanih eksperimentalno določenih koncentracij IC_{50}. V dodatku B podrobneje predstavimo farmakokinetični model, s katerim simuliramo časovni potek zdravil v krvi. V prilogah 1, 2 in 3 pa so predstavljeni izvirni znanstveni prispevki Doboviška in sodelavcev [18, 19] ter Fajmuta in sodelavcev [25], na katerih temelji pričujoče doktorsko delo.

2 Matematični model

V tem poglavju bomo predstavili izvirni matematični model, s katerim smo v naših raziskavah [18, 19, 25] (priloge 1, 2, 3) preučevali vpliv NSAR na pojav aspirinske intolerance. Osnovno ogrodje modela sestoji iz šestih diferencialnih enačb prvega reda, s katerimi opišemo časovni razvoj koncentracije AA in njenih produktov: [*AA*], [*piPGs*], [*aiPGs*], [LTA_4], [LTB_4] in [LTC_4].

$$\frac{d[AA]}{dt} = v_0 - v_1 - v_3 - v_5 \tag{1}$$

$$\frac{d[piPGs]}{dt} = v_1 - v_2 \tag{2}$$

$$\frac{d[aiPGs]}{dt} = v_3 - v_4 \tag{3}$$

$$\frac{d[LTA_4]}{dt} = v_5 - v_6 - v_8 \tag{4}$$

$$\frac{d[LTB_4]}{dt} = v_8 - v_9 \tag{5}$$

$$\frac{d[LTC_4]}{dt} = v_6 - v_7 \tag{6}$$

S simboli v_i, kjer je i = 1 … 9, so označene hitrosti posameznih encimskih reakcij v presnovi AA, kot je prikazano na sliki 1.

V modelu smo upoštevali konstanten dotok AA, v_0 = 0,70 μMs^{-1}. Za to vrednost parametra v_0 dosežejo vse modelne spremenljivke in ostali tokovi stacionarne vrednosti pri fiziološko smiselnih vrednostih parametrov v_{max1}, v_{max3} in v_{max6}, na osnovi katerih z modelom ločimo neastmatike, aspirinsko-tolerantne astmatike in tri različne populacije aspirinsko-intolerantnih astmatikov. Za navedeno vrednost parametra v_0 je [*AA*] približno 10 μM, kar je tudi tipična vrednost, o kateri poročajo v več eksperimentalnih študijah [28, 32, 49]**.**

Aktivnost encima PGHS2 opišemo z Michaelis-Mentenino kinetiko [13, 71]:

$$v_1 = \frac{v_{\text{maks}1}[AA]}{K_1\alpha_1 + [AA]}, \tag{7}$$

kjer je $v_{\text{maks}1}$ maksimalna hitrost reakcije, K_1 Michaelis-Mentenina konstanta in $[AA]$ koncentracija AA. α_1 opiše inhibicijo encima PGHS2 z NSAR:

$$\alpha_1 = 1 + \frac{[NSAR]}{K_{\text{I}1}}, \tag{8}$$

kjer je $K_{\text{I}1}$ inhibitorna ravnotežna konstanta inhibicije encima PGHS2 z NSAR, $[NSAR]$ pa koncentracija zdravila. Pri tem je upoštevana kompetitivna reverzibilna inhibicija, ki je podrobneje opisana v dodatku A2.

Tok piPGs iz celice opišemo z linearno kinetiko [28]:

$$v_2 = k_2[piPGs], \tag{9}$$

pri čemer je $[piPGs]$ koncentracija piPGs, k_2 pa hitrostna konstanta.

Aktivnost encima PGHS1 opišemo z Michaelis-Mentenino kinetiko [13, 71]:

$$v_3 = \frac{v_{\text{maks}3}[AA]}{K_3\alpha_3 + [AA]}. \tag{10}$$

$v_{\text{maks}3}$ je maksimalna hitrost reakcije, K_3 Michaelis-Mentenina konstanta in [AA] koncentracija AA. α_3 opiše inhibicijo PGHS1 z NSAR:

$$\alpha_3 = 1 + \frac{[NSAR]}{K_{\text{I}3}}, \tag{11}$$

kjer je $K_{\text{I}3}$ inhibitorna ravnotežna konstanta inhibicije encima PGHS1 z NSAR, $[NSAR]$ pa koncentracija zdravila. Podobno kot pri opisu inhibicije encima PGHS2 opišemo tudi inhibicijo encima PGHS1 s kompetitivno reverzibilno inhibicijo (dodatek A2).

Tok aiPGs iz celice opišemo z linearno kinetiko [28]:

$$v_4 = k_4[aiPGs], \tag{12}$$

pri čemer je [*aiPGs*] koncentracija aiPGs, k_4 pa hitrostna konstanta.

Aktivnost encima 5-LOX opiše enačba [1]:

$$v_5 = \frac{v_{\text{maks }5}[AA]}{K_5\alpha_5 + [AA]}, \tag{13}$$

kjer je K_5 ravnotežna disociacijska konstanta za kompleks 5-LOX·AA. Inhibicijo encima 5-LOX z aiPGs pa opiše α_5:

$$\alpha_5 = 1 + \frac{[aiPGs]}{K_{I5}}, \tag{14}$$

kjer je [*aiPGs*] koncentracija aiPGs in K_{I5} inhibitorna ravnotežna konstanta inhibicije encima 5-LOX z aiPGs. Tudi tukaj je upoštevana kompetitivna reverzibilna inhibicija. Kot smo opisali že v uvodu, inhibicija 5-LOX ne poteče z direktno vezavo PGE_2 oz. aiPGs na ta encim, temveč z vezavo PGE_2 na EP receptor na celični membrani, IL-10 pa nato deaktivira protein FLAP, kar prepreči aktivacijo encima 5-LOX. Ker vsi koraki tega mehanizma niso dovolj podrobno raziskani, smo predpostavili, da je inhibicija encima 5-LOX sorazmerna kar s koncentracijo PGE_2 v celici.

Aktivnost encima LTC_4S je podana z enačbo [29]:

$$v_6 = \frac{v_{\text{maks }6}[LTA_4]}{A + B[LTA_4] + C[LTA_4]^2}, \tag{15}$$

kjer je [LTA_4] koncentracija LTA_4, A, B in C pa so konstante. V tej reakciji je LTA_4 substrat, ki pri nizkih koncentracijah aktivira encim LTC_4S, pri visokih pa ga inhibira. Pri slednjem gre za t. i. avtoinhibitorni učinek.

Tok LTC_4 iz celice opišemo z linearno kinetiko [55]:

$$v_7 = k_7[LTC_4], \tag{16}$$

kjer je $[LTC_4]$ koncentracija LTC_4, k_7 pa hitrostna konstanta.

Aktivnost encima LTA_4H opiše enačba [31]:

$$v_8 = \frac{v_{\text{maks}\,8}\left[LTA_4\right]}{K_8 + \left[LTA_4\right]}, \tag{17}$$

kjer je $v_{\text{maks}8}$ maksimalna hitrost reakcije, K_8 Michaelis-Mentenina konstanta in $[LTA_4]$ koncentracija LTA_4.

Tok LTB_4 iz celice je opisan z enačbo [42]:

$$v_9 = \frac{v_{\text{maks}\,9}\left[LTB_4\right]}{K_9 + \left[LTB_4\right]}, \tag{18}$$

kjer je $v_{\text{maks}9}$ maksimalna hitrost reakcije, K_9 Michaelis-Mentenina konstanta in $[LTB_4]$ koncentracija LTB_4.

Časovne poteke koncentracij različnih zdravil v krvni plazmi opišemo s standardnim farmakokinetičnim modelom, ki je podrobneje predstavljen v dodatku B. V modelu sta upoštevani fazi absorpcije in eliminacije zdravila v oz. iz krvne plazme:

$$[C](t) = \frac{D^{(\mathrm{j})} k_a^{\,(\mathrm{j})}}{(V/F)^{(\mathrm{j})}\left(k_a^{\,(\mathrm{j})} - k^{(\mathrm{j})}\right)}\left(e^{-k^{(\mathrm{j})}t} - e^{-k_a^{\,(\mathrm{j})}t}\right). \tag{19}$$

V enačbi (19) je $[C]$ koncentracija zdravila, $D^{(\mathrm{j})}$ doza zdravila, $F^{(\mathrm{j})}$ delež absorbiranega zdravila, $V^{(\mathrm{j})}$ navidezni volumen porazdelitve zdravila, $k_{\mathrm{a}}{}^{(\mathrm{j})}$ hitrostna konstanta absorpcije zdravila v krvno plazmo in $k^{(\mathrm{j})}$ hitrostna konstanta eliminacije zdravila iz krvne plazme. Z indeksom (j) smo označili farmakokinetične parametre za posamezno zdravilo. V primerih za aspirin (j = ASA), ibuprofen (j = IBU) in celecoxib (j = CEL) je $C = NSAR$, za ABT-761 (j = ABT) pa je $C = 5\text{-}LOXIB$.

Kot kvantitativni kriterij za napoved bronhokonstrikcije pri aspirinski intoleranci uporabimo razmerje $[aiPGs]/[LTC_4]$, ki ga krajše označimo z Rf:

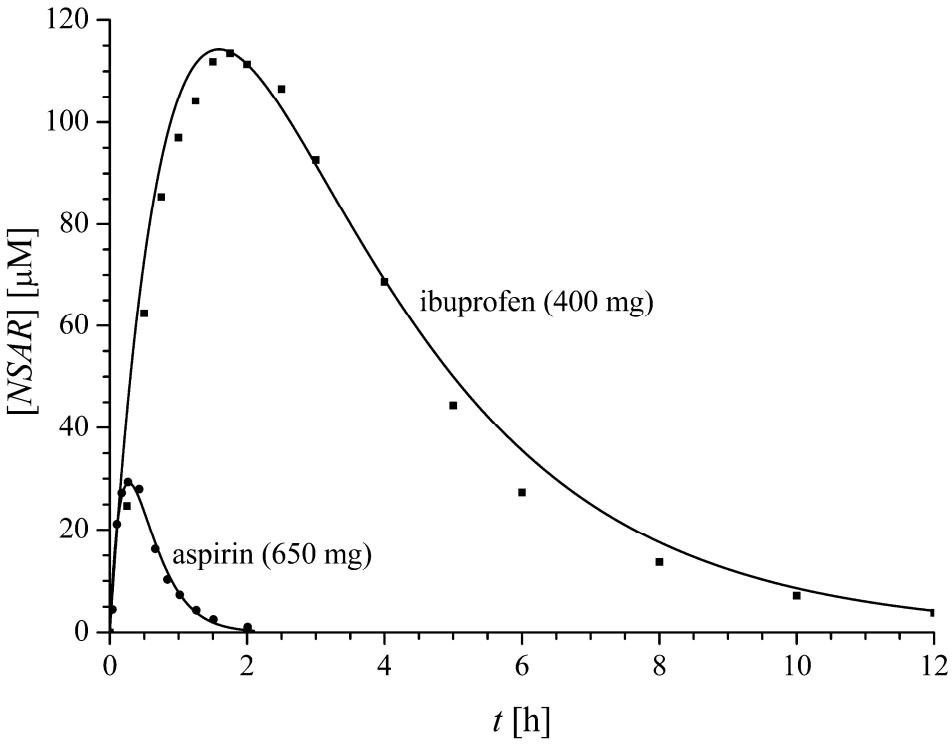
$$Rf = \frac{\left[aiPGs\right]}{\left[LTC_4\right]}. \tag{20}$$

V enačbi (20) je [*aiPGs*] koncentracija aiPGs, [LTC_4] pa koncentracija LTC_4. V modelu upoštevamo pogoj $Rf \leq 1$ [65] kot pogoj, s katerim napovemo možnost nastanka bronhokonstrikcije pri aspirinsko-intolerantnih astmatikih.

Vrednosti večine modelnih parametrov smo povzeli direktno po literaturi, za nekatere parametre pa smo njihove vrednosti ocenili z izračunom, kot je to podrobneje opisano v izvirnem znanstvenem članku [18], ki je priložen v prilogi 1. Opisi in vrednosti kinetičnih parametrov so podani v tabeli 1. Vrednosti modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6} so različne za neastmatike ter aspirinsko-tolerantne in aspirinsko-intolerantne astmatike ter so opisane v poglavju 3.1 − Modelna stanja.

V modelnih simulacijah uporabljamo farmakokinetične podatke za pet različnih zdravil. Tri različne NSAR, aspirin, ibuprofen in celecoxib ter dva različna inhibitorja encima 5-LOX − sintetični analog PGE_2 − nocloprost in ABT-761. Inhibitorne konstante NSAR za encima PGHS1 in PGHS2 ter inhibitorne konstante inhibitorjev 5-LOX smo izračunali s Cheng-Prussoevo enačbo iz eksperimentalno določenih vrednosti IC_{50}. Enačba je izpeljana v dodatku A3, primeri izračunov pa so opisani v članku [18] v prilogi 1. Vrednosti farmakokinetičnih parametrov $(V/F)^{(j)}$, $k_a^{(j)}$ in $k^{(j)}$ za posamezna zdravila smo povzeli ali direktno po literaturi ali pa smo jih določili s prilagajanjem enačbe (19) k izmerjenim časovnim potekom koncentracije zdravil v krvni plazmi človeka. Na sliki 2 je prikazan primer prilagojene funkcije (19) k izmerjenima časovnima potekoma koncentracije aspirina [62] in ibuprofena [40]. Prilagajanje enačbe (19) k eksperimentalnim podatkom [40, 62] smo izvedli v programih Origin 6.1 (OriginLab Corporation, 2000) [51] ali Berkeley Madonna 8.0.1 (Macey in Oster, 2000) [43]. Opisi in vrednosti farmakokinetičnih parametrov so podani v tabeli 2.

V simulacijah smo sistem enačb (1)−(6) v vsakem izmed modelnih stanj numerično integrirali iz začetnega stacionarnega stanja, ki je določeno v odsotnosti zdravila. Integriranje smo izvedli z numerično integracijsko metodo s spremenljivim integracijskim korakom v programu Berkeley Madonna 8.0.1 (Macey in Oster, 2000) [43].



Slika 2. Časovna poteka koncentracije aspirina in ibuprofena v krvni plazmi človeka. *Polni črni kvadrati:* izmerjen časovni potek koncentracije ibuprofena v krvni plazmi človeka [40]. *Polni črni krogi:* izmerjen časovni potek koncentracije aspirina v krvni plazmi človeka [62]. *Polna črna črta:* funkcija (19), prirejena k eksperimentalnim podatkom iz [40, 62].

Tabela 1. Opis in vrednosti modelnih parametrov, ki smo jih uporabili v raziskavah [18, 19, 25].

Parameter	Vrednost in enota	Opis parametra	Referenca
	Reakcija 1		
$v_{maks\,1}$	poglavje 3.1	maksimalna hitrost reakcije encima PGHS2	poglavje 3.1
K_1	2,5 μM	Michaelis-Mentenina konstanta	[49]
$K_{I1}^{(ASA)}$	85 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS2 za aspirin	[52]
$K_{I1}^{(IBU)}$	60 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS2 za ibuprofen	[52]
$K_{I1}^{(CEL)}$	0,07 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS2 za celecoxib	[27]
	Reakcija 2		
k_2	0,0028 s^{-1}	hitrostna konstanta toka piPGs iz celice	[28]
	Reakcija 3		
$v_{maks\,3}$	poglavje 3.1	maksimalna hitrost reakcije encima PGHS1	poglavje 3.1
K_3	3,0 μM	Michaelis-Mentenina konstanta	[49]
$K_{I3}^{(ASA)}$	4,0 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS1 za aspirin	[52]
$K_{I3}^{(IBU)}$	1,3 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS1 za ibuprofen	[52]
$K_{I3}^{(CEL)}$	0,2 μM	inhibitorna ravnotežna konstanta inhibicije encima PGHS1 za celecoxib	[27]
	Reakcija 4		
k_4	0,0028 s^{-1}	hitrostna konstanta toka aiPGs iz celice	[28]
	Reakcija 5		
$v_{maks\,5}$	86 μMs^{-1}	maksimalna hitrost reakcije encima 5-LOX	[53]
K_5	25,4 μM	ravnotežna disociacijska konstanta za kompleks 5-LOX·AA	[1]
K_{I5}	0,03 μM	inhibitorna ravnotežna konstanta inhibicije encima 5-LOX za aiPGs	[32]
$K_{I5}^{(ABT)}$	0,018 μM	inhibitorna ravnotežna konstanta inhibicije encima 5-LOX za ABT-761	[7]
	Reakcija 6		
$v_{maks\,6}$	poglavje 3.1	maksimalna hitrost reakcije encima LTC_4S	poglavje 3.1
A	56 μM	konstanta	[29]
B	1,4	konstanta	[29]
C	0,17 μM^{-1}	konstanta	[29]
	Reakcija 7		
k_7	0,0015 s^{-1}	hitrostna konstanta toka LTC_4 iz celice	[55]
	Reakcija 8		
$v_{maks\,8}$	3,7 μMs^{-1}	maksimalna hitrost encima LTA_4H	[31]
K_8	27 μM	Michaelis-Mentenina konstanta	[31]
	Reakcija 9		
$v_{maks\,9}$	5,74 μMs^{-1}	maksimalen tok LTB_4 iz celice	[42]
K_9	239 μM	Michaelis-Mentenina konstanta	[42]
	Ostali parametri		
v_0	0,70 μMs^{-1}	pritok AA	[28, 32, 49]

Tabela 2. Opis in vrednosti farmakokinetičnih parametrov za različna zdravila, ki smo jih uporabili v raziskavah [18, 19, 25].

Parameter	Vrednost in enota	Opis parametra	Referenca
	aspirin		
$k^{(\mathrm{ASA})}$	$5{,}5 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta eliminacije	[62]
$k_a^{(\mathrm{ASA})}$	$23 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta absorpcije	[62]
$(V/F)^{(\mathrm{ASA})}$	74 L	razmerje med deležem absorbiranega zdravila in navideznim volumnom porazdelitve	[62]
	ibuprofen		
$k^{(\mathrm{IBU})}$	$1{,}0 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta eliminacije	[40]
$k_a^{(\mathrm{IBU})}$	$2{,}8 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta absorpcije	[40]
$(V/F)^{(\mathrm{IBU})}$	8,4 L	razmerje med deležem absorbiranega zdravila in navideznim volumnom porazdelitve	[40]
	celecoxib		
$k^{(\mathrm{CEL})}$	$4{,}4 \cdot 10^{-5}\ \mathrm{s}^{-1}$	hitrostna konstanta eliminacije	[56]
$k_a^{(\mathrm{CEL})}$	$2{,}0 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta absorpcije	[56]
$(V/F)^{(\mathrm{CEL})}$	242 L	razmerje med deležem absorbiranega zdravila in navideznim volumnom porazdelitve	[56]
	sintetični analog* PGE_2 – *nocloprost		
$k^{(\mathrm{PGE}_2)}$	$3{,}3 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta eliminacije	[76]
$k_a^{(\mathrm{PGE}_2)}$	$8{,}1 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta absorpcije	[76]
$(V/F)^{(\mathrm{PGE}_2)}$	13 L	razmerje med deležem absorbiranega zdravila in navideznim volumnom porazdelitve	[76]
	ABT-761		
$k^{(\mathrm{ABT})}$	$1{,}4 \cdot 10^{-5}\ \mathrm{s}^{-1}$	hitrostna konstanta eliminacije	[83, 84]
$k_a^{(\mathrm{ABT})}$	$3{,}2 \cdot 10^{-4}\ \mathrm{s}^{-1}$	hitrostna konstanta absorpcije	[83, 84]
$(V/F)^{(\mathrm{ABT})}$	60 L	razmerje med deležem absorbiranega zdravila in navideznim volumnom porazdelitve	[83, 84]

3 Rezultati in diskusija

3.1 Modelna stanja

V modelnih simulacijah uporabimo eksperimentalne podatke [15, 57, 58, 74] o različnih ekspresijah encimov PGHS1, PGHS2 in LTC_4S pri neastmatikih, aspirinsko-tolerantnih astmatikih in aspirinsko-intolerantnih astmatikih kot vstopne podatke za matematični model. Predpostavljamo, da je ekspresija encimov PGHS1, PGHS2 in LTC_4S sorazmerna z njihovo totalno koncentracijo v celici. Tako ekspresija encimov vpliva direktno na maksimalno aktivnost encimov. Na osnovi te predpostavke je modelna stanja možno definirati s tremi prostimi modelnimi parametri v_{maks1}, v_{maks3} in v_{maks6}. V modelu ločimo naslednja modelna stanja: modelno stanje, s katerim opišemo neastmatike (NA), modelno stanje, s katerim opišemo aspirinsko-tolerantne astmatike (ATA) in tri različna modelna stanja, s katerimi opišemo tri različne populacije aspirinsko-intolerantnih astmatikov $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. Celotna analiza zajema torej pet modelnih stanj: NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. V nadaljevanju bomo podrobneje opisali postopek določitve vrednosti parametrov v_{maks1}, v_{maks3} in v_{maks6} v posameznih stanjih.

Izhodiščni oz. referenčni vrednosti modelnih parametrov v_{maks1} in v_{maks6} sta povzeti po literaturi. Vrednost parametra v_{maks1} = 0,65 μMs^{-1} je izračunana po enačbi $v_{maks1} = k_1[PGHS2]_{TOT}$, kjer je $k_1 = 13\ s^{-1}$ [71] in $[PGHS2]_{TOT}$ = 50 nM [71], vrednost parametra v_{maks6} pa je 0,23 μMs^{-1} [29]. Vrednost parametra v_{maks3} je določena v simulacijah. Pri tem uporabimo eksperimentalne rezultate iz [65], kjer so avtorji merili koncentracijo PGE_2 in levkotrienov v krvi neastmatikov, aspirinsko-tolerantnih astmatikov in aspirinsko-intolerantnih astmatikov pred in po provokacijskem testu z aspirinom. Iz rezultatov meritev [65] najprej izračunamo vrednost razmerja *Rf* za ATA, ki je med 13 in 26 pred provokacijskim testom in med 2,3 in 8,7 po provokacijskem testu. Ta dva intervala vrednosti *Rf* uporabimo v simulacijah za določitev vrednosti modelnega parametra v_{maks3}. Pri tem v simulacijah uporabimo dozo 650 mg aspirina. S simulacijami ugotovimo, da se z modelom napovedane vrednosti *Rf* pred in po doziranju aspirina z ustreznimi eksperimentalno določenimi vrednostmi *Rf* za aspirinsko-tolerantne astmatike najbolje ujemajo pri vrednosti parametra v_{maks3} = 0,096 μMs^{-1}. Pri tej vrednosti parametra v_{maks3} je namreč izračunana vrednost *Rf* pred doziranjem aspirina enaka 26, po doziranju pa 7,1. Obe napovedani vrednosti *Rf* se tako v okviru merskih napak ujemata z eksperimentalno določenimi vrednostmi *Rf*. Vrednost parametra v_{maks3} = 0,096 μMs^{-1} je torej določena s primerjavo modelnih simulacij in eksperimentalnih rezultatov, izmerjenih za

aspirinsko-tolerantne astmatike. Študije, iz katerih povzamemo referenčni vrednosti parametrov v_{maks1} = 0,65 μMs^{-1} in v_{maks6} = 0,23 μMs^{-1}, pa so izvedene na encimih PGHS2 in LTC_4S in se ne nanašajo specifično na katero izmed populacij astmatikov ali neastmatikov, zato smo celoten set parametrov v_{maks1} = 0,65 μMs^{-1}, v_{maks3} = 0,096 μMs^{-1} in v_{maks6} = 0,23 μMs^{-1} uporabili za definicijo modelnega stanja ATA.

Pri definiciji modelnega stanja NA izhajamo iz eksperimentalnih podatkov, objavljenih v [15, 74], kjer poročajo, da je ekspresija encima LTC_4S pri neastmatikih približno 4-krat nižja kot pri aspirinsko-tolerantnih astmatikih. Modelno stanje NA je tako definirano z enakima vrednostima modelnih parametrov v_{maks1} in v_{maks3} kot stanje ATA, vendar s 4-krat nižjo vrednostjo parametra v_{maks6} v primerjavi z modelnim stanjem ATA.

Stanja $AIA^{(i)}$ so prav tako definirana na osnovi eksperimentalnih podatkov iz literature. Definiramo jih na naslednji način.

$AIA^{(1)}$: Ekspresija encima PGHS1 je pri aspirinsko-intolerantnih astmatikih 3-krat nižja kot pri aspirinsko-tolerantnih astmatikih [58]. Modelno stanje $AIA^{(1)}$ je zato definirano z enakima vrednostima parametrov v_{maks1} in v_{maks6} kot stanje ATA in s 3-krat nižjo vrednostjo parametra v_{maks3} kot stanje ATA.

$AIA^{(2)}$: Ekspresija encima LTC_4S pri aspirinsko-intolerantnih astmatikih je 5-krat višja kot pri aspirinsko-tolerantnih astmatikih [15, 74]. Modelno stanje $AIA^{(2)}$ je tako definirano z enakima vrednostima parametrov v_{maks1} in v_{maks3} kot stanje ATA ter s 5-krat višjo vrednostjo parametra v_{maks6} kot stanje ATA.

$AIA^{(3)}$: Ekspresija encima PGHS2 pri aspirinsko-intolerantnih astmatikih je 6-krat nižja kot pri aspirinsko-tolerantnih astmatikih [57]. Modelno stanje $AIA^{(3)}$ je definirano z enakima vrednostima parametrov v_{maks3} in v_{maks6} kot stanje ATA in s 6-krat nižjo vrednostjo parametra v_{maks1} kot stanje ATA. Vrednosti modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6} v modelnih stanjih NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ so podane v tabeli 3.

Tabela 3. Vrednosti modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6} v modelnih stanjih NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ [18].

Parameter	**Modelno stanje**				
	NA	**ATA**	**$AIA^{(1)}$**	**$AIA^{(2)}$**	**$AIA^{(3)}$**
v_{maks1} [μMs^{-1}]	0,65	0,65	0,65	0,65	0,11
v_{maks3} [μMs^{-1}]	0,096	0,096	0,032	0,096	0,096
v_{maks6} [μMs^{-1}]	0,057	0,23	0,23	1,15	0,23

3.2 Modelne napovedi

3.2.1 Verifikacija modela

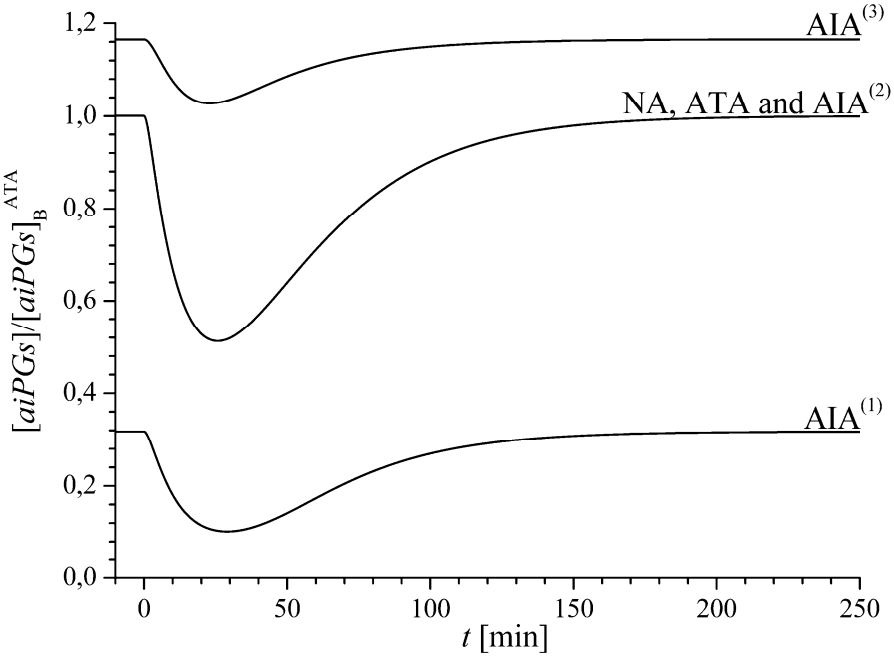
V tem poglavju bomo izvedli verifikacijo modela, s katero želimo preučiti, ali je referenčno modelno stanje ATA − in s tem tudi druga modelna stanja − definirano dovolj natančno, da lahko z modelom opišemo eksperimentalne rezultate, pridobljene iz literature [65]. V ta namen bomo analizirali napovedane časovne poteke modelnih spremenljivk [*aiPGs*] in [LTC_4] ter njunega razmerja *Rf* ob odsotnosti in prisotnosti NSAR ter modelno napoved časa maksimalnega transporta levkotrienov iz celice po doziranju NSAR. Te modelne napovedi lahko direktno primerjamo z eksperimentalnimi rezultati, objavljenimi v literaturi [65]. Vsi izračuni, ki so predstavljeni v nadaljevanju, so izvedeni pri dozi 650 mg aspirina.

Osnova za verifikacijo modela so modelne napovedi časovnih potekov [*aiPGs*] in [LTC_4] pred in po doziranju aspirina v modelnih stanjih NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, ki so prikazani na slikah 3 in 4. Na teh slikah sta vrednosti [*aiPGs*] in [LTC_4] izraženi glede na koncentraciji aiPGs in LTC_4 v stanju ATA pred doziranjem aspirina, ki ju imenujemo bazalni koncentraciji in ju označimo z $[aiPGs]_{\mathrm{B}}^{\mathrm{ATA}}$ in $[LTC_4]_{\mathrm{B}}^{\mathrm{ATA}}$.

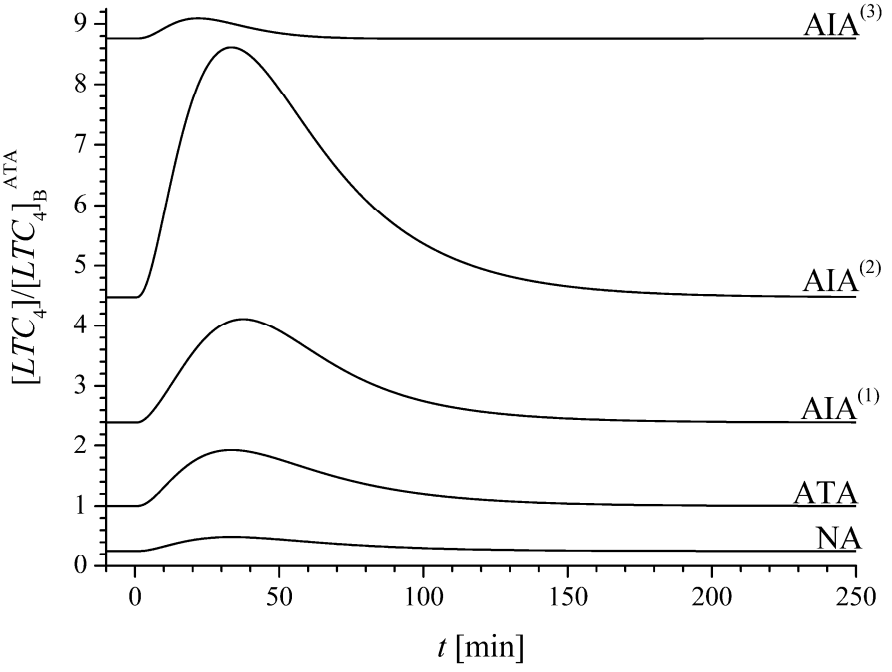
Na sliki 3 vidimo, da se časovni poteki [*aiPGs*] v modelnih stanjih NA, ATA in $AIA^{(2)}$ med seboj ne razlikujejo in so enaki pred in po doziranju aspirina. V teh treh stanjih se po doziranju aspirina koncentracija aiPGs tudi najbolj zniža glede na njeno bazalno vrednost. Časovni poteki [*aiPGs*] so v teh modelnih stanjih enaki zaradi enakih maksimalnih aktivnosti encimov PGHS1 in PGHS2. Maksimalna aktivnost encima LTC_4S je v vseh treh stanjih različna, kar kaže, da maksimalna aktivnost LTC_4S ne vpliva na [*aiPGs*] v bazalnem stanju in ne po doziranju aspirina. V stanju $AIA^{(1)}$ je [*aiPGs*] ves čas najnižja, kar je posledica 3-krat nižje aktivnosti encima PGHS1 v primerjavi z drugimi modelnimi stanji. V stanju $AIA^{(3)}$ pa je [*aiPGs*] ves čas najvišja, kar je posledica 6-krat nižje maksimalne aktivnosti PGHS2 v primerjavi z drugimi modelnimi stanji.

Na sliki 4 vidimo, da je [LTC_4] v stanjih NA in ATA znižana v primerjavi s stanji $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, kar je skladno z eksperimentalnimi opažanji [65]. V stanju NA se [*aiPGs*] po doziranju aspirina zniža, toda [LTC_4] se po doziranju aspirina v tem stanju bistveno ne poviša. To je posledica 4-krat nižje maksimalne aktivnosti encima LTC_4S v stanju NA v primerjavi s stanji ATA, $AIA^{(1)}$ in $AIA^{(3)}$ ter kar 20-krat nižje maksimalne aktivnosti LTC_4S v stanju NA kot v stanju $AIA^{(2)}$. Bazalna [LTC_4] v stanju ATA je približno 5-krat višja kot v stanju NA in približno 2-, 4- in 9-krat nižja kot v stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. V

stanju AIA$^{(1)}$ je bazalna [LTC_4] povišana glede na stanji NA in ATA zaradi nizke bazalne [*aiPGs*] in s tem šibke inhibicije encima 5-LOX.



Slika 3. Časovni poteki $[aiPGs]/[aiPGs]_B^{ATA}$ pred ($t < 0$) in po ($t > 0$) aplikaciji 650 mg aspirina, v modelnih stanjih NA, ATA, AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ [18].



Slika 4. Časovni poteki $[LTC_4]/[LTC_4]_B^{ATA}$ pred ($t < 0$) in po ($t > 0$) doziranju 650 mg aspirina, v modelnih stanjih NA, ATA, AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ [18].

V stanju $AIA^{(2)}$ je bazalna [LTC_4] povišana zaradi višje maksimalne aktivnosti LTC_4S, v stanju $AIA^{(3)}$ pa zaradi nizke maksimalne aktivnosti PGHS2. Slednje vodi do večje presnove AA po lipoksigenazni poti in s tem posledično tudi do višje [LTC_4]. Po doziranju aspirina se v vseh modelnih stanjih razen v stanju $AIA^{(3)}$ [LTC_4] poviša na približno 2-kratno vrednost nad bazalno. V stanju $AIA^{(3)}$ je odziv sistema na zdravilo precej šibek v primerjavi z ostalimi stanji.

Iz te analize ugotavljamo, da povišana ekspresija encima LTC_4S poviša bazalno [LTC_4], ne vpliva pa na bazalno [*aiPGs*] ali na [*aiPGs*] po doziranju NSAR. Ekspresiji encimov PGHS1 in PGHS2 vplivata na bazalni [LTC_4] in [*aiPGs*]. Znižana ekspresija PGHS1 zniža bazalno [*aiPGs*] in poviša bazalno [LTC_4], znižana ekspresija PGHS2 pa obe bazalni koncentraciji poviša. Poleg tega sprememba v ekspresiji PGHS2 precej oslabi odziv sistema na zdravilo v stanju $AIA^{(3)}$.

Eden izmed rezultatov, o katerem poročajo v študiji [65], je čas maksimalnega izmerjenega toka levkotrienov iz celic. V raziskavi poročajo, da je bil tok levkotrienov iz celic maksimalen približno 20 minut po pričetku merjenja. Ta rezultat je neodvisen od tega, ali so eksperiment izvedli na krvi neastmatikov, aspirinsko-tolerantnih astmatikov ali aspirinsko-intolerantnih astmatikov, ki so predhodno opravili provokacijski test z aspirinom, ali ne. Z našim matematičnim modelom je možno podrobneje analizirati ta eksperimentalni rezultat. V modelu je tok LTC_4 iz celice v_7 linearno odvisen od [LTC_4], kar pomeni, da bo tok maksimalen v trenutku, ko bo tudi [LTC_4] maksimalna.

V študiji [18] (priloga 1) smo podrobneje analizirali časovne intervale, v katerih se po doziranju aspirina pojavijo minimumi [*aiPGs*] in maksimumi [LTC_4]. Rezultati simulacij so podani v tabeli 4 in v splošnem kažejo, da je z modelom napovedan čas maksimalnega toka LTC_4 iz belih krvnih celic po doziranju aspirina primerljiv z izmerjenim časom 20 minut [65]. Podrobna analiza pokaže, da v vseh modelnih stanjih, razen v $AIA^{(3)}$, minimumi [*aiPGs*] nastopijo pred maksimumi [LTC_4]. Minimumi [*aiPGs*] se v različnih modelnih stanjih pojavijo od 23 do 29 minut po doziranju aspirina, maksimumi [LTC_4] pa od 21 do 37 minut po doziranju aspirina. Ti rezultati so v skladu s splošno sprejetim mnenjem, da je znižana koncentracija aiPGs, do katere pride zaradi inhibitornega učinka NSAR na PGHS1 in PGHS2, ključen dogodek, ki preusmeri presnovo AA po lipoksigenazni poti in s tem poviša produkcijo vnetnega mediatorja LTC_4 [2, 74].

Tabela 4. Časovna analiza minimumov [*aiPGs*] in maksimumov [LTC_4] v modelnih stanjih NA, ATA, AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ po doziranju 650 mg aspirina [18]**.** Modelne napovedi so odčitane iz slik 3 in 4, eksperimentalni rezultat v zadnjem stolpcu tabele pa je povzet iz študije [65].

Dogodek	**Modelne napovedi: *t* [min]**					**Eksp. rezultat:**
	NA	ATA	AIA$^{(1)}$	AIA$^{(2)}$	AIA$^{(3)}$	***t* [min]**
minimum [*aiPGs*]	25	25	29	26	23	/
maksimum [LTC_4]	33	33	37	33	21	20

Z modelom izvedemo tudi podrobno analizo vrednosti [*aiPGs*] in [LTC_4] v vseh modelnih stanjih. Med seboj primerjamo različna napovedana razmerja [*aiPGs*] in [LTC_4] v posameznih modelnih stanjih in ustrezna razmerja, ki jih izračunamo iz eksperimentalnih podatkov, objavljenih v [65]. V tej analizi smo upoštevali tudi merske napake. Npr., če je izmerjena vrednost bazalne koncentracije levkotrienov za aspirinsko-tolerantne astmatike enaka $[LTC_4]_B^{ATA} = 40{,}2 \pm 9{,}6$ pg mL^{-1} [65], bazalna vrednost za neastmatike pa $[LTC_4]_B^{NA} = 1{,}5 \pm 0{,}4$ pg mL^{-1} [65], potem je minimalna vrednost razmerja bazalnih koncentracij $[LTC_4]_B^{ATA} / [LTC_4]_B^{NA}$ izračunana kot 30,6/1,9 ≅ 16, maksimalna pa kot 49,6/1,1 ≅ 45. Ob upoštevanju merskih napak ima torej razmerje $[LTC_4]_B^{ATA} / [LTC_4]_B^{NA}$ vrednosti med 16 in 45. Vsa razmerja, izračunana iz eksperimentalnih podatkov, povzetih po študiji [65], so izračunana na zgoraj opisan način. V tabeli 5 je prikazana primerjava modelnih napovedi razmerij [LTC_4] in [*aiPGs*] za modelni stanji NA in ATA z ustreznimi razmerji, določenimi iz eksperimentalnih podatkov [65].

Rezultati v tabeli 5 kažejo, da so razmerja, ki jih napovemo z modelom, enakega velikostnega reda kot razmerja, izračunana iz eksperimentalnih podatkov [65]. Do večjih odstopanj prihaja v primerih razmerij $[LTC_4]_B^{ATA} / [LTC_4]_B^{NA}$, $[aiPGs]_B^{ATA} / [aiPGs]_B^{NA}$ in $[aiPGs]_A^{ATA} / [aiPGs]_A^{NA}$. V prvem primeru je z modelom napovedana vrednost precej nižja kot pa ustrezna izmerjena vrednost. V stanju NA naš model namreč napove precej višjo [LTC_4], kot pa je dejansko izmerjena, zato ima razmerje $[LTC_4]_B^{ATA} / [LTC_4]_B^{NA}$ nižjo vrednost. Z modelom je sicer mogoče doseči boljše ujemanje vseh razmerij, izračunanih za [LTC_4], vendar pa bi morali v tem primeru uporabiti še za nekaj krat nižjo vrednost parametra v_{maks6} v stanju NA, kot pa je dejansko uporabljena. To bi bilo v nasprotju z eksperimentalnimi podatki [15, 74], ki kažejo, da je ekspresija LTC_4S pri neastmatikih 4-krat nižja kot pri aspirinsko-tolerantnih astmatikih. Slednje smo upoštevali tudi v naših simulacijah. V primerih $[aiPGs]_B^{ATA} / [aiPGs]_B^{NA}$ in $[aiPGs]_A^{ATA} / [aiPGs]_A^{NA}$ prav tako pride do manjših odstopanj, ker

sta vrednosti parametrov v_{maks3} in v_{maks1} v modelnih stanjih NA in ATA enaki. V literaturi ni zaslediti rezultatov raziskav, ki bi poročali, da sta ekspresiji PGHS1 in PGHS2 pri neastmatikih in aspirinsko-tolerantnih astmatikih različni. Ravno nasprotno, v študiji [58] so pokazali, da so bistvene razlike v ekspresiji PGHS1 le med aspirinsko-tolerantnimi in aspirinsko-intolerantnimi astmatiki. Modelni stanji NA in ATA se tako v naših simulacijah med seboj razlikujeta le po ekspresiji encima LTC_4S, ki pa ne vpliva na modelne napovedi $[aiPGs]$, zato sta tudi vrednosti razmerij $[aiPGs]_B^{ATA}/[aiPGs]_B^{NA}$ in $[aiPGs]_A^{ATA}/[aiPGs]_A^{NA}$ v teh dveh modelnih stanjih enaki.

Tabela 5. Primerjava razmerij $[LTC_4]$ in $[aiPGs]$ v modelnih stanjih NA in ATA. Rezultati so izračunani v bazalnem stanju in po doziranju 650 mg aspirina [18]. Modelne napovedi primerjamo z ustreznimi vrednostmi, ki so določene iz eksperimentalnih podatkov [65]. Spodnja indeksa imata naslednji pomen: B − koncentracija v bazalnem stanju pred doziranjem aspirina in A − maksimalna $[LTC_4]$ oz. minimalna $[aiPGs]$ po doziranju aspirina. Zgornji indeks označuje modelni stanji NA in ATA.

Razmerje	**Modelne napovedi**	**Eksperimentalni rezultati**
$[LTC_4]_B^{ATA}/[LTC_4]_B^{NA}$	3,9	16−45
$[LTC_4]_A^{NA}/[LTC_4]_B^{NA}$	1,9	3,1−8,3
$[LTC_4]_A^{ATA}/[LTC_4]_B^{ATA}$	1,9	0,80−2,9
$[LTC_4]_A^{ATA}/[LTC_4]_A^{NA}$	3,9	4,3−15
$[aiPGs]_B^{ATA}/[aiPGs]_B^{NA}$	1,0	2,4−4,0
$[aiPGs]_A^{NA}/[aiPGs]_B^{NA}$	0,51	0,10−0,26
$[aiPGs]_A^{ATA}/[aiPGs]_B^{ATA}$	0,51	0,26−0,50
$[aiPGs]_A^{ATA}/[aiPGs]_A^{NA}$	1,0	4,1−11

V tabeli 6 je predstavljena primerjava razmerij $[LTC_4]$ in $[aiPGs]$ v stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ s stanjema NA in ATA. Iz rezultatov lahko naredimo dva splošna zaključka: 1) vrednosti modelno napovedanih razmerij $[LTC_4]$ v stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ so enakega velikostnega reda kot ustrezne vrednosti, izračunane iz eksperimentalnih podatkov [65], in 2) samo v stanju $AIA^{(1)}$ je velikostni red napovedanih razmerij $[aiPGs]$ primerljiv z redom velikosti razmerij, izračunanih iz eksperimentalnih podatkov [65]. V stanjih $AIA^{(2)}$ in $AIA^{(3)}$ so napovedane vrednosti razmerij $[aiPGs]$ nekoliko večje. Iz te analize vidimo, da model v vseh treh modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ ustrezno napove visoke koncentracije LTC_4 v primerjavi z modelnima stanjema NA in ATA, vendar pa je samo v

modelnem stanju AIA$^{(1)}$ [*aiPGs*] hkrati tudi nižja kot v modelnih stanjih NA in ATA. Podobno kvalitativno obnašanje sistema, kot je prisotno v modelnem stanju AIA$^{(1)}$, kaže tudi eksperimentalna študija [65].

Tabela 6. Primerjava [LTC_4] in [*aiPGs*] v modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ s stanjema NA in ATA Rezultati so izračunani v bazalnem stanju in po doziranju 650 mg aspirina [18]. Modelne napovedi primerjamo z ustreznimi razmerji, določenimi iz eksperimentalnih podatkov [65]. Spodnja indeksa imata naslednji pomen: B − koncentracija v bazalnem stanju pred aplikacijo aspirina in A − maksimalna [LTC_4] oz. minimalna [*aiPGs*] po doziranju aspirina. Zgornji indeks označuje modelna stanja AIA$^{(1)}$, AIA$^{(2)}$, AIA$^{(3)}$, NA in ATA.

Razmerje	AIA$^{(1)}$	AIA$^{(2)}$	AIA$^{(3)}$	Eksperimentalni podatki
$[LTC_4]_B^{AIA^{(i)}} / [LTC_4]_B^{NA}$	9,5	8,0	35	25−58
$[LTC_4]_B^{AIA^{(i)}} / [LTC_4]_B^{ATA}$	2,4	4,5	8,8	0,94−2,1
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_B^{AIA^{(i)}}$	1,7	1,9	1,0	3,2−5,8
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_A^{NA}$	8,4	18	19	22−46
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_A^{ATA}$	2,1	4,5	4,7	2,2−6,9
$[aiPGs]_B^{AIA^{(i)}} / [aiPGs]_B^{NA}$	0,32	1,0	1,2	0,037−0,15
$[aiPGs]_B^{AIA^{(i)}} / [aiPGs]_B^{ATA}$	0,32	1,0	1,2	0,013−0,045
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_B^{AIA^{(i)}}$	0,32	0,51	0,88	0,27−2,5
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_A^{NA}$	0,20	1,0	2,0	0,16−0,90
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_A^{ATA}$	0,20	1,0	2,0	0,025−0,12

Nazadnje preverimo še vrednosti razmerja *Rf*. V tabeli 7 so za modelna stanja NA, ATA , AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ podane modelne napovedi bazalnih vrednosti *Rf* ter modelne napovedi vrednosti *Rf* po simulaciji doziranja treh različnih doz aspirina: 65 mg, 650 mg in 6500 mg. Napovedane vrednosti *Rf* primerjamo z vrednostmi, ki smo jih določili iz eksperimentalnih podatkov [65]. Pred doziranjem aspirina je v vseh modelnih stanjih $Rf > 1$. Vidimo, da je vrednost *Rf* v stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ nižja v primerjavi z modelnima stanjema NA in ATA. Z modelom napovedane bazalne vrednosti *Rf* v stanjih NA in ATA se v okviru merske napake ujemajo z eksperimentalnimi rezultati [65], v stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ pa so napovedane bazalne vrednosti *Rf* nekoliko višje kot tiste, določene iz eksperimentalnih podatkov [65]. Po doziranju 65 mg aspirina opazimo, da se *Rf* v vseh modelnih stanjih nekoliko zniža. Po doziranju 650 mg aspirina so vrednosti *Rf* v vseh stanjih

Tabela 7. Primerjava modelnih napovedi vrednosti razmerja *Rf* v stanjih NA, ATA, AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ [18] z vrednostimi *Rf*, ki so izračunane iz eksperimentalnih rezultatov [65]. Rezultati so izračunani v bazalnem stanju in po doziranju treh različnih doz aspirina: 65mg, 650 mg in 6500 mg. Spodnja indeksa B in A imata naslednji pomen: B – vrednost razmerja *Rf* v bazalnem stanju in A – minimalna vrednost razmerja *Rf*, določena po doziranju aspirina.

Rf	$D^{(ASA)}$	**Modelne napovedi**					**Eksperimentalni podatki**		
	[mg]	**NA**	**ATA**	**AIA$^{(1)}$**	**AIA$^{(2)}$**	**AIA$^{(3)}$**	**NA**	**ATA**	**AIA**
$([aiPGs]/[LTC_4])_B$	/	104	26	3,5	5,9	3,5	104−249	13−26	0,16−0,63
$([aiPGs]/[LTC_4])_A$	65	85	21	2,8	4,8	3,4	/	/	/
$([aiPGs]/[LTC_4])_A$	650	28	7,1	0,66	1,6	2,9	3,2−8,7	2,3−8,7	0,029−0,070
$([aiPGs]/[LTC_4])_A$	6500	0,88	0,22	0,0075	0,050	0,71	/	/	/

še nižje, v stanju $AIA^{(1)}$ pa je pri tej dozi vrednost *Rf* že manjša od ena. Pri dozi 6500 mg aspirina je vrednost *Rf* manjša od ena v vseh modelnih stanjih. Iz teh rezultatov vidimo, da doza NSAR vpliva na razmerje med koncentracijama LTC_4 in aiPGs ter da je tveganje za pojav bronhokonstrikcije pri aspirinsko-intolerantnih astmatikih odvisno od doze NSAR, kar je v skladu s kliničnimi opažanji [73].

Verifikacija modela, ki smo jo izvedli v tem poglavju, kaže, da model kvalitativno konsistentno opiše eksperimentalna opažanja in da tudi v kvantitativnem pogledu ni večjih odstopanj med modelnimi napovedmi in eksperimentalnimi podatki, objavljenimi v študiji [65]. V večini primerov so vrednosti izračunanih rezultatov enakega velikostnega reda kot vrednosti izmerjenih. V nekaterih primerih gre celo za ujemanje znotraj napak pri merjenju. Večja odstopanja se pojavljajo le v nekaj primerih.

Modelno stanje ATA je izbrano kot referenčno modelno stanje, glede na katerega so na osnovi razpoložljivih eksperimentalnih podatkov iz literature [15, 57, 58, 74] definirana ostala modelna stanja NA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. V modelnem stanju ATA je vrednost modelnega parametra v_{maks3} določena s prilagajanjem modelnih napovedi razmerja *Rf* k eksperimentalno določenim vrednostim tega razmerja za aspirinsko-tolerantne astmatike pred in po inhalatornem provokacijskem testu z aspirinom [65]. Dobro kvantitavno ujemanje modelnih napovedi razmerja *Rf* v modelnem stanju ATA z eksperimentalno določenimi vrednostmi *Rf* iz [65] nas tako ne sme presenečati. Toda − vrednosti vseh treh modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6} v ostalih štirih modelnih stanjih NA ter $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ so določene na osnovi meritev iz več različnih eksperimentalnih študij [15, 57, 58, 74]. Te eksperimentalne podatke uporabimo kot vstopne podatke za model, katerega modelne napovedi pa nato verificiramo na meritvah koncentracij PGE_2 in LTC_4 iz eksperimentalne študije [65]. V verifikaciji modela tako uporabljamo podatke iz petih eksperimentalnih študij različnih avtorjev. Bolj kot podrobno kvantitativno ujemanje modelnih napovedi in eksperimentalnih rezultatov se nam zdi pomembno dejstvo, da modelne napovedi v različnih modelnih stanjih − ob prisotnosti ali odsotnosti NSAR − kažejo podobne lastnosti sistema, kot jih lahko opazimo pri eksperimentalnih biokemijskih in kliničnih študijah [65, 73].

3.2.2 Senzitivnostna analiza

Eksperimentalne in teoretične študije [34, 44, 46, 48] kažejo, da so za biološke celice značilne interindividualne razlike. To pomeni, da se celice znotraj iste populacije, ki v organizmu opravljajo enako fiziološko funkcijo, med seboj nekoliko razlikujejo po lastnostih, kot so ekspresija encimov, kinetične lastnosti encimov, število ionskih kanalov na celični membrani, število receptorjev na celični membrani itd. Zaradi teh razlik se celice na zunanje vplive odzovejo precej različno, vendar pa je v kvalitativnem smislu odziv celic na zunanji vpliv še vedno enak [44]. Biološke celice so torej dovolj "trdožive" oz. robustne, da se − navkljub manjšim medsebojnim razlikam − v splošnem še enako odzovejo na zunanje vplive, zato je smiselno pričakovati, da bo tudi matematični model, s katerim opišemo dogajanje v celici, vsaj do neke mere kazal podobne lastnosti kot realne celice. Želimo torej, da bi bil tudi model ustrezno robusten.

Pri modeliranju celičnih procesov opišemo lastnosti celice z modelnimi parametri, katerih vrednosti so običajno določene eksperimentalno kot povprečne vrednosti več meritev. Tako določeni modelni parametri dajejo opis povprečne celice. Interindividualne razlike pa kažejo, da se celice med seboj razlikujejo. V okviru matematičnega modeliranja to pomeni, da se vrednosti modelnih parametrov od celice do celice spreminjajo v okolici eksperimentalno določenih vrednostih znotraj fiziološko še smiselnih intervalov. Če je model ustrezno robusten, tedaj smiselne spremembe v vrednostih modelnih parametrov ne smejo voditi do bistveno drugačnega kvalitativnega rezultata.

Robustnost modela preučimo s senzitivnostno analizo [35, 39, 44]. Pri tem spreminjamo vrednosti enega ali več izbranih modelnih parametrov in opazujemo, kako majhne spremembe v vrednostih parametrov vplivajo na vrednosti ene ali več izbranih modelnih spremenljivk v stacionarnem stanju. Mera za robustnost ali senzitivnost modela je senzitivnostni koeficient R_X^Y, ki je podan kot razmerje $(\Delta Y / Y)/(\Delta X / X)$, kjer je $\Delta Y/Y$ relativna sprememba stacionarne vrednosti modelne spremenljivke Y in $\Delta X/X$ majhna relativna sprememba modelnega parametra X. Tako določen senzitivnostni koeficient npr. pove, za koliko odstotkov se spremeni stacionarna vrednost spremenljivke, če vrednost parametra spremenimo za en odstotek.

V naši študiji [19] (priloga 2) smo z modelom preučili interindividualne razlike v ekspresiji encimov PGHS1, PGHS2 in LTC_4S znotraj treh populacij aspirinsko-intolerantnih astmatikov, ki jih opišemo z modelnimi stanji $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. Za vsa tri modelna

stanja smo izvedli senzitivnostno analizo za razmerje *Rf* glede na spremembe v vrednostih modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6}. Ustrezni senzitivnostni koeficienti so podani kot:

$$R_{v_{\text{maks i}}}^{Rf} = \frac{\Delta Rf / Rf}{\Delta v_{\text{maks i}} / v_{\text{maks i}}} \qquad i = 1, 3, 6, \qquad (22)$$

kjer je $\Delta Rf/Rf$ relativna sprememba stacionarne vrednosti razmerja *Rf*, $\Delta v_{\text{maks i}}/v_{\text{maksi}}$ pa majhna relativna sprememba ($\Delta v_{\text{maks i}}/v_{\text{maks i}} = 0{,}01$) vrednosti modelnih parametrov v_{maks1}, v_{maks3} in v_{maks6}. Senzitivnostne koeficiente smo izračunali za dva primera: v odsotnosti zdravila in ob prisotnosti zdravila v sistemu v odvisnosti od izbranih doz aspirina in ibuprofena. V zadnjem primeru smo pri izračunu senzitivnostnih koeficientov upoštevali stacionarne koncentracije zdravila, ki ustrezajo maksimalnim koncentracijam zdravila v krvi za izbrane doze NSAR.

Rezultati kažejo, da je vrednost senzitivnostnega koeficienta $R_{v_{\text{maks 6}}}^{Rf}$ neodvisna od tipa in doze NSAR. Vrednost $R_{v_{\text{maks 6}}}^{Rf}$ je približno −0,9 v vseh modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ pred in po doziranju NSAR. To pomeni, da se vrednost razmerja *Rf* v modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ zniža za 0,9 %, če se vrednost parametra v_{maks6} poviša za 1 %. Vrednosti senzitivnostnih koeficientov $R_{v_{\text{maks1}}}^{Rf}$ in $R_{v_{\text{maks 3}}}^{Rf}$ v modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ v odvisnosti od izbranih doz aspirina so prikazane na slikah 5a in 5b, za izbrane doze ibuprofena pa na slikah 6a in 6b. Rezultati kažejo naslednje.

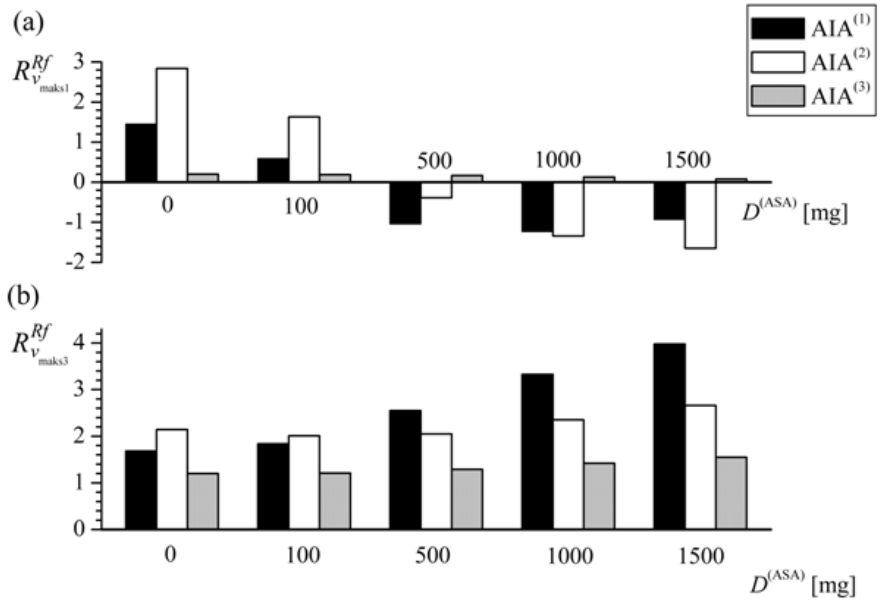
i) Kadar zdravilo v sistemu ni prisotno, je na spremembe v vrednostih modelnih parametrov v_{maks1} in v_{maks3} najbolj občutljivo modelno stanje AIA$^{(2)}$, najmanj pa stanje AIA$^{(3)}$ (glej slike 5a, b in 6a, b za $D^{(\text{ASA})} = D^{(\text{IBU})} = 0$).

ii) V modelnih stanjih AIA$^{(1)}$ in AIA$^{(2)}$ se pri dozah aspirina, ki so večje od 500 mg, spremeni predznak senzitivnostnega koeficienta $R_{v_{\text{maks1}}}^{Rf}$. V modelnem stanju AIA$^{(3)}$ je vrednost $R_{v_{\text{maks1}}}^{Rf}$ pri vseh izbranih dozah aspirina pozitivna, zelo blizu nič in se v odvisnosti od doze aspirina bistveno ne spreminja (slika 5a).

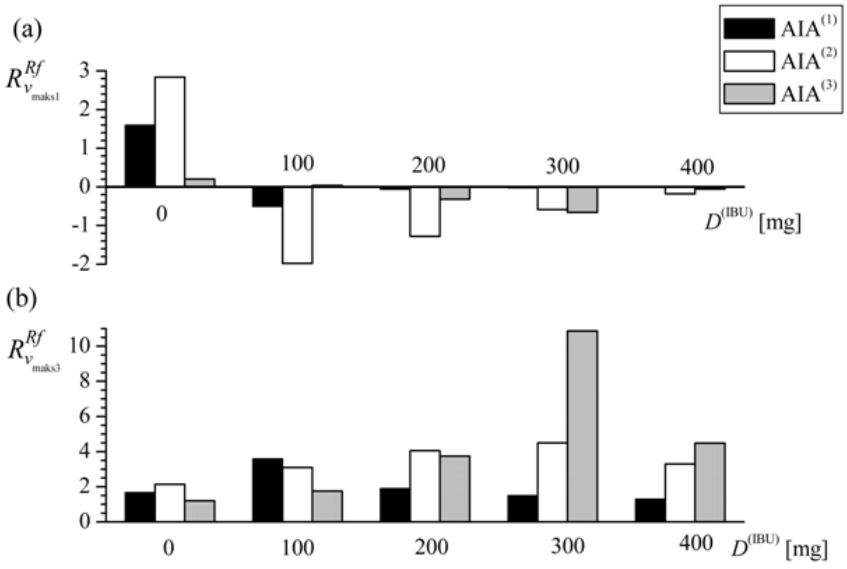
iii) V vseh treh modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ se predznak $R_{v_{\text{maks1}}}^{Rf}$ spremeni pri dozah, večjih od 200 mg ibuprofena (slika 6a).

iv) V modelnem stanju AIA$^{(1)}$ vrednost senzitivnostnega koeficienta $R_{v_{\text{maks 3}}}^{Rf}$ narašča v odvisnosti od doze aspirina, v modelnih stanjih AIA$^{(2)}$ in AIA$^{(3)}$ pa je ta vrednost približno dva oz. ena in se v odvisnosti od doze aspirina bistveno ne spreminja (slika 5b).

v) V vseh treh modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ se vrednost $R^{Rf}_{v_{maks\,3}}$ bistveno spreminja v odvisnosti od doze ibuprofena. Še posebej izstopa vrednost $R^{Rf}_{v_{maks\,3}}$ v modelnem stanju AIA$^{(3)}$, za dozo 300 mg ibuprofena, ki je precej visoka in znaša približno 10.



Slika 5. Vrednosti senzitivnostnih koeficientov a) $R^{Rf}_{v_{maks\,1}}$ in b) $R^{Rf}_{v_{maks\,3}}$ v odvisnosti od doze aspirina [19].



Slika 6. Vrednosti senzitivnostnih koeficientov a) $R^{Rf}_{v_{maks\,1}}$ in b) $R^{Rf}_{v_{maks\,3}}$ v odvisnosti od doze ibuprofena [19].

V večini primerov, ki smo jih preučili s senzitivnostno analizo, so vrednosti senzitivnostnih koeficientov $R^{Rf}_{v_{maks3}}$, $R^{Rf}_{v_{maks1}}$ in $R^{Rf}_{v_{maks6}}$ med 2 in 5. To pomeni, da se pod vplivom majhnih sprememb v ekspresiji encimov PGHS1, PGHS2 in LTC_4S, ki znašajo 1 %, v modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ stacionarne vrednosti razmerja *Rf* spremenijo kvečjemu za 5 %. S tem se tudi kvalitativno obnašanje modela v posameznih modelnih stanjih ne bo bistveno spremenilo. Za majhne spremembe parametrov model torej kaže ustrezno robustnost. Za večje spremembe vrednosti parametrov, denimo 10 %, pa bi se vrednost razmerja *Rf* v modelnih stanjih lahko spreminjala tudi do 50 %, kar pa bi že lahko vodilo tudi do kvalitativno drugačnega rezultata. Kot kažejo izračuni, je model še posebej občutljiv na spremembe parametra v_{maks3} pri dozi okrog 300 mg ibuprofena, kjer je vrednost $R^{Rf}_{v_{maks3}}$ približno 10. To kaže, da bi pri spremembi parametra v_{maks3} za 10 % vrednost *Rf* lahko bila kar za 100 % različna.

Opisali smo že, da pogoj *Rf* = 1 predstavlja osrednji kriterij za napoved bronhokonstrikcije in − kot bomo pokazali v nadaljevanju − tudi za izračun mejnih doz NSAR, pri katerih je povečano tveganje za pojav bronhokonstrikcije. V obzir moramo vzeti, da je pogoj *Rf* = 1, ki ga kot kriterij za napoved bronhokonstrikcije predlagajo v študiji [65] in ga uporabljamo tudi v naših študijah [18, 19, 25], le okvirno določen na manjši skupini aspirinsko-intolerantnih astmatikov, za katere ekspresije encimov PGHS1, PGHS2 in LTC_4S niso bile posebej določene. Glede na to, da lahko model ob večjih spremembah parametrov v_{maks1}, v_{maks3} in v_{maks6} napove precej različne rezultate tudi znotraj istih modelnih stanj, bi za večjo zanesljivost rezultatov potrebovali natančno določene ekspresije encimov PGHS1, PGHS2 in LTC_4S, hkrati pa za posamezne populacije aspirinsko-intolerantnih astmatikov tudi sistematično določene mejne vrednosti razmerja *Rf* ali drugih kriterijev, pri katerih lahko pričakujemo pojav bronhokonstrikcije in drugih kliničnih znakov aspirinske intolerance.

3.2.3 Uporaba modela

3.2.3.1 Izračun mejnih doz za aspirin in ibuprofen

Izračunane vrednosti *Rf* v tabeli 7 kažejo, da vrednost *Rf* pada v odvisnosti od doze aspirina in da se pri določeni dozi v vseh modelnih stanjih vrednost *Rf* zniža pod vrednost 1. Ker vrednost *Rf* = 1 predstavlja mejno vrednost, pri kateri se pojavi bronhokonstrikcija [65], je s simulacijami smiselno določiti mejno dozo NSAR, ki ustreza pogoju *Rf* = 1. Mejna doza je teoretična ocena za najvišjo dozo NSAR, ki jo še smemo dozirati aspirinsko-intolerantnim

astmatikom, ne da bi tvegali pojav bronhokonstrikcije. Tudi v literaturi [63, 69, 73, 78] poročajo o mejnih dozah, za katere so pri aspirinsko-intolerantnih astmatikih opazili bronhokonstrikcijo ali druge simptome aspirinske intolerance. Mejne doze za aspirin znašajo med 325 mg in 650 mg [69], za ibuprofen pa so manjše od 400 mg [78]. V tabeli 8 so predstavljene napovedane vrednosti mejnih doz za aspirin ($D_{\text{mejna}}^{(\text{ASA})}$) in ibuprofen ($D_{\text{mejna}}^{(\text{IBU})}$) v vseh modelnih stanjih, ki jih primerjamo z maksimalnimi izmerjenimi mejnimi dozami za aspirin in ibuprofen.

Tabela 8. Vrednosti mejnih doz za aspirin ($D_{\text{mejna}}^{(\text{ASA})}$) in ibuprofen ($D_{\text{mejna}}^{(\text{IBU})}$) v modelnih stanjih NA, ATA, AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$ [18, 19]**.** V drugi in četrti vrstici tabele so mejne doze primerjane z maksimalnimi vrednostmi mejnih doz za aspirin (650 mg) [69] in ibuprofen (400 mg) [78].

Modelno stanje	**NA**	**ATA**	**AIA$^{(1)}$**	**AIA$^{(2)}$**	**AIA$^{(3)}$**
$D_{\text{mejna}}^{(\text{ASA})}$ [mg]	6110	2925	455	1040	5135
$D_{\text{mejna}}^{(\text{ASA})}$ / 650 mg	9,4	4,5	0,70	1,6	7,9
$D_{\text{mejna}}^{(\text{IBU})}$ [mg]	144	83	16	35	175
$D_{\text{mejna}}^{(\text{IBU})}$ / 400 mg	0,36	0,21	0,040	0,090	0,44

Izračunane mejne doze za aspirin v stanjih NA, ATA in AIA$^{(3)}$ so 9,4-, 4,5- in 7,9-krat večje od maksimalne izmerjene mejne doze 650 mg [69]. V modelnih stanjih AIA$^{(1)}$ in AIA$^{(2)}$ sta izračunani mejni dozi aspirina primerljivi z eksperimentalno določeno maksimalno mejno dozo. V stanju AIA$^{(1)}$ je napovedana mejna doza celo znotraj intervala eksperimentalno določenih mejnih doz, o katerih poročajo [69]. Mejne doze za ibuprofen so v vseh modelnih stanjih precej nižje kot maksimalna mejna doza 400 mg, o kateri poročajo v študiji [78]. Izračunane doze za ibuprofen so podobno kot za aspirin nekoliko višje v modelnih stanjih NA, ATA in AIA$^{(3)}$ in nizke v modelnih stanjih AIA$^{(1)}$ in AIA$^{(2)}$. Ta rezultat kaže, da sta stanji AIA$^{(1)}$ in AIA$^{(2)}$ izmed vseh modelnih stanj najbolj občutljivi na vpliv NSAR. Populacija AIA$^{(1)}$ je občutljiva na doze, večje od 455 mg aspirina in 16 mg ibuprofena, populacija AIA$^{(2)}$ pa na nekoliko višje doze aspirina (1040 mg) in dokaj nizke doze ibuprofena (35 mg). Spremenjeni ekspresiji encimov PGHS1 in LTC_4S bi tako lahko bili bistvenega pomena pri razvoju bronhokonstrikcije pri aspirinsko-intolerantnih astmatičnih bolnikih.

Rezultati v tabeli 8 kažejo, da so različne populacije aspirinsko-intolerantnih astmatičnih bolnikov različno občutljive na aspirin in ibuprofen. O tem poročajo tudi v

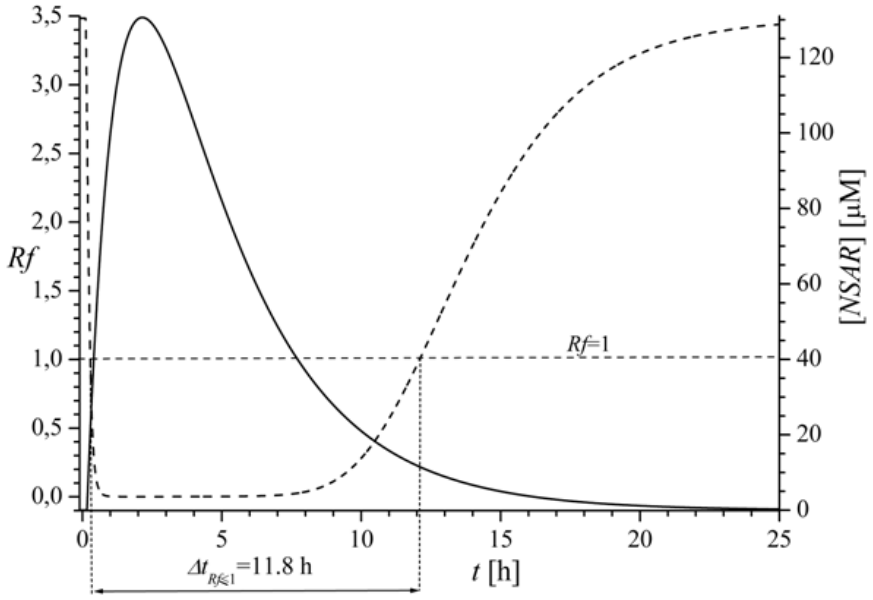
kliničnih študijah [41]. Če izberemo kriterij, da bodo aspirinsko-intolerantni bolniki znotraj neke populacije doživeli bronhokonstrikcijo tedaj, ko je napovedana mejna doza manjša ali enaka eksperimentalno določeni mejni dozi, potem iz naših rezultatov sledi, da je na aspirin občutljiva samo populacija, ki jo opišemo z modelnim stanjem $AIA^{(1)}$, na ibuprofen pa so občutljive vse tri populacije $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. Ta rezultat je v skladu s poročili iz klinične prakse, da je ibuprofen tisti NSAR, ki najpogosteje sproži simptome aspirinske intolerance [61]. V študiji [61] poročajo, da so bolnike, ki pri dozi 1000 mg aspirina niso doživeli bronhokonstrikcije, obravnavali kot aspirinsko-tolerantne. Po tem kriteriju je populacija $AIA^{(1)}$ občutljiva na aspirin, populacija $AIA^{(2)}$ pa predstavlja mejni primer. Populacija $AIA^{(3)}$ tudi po tem kriteriju ni občutljiva na aspirin v smislu razvoja bronhokonstrikcije. V naših raziskavah smo modelno stanje $AIA^{(3)}$ definirali na osnovi eksperimentalnih podatkov o znižani ekspresiji encima PGHS2 [57], ki pa so bili izmerjeni na aspirinsko-intolerantnih astmatikih, ki so po zaužitju NSAR kazali samo simptome vnetja zgornjih dihalnih poti.

Na osnovi tega vedenja in rezultatov za mejne doze aspirina in ibuprofena, ki so prikazani v tabeli 8, lahko zaključimo naslednje: i) ob doziranju ibuprofena je veliko tveganje, da se bronhokonstrikcija pojavi pri vseh treh populacijah $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, saj se pri vseh treh populacijah vrednost *Rf* zniža pod vrednost ena pri dozah ibuprofena, ki so manjše od izmerjenih mejnih doz ali tipičnih terapevtskih doz ibuprofena, ii) ob doziranju aspirina se bronhokonstrikcija lahko pojavi pri populaciji $AIA^{(1)}$ pri običajnih terapevtskih dozah aspirina ob nekoliko višjih dozah, okrog 1000 mg, pa tudi pri populaciji $AIA^{(2)}$ in iii) pri populaciji $AIA^{(3)}$ se lahko bronhokonstrikcija pojavi pri zelo visokih dozah aspirina, okrog 5000 mg, kar pa niso več smiselne terapevtske doze. Pri običajnih terapevtskih dozah aspirina bi se pri tej populaciji lahko pojavili drugi simptomi aspirinske intolerance, npr. vnetje grla in nosu.

3.2.3.2 Modelne napovedi pojava in trajanja bronhokonstrikcije

Z modelom je mogoče napovedati, po kolikem času od zaužitja NSAR lahko pričakujemo pojav bronhokonstrikcije in kako dolgo bi le-ta lahko trajala. V skladu z eksperimentalnimi rezultati, predstavljenimi v študiji [65], predpostavljamo, da se bronhokonstrikcija pojavi pri mejni vrednosti $Rf = 1$ in traja dokler je $Rf \leq 1$. Vrednost *Rf* je torej osrednji kvantitativni kriterij za izračun modelnih napovedi časa pojava bronhokonstrikcije po doziranju NSAR in časovnega intervala trajanja bronhokonstrikcije. V nadaljevanju bomo za primer modelnega stanja $AIA^{(1)}$ in dozo 400 mg ibuprofena podrobneje pokazali, kako je v okviru našega matematičnega modela možno ustrezno napovedati oba

časa. Na sliki 7 sta prikazana časovna poteka koncentracije ibuprofena v krvni plazmi in ustrezen časovni potek razmerja *Rf* .



Slika 7. Časovni potek koncentracije ibuprofena v krvni plazmi in ustrezen časovni potek razmerja *Rf* za dozo 400 mg ibuprofena v modelnem stanju AIA$^{(1)}$. *Črna neprekinjena krivulja*: časovni potek koncentracije ibuprofena v krvni plazmi pri dozi 400 mg. *Črna črtasta krivulja*: časovni potek *Rf* za 400 mg ibuprofena v modelnem stanju AIA$^{(1)}$. Z vodoravno črtkano črto je na grafu označena vrednost $Rf = 1$. Pod grafom je označen časovni interval $\Delta t_{Rf\leq 1}$, znotraj katerega je vrednost $Rf \leq 1$.

V bazalnem stanju pred doziranjem zdravila je vrednost $Rf = 3{,}5$. Po doziranju zdravila se v fazi absorpcije zdravila v kri vrednost *Rf* najprej zniža do minimalne vrednosti, nato pa v fazi eliminacije zdravila iz krvi ponovno narašča nazaj na bazalno vrednost. Za primer, prikazan na sliki 7, se vrednost *Rf* zniža do ena približno ob času $t_{Rf=1} = 0{,}16$ h po doziranju 400 mg ibuprofena in ostane pod to vrednostjo še približno $\Delta t_{Rf\leq 1} = 11{,}8$ h. Modelno napoved časa $t_{Rf=1}$ uporabimo pri napovedi časa pojava bronhokonstrikcije (t_B) po doziranju NSAR. V literaturi [8, 61, 73] poročajo, da se bronhokonstrikcija pojavi med 0,5 h do 1,5 h po zaužitju NSAR. Čas od zaužitja NSAR do pojava oz. razvoja bronhokonstrikcije (t_B) grobo ocenimo kot vsoto $t_{Rf=1}$ in eksperimentalno določenega časa od stimulacije gladkih mišičnih celic dihalnih poti z LTC_4 do njihove kontrakcije. Iz rezultatov, objavljenih v študij [70], se da oceniti, da ta čas znaša približno 15 min. Na tak način ocenjena vrednost t_B znaša za primer

na sliki 7 približno 0,80 h in je enakega velikostnega reda kot klinično opažen čas, o katerem poročajo v študiji [73], od 0,5 h do 1 h.

V tabeli 9 so prikazani rezultati simulacij, v katere smo zajeli vsa modelna stanja $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ ter pri tem določili vrednosti $t_{Rf=1}$ in $\Delta t_{Rf\leq 1}$ za 1000 mg aspirina ter 200 in 400 mg ibuprofena. Z vrednostjo $t_{Rf=1}$ smo nato ocenili še čas t_B. Analizo za aspirin smo izvedli samo v stanju $AIA^{(1)}$, saj je le populacija $AIA^{(1)}$ občutljiva na 1000 mg aspirina, za ibuprofen pa je analiza izvedena v vseh treh modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$.

Tabela 9. Modelne napovedi $t_{Rf=1}$ in $\Delta t_{Rf\leq 1}$ v modelnem stanju $AIA^{(1)}$ za 1000 mg aspirina in v modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ za 200 mg in 400 mg ibuprofena. Za vsako dozo in ustrezno stanje je izračunan tudi čas od zaužitja zdravila do pojava bronhokonstrikcije t_B.

	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
	1000 mg aspirina		
$t_{Rf=1}$[h]	0,22	/	/
$\Delta t_{Rf\leq 1}$ [h]	0,89	/	/
t_B [h]	0,47	/	/
	200 mg ibuprofena		
$t_{Rf=1}$ [h]	0,22	0,32	1,2
$\Delta t_{Rf\leq 1}$ [h]	9,8	7,5	1,8
t_B [h]	0,47	0,57	1,5
	400 mg ibuprofena		
$t_{Rf=1}$ [h]	0,16	0,23	0,55
$\Delta t_{Rf\leq 1}$ [h]	11,8	9,6	4,9
t_B [h]	0,41	0,48	0,80

Naše modelne napovedi časa t_B so v večini obravnavanih primerov, predstavljenih v tabeli 9, enakega velikostnega reda kot klinično opažen čas pojava bronhokonstrikcije, o katerem poročajo v študiji [73]. Do večjih odstopanj prihaja v primeru modelnega stanja $AIA^{(3)}$ za 200 mg ibuprofena, kjer je napovedana vrednost $t_B = 1,5$ h.

Z modelom napovedane časovne intervale $\Delta t_{Rf\leq 1}$ lahko uporabimo tudi kot teoretične ocene za čas trajanja bronhokonstrikcije. Avtorji v študiji [69] poročajo, da bronhokonstrikcija traja do 9,2 h. Naše teoretične napovedi $\Delta t_{Rf\leq 1}$, izračunane v modelnih stanjih $AIA^{(1)}$ in $AIA^{(2)}$ za 200 mg in 400 mg ibuprofena, se nahajajo med 7,5 h do 11,8 h, kar je enakega velikostnega reda kot čas trajanja bronhokonstrikcije, opažen v kliničnih raziskavah [69]. Za modelno stanje $AIA^{(1)}$ pri 1000 mg aspirina in v modelnem stanju $AIA^{(3)}$ za 200 mg in 400 mg ibuprofena pa je z modelom napovedan čas trajanja bronhokonstrikcije

precej krajši kot eksperimentalno opažen. V obzir moramo vzeti, da na dolžino časovnega intervala $\Delta t_{Rf\leq 1}$ poleg doze NSAR vplivajo še farmakokinetične lastnosti NSAR (hitrost absorpcije NSAR v krvno plazmo in hitrost eliminacije NSAR iz krvne plazme) in da je, kot kažejo rezultati naših simulacij, dolžina časovnega intervala $\Delta t_{Rf\leq 1}$ odvisna tudi od posamezne populacije aspirinsko-intolerantnih astmatikov.

Farmakokinetika aspirina je denimo mnogo hitrejša kot pri ibuprofenu, zato je tudi pri višjih dozah aspirina smiselno pričakovati krajši interval $\Delta t_{Rf\leq 1}$ kot pri ibuprofenu. Po drugi strani pa bi pri aspirinu morali upoštevati ireverzibilno inhibicijo encimov PGHS1 in PGHS2. To je najverjetneje glavni razlog, da se modelna napoved časovnega intervala $\Delta t_{Rf\leq 1}$ pri dozi 1000 mg aspirina v stanju $AIA^{(1)}$ tako bistveno razlikuje od časa trajanja bronhokonstrikcije iz klinične študije [69], saj v našem modelu upoštevamo reverzibilno inhibicijo. Dokaj velike razlike med eksperimentalnimi rezultati [69, 73] in modelnimi napovedmi, izračunanih za dozi 200 mg in 400 mg ibuprofena v modelnem stanju $AIA^{(3)}$, so predvsem posledica dejstva, da spremenjena ekspresija encima PGHS2 zmanjša odziv sistema na zdravilo tako, da so za modelno stanje $AIA^{(3)}$ značilne dokaj visoke mejne doze NSAR v primerjavi s stanjema $AIA^{(1)}$ in $AIA^{(2)}$. Na drugi strani pa so pomembna tudi dejstva, ki izhajajo iz farmakokinetičnih lastnosti zdravil, in sicer da sta hitrost absorpcije NSAR v krvno plazmo in maksimalna koncentracija NSAR v krvni plazmi odvisni od doze. Najlažje to konkretno pojasnimo na dveh primerih. Npr. v stanju $AIA^{(1)}$ je mejna doza za ibuprofen 16 mg. Maksimalna koncentracija ibuprofena v krvni plazmi pri tej dozi je mejna koncentracija, pri kateri se vrednost *Rf* v stanju $AIA^{(1)}$ zniža iz 3,5 na 1. Omenili smo, da je hitrost absorpcije zdravila odvisna od doze zdravila. Če v stanju $AIA^{(1)}$, v katerem je mejna doza ibuprofena relativno nizka (16 mg), doziramo dozo 200 mg ibuprofena, kar je precej več od mejne doze, bo koncentracija zdravila naraščala mnogo hitreje in mnogo višje, kot pa se to dogaja pri dozi 16 mg. Tako bo koncentracija zdravila pri dozi 200 mg v dokaj kratkem času dosegla mejno koncentracijo, kar pomeni, da bo $t_{Rf=1}$ kratek. Po drugi strani bo potekel daljši čas, preden se bo koncentracija zdravila v fazi absorpcije od mejne koncentracije dvignila do maksimalne in se nato v fazi eliminacije iz maksimalne koncentracije ponovno spustila nazaj do mejne, pri kateri je $Rf = 1$. To pa pomeni relativno dolg interval $\Delta t_{Rf\leq 1}$. Temu nasproten primer je simulacija, kjer smo v stanju $AIA^{(3)}$, v katerem je mejna doza ibuprofena 175 mg, dozirali dozo 200 mg ibuprofena. V tem primeru absorpcija NSAR v krvno plazmo ne bo bistveno hitrejša kot pri mejni dozi 175 mg, narasla pa bo le malo čez mejno koncentracijo, pri kateri je $Rf = 1$. To pomeni, da bo čas $t_{Rf=1}$ relativno dolg, časovni interval $\Delta t_{Rf\leq 1}$ pa kratek. Podobno

razložimo tudi primer simulacije, kjer smo v stanju $AIA^{(3)}$ dozirali dozo 400 mg ibuprofena. Ker pa je v tem primeru izbrana doza vendarle nekoliko višja kot mejna doza, je temu primeren krajši čas $t_{Rf=1}$ in ustrezno daljši časovni interval $\Delta t_{Rf\leq 1}$. Iz te razprave lahko zaključimo, da pri dozah, ki so le nekoliko višje od mejnih doz, bronhokonstrikcija nastopi po nekoliko daljšem času in ne traja dolgo. Iz modelnih napovedi, izračunanih za ibuprofen v vseh treh modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, pa vidimo, da povečana doza ibuprofena iz 200 mg na 400 mg skrajša čas $t_{Rf=1}$ in hkrati podaljša časovni interval $\Delta t_{Rf\leq 1}$. Iz tega bi lahko sklepali, da bi se pri višji dozi NSAR bronhokonstrikcija pojavila prej in bi trajala dlje časa.

3.2.3.3 Strategije varnega doziranja NSAR

V znanstveni literaturi lahko zasledimo raziskave, v katerih poročajo o možnih načinih ali strategijah, ki bi aspirinsko-intolerantnim astmatikom lahko omogočile varno doziranje NSAR [8, 63, 65, 73, 74, 78]. Iz kliničnega vidika so te strategije pomembne v primerih, ko je aspirinsko-intolerantnim astmatikom potrebno lajšati bolečine pri kroničnih vnetnih obolenjih sklepov, glavobolih in drugih zdravstvenih težavah, kjer je potrebna stalna ali občasna terapija z NSAR [63, 73, 74].

Ena izmed takšnih strategij je t. i. desenzibilizacija [63, 73, 74, 78]. Pri tej strategiji se − ob vsakodnevnem uživanju manjših doz NSAR skozi obdobje nekaj dni − vzpostavi toleranca na NSAR. Zakaj se le-ta vzpostavi, raziskovalci še ne razumejo popolnoma, vendar pa strategijo že uspešno uporabljajo v klinični praksi [73]. V naših raziskavah in v tem doktorskem delu se s preučevanjem te strategije nismo podrobneje ukvarjali, na tem mestu jo le omenimo.

Druga možnost je uporaba t. i. selektivnih NSAR [30, 73, 74, 81, 87]. Splošno znano je, da običajni (neselektivni) NSAR, kot sta aspirin in ibuprofen, inhibirajo oba encima PGHS1 in PGHS2, pri čemer je inhibicija encima PGHS1 precej močnejša kot inhibicija PGHS2. To je mogoče ugotoviti iz inhibitornih konstant K_{I3} in K_{I1} encimov PGHS1 in PGHS2 za aspirin in ibuprofen, ki so podane v tabeli 1 (glej poglavje Matematični model). Nižja vrednost konstante pomeni močnejši inhibitorni učinek NSAR na encim. Kot lahko vidimo iz tabele 1, sta vrednosti inhibitornih konstant za encim PGHS1 (K_{I3}) v primeru aspirina in ibuprofena precej nižji kot pa ustrezni vrednosti inhibitornih konstant za encim PGHS2 (K_{I1}). Običajni NSAR torej bolj zavirajo produkcijo aiPGs kot pa produkcijo piPGs. Iz tega bi sklepali, da aspirinska intoleranca nastopi kot stranski učinek inhibicije PGHS1. V

klinično prakso so tako pričeli uvajati selektivne NSAR, ki so veliko močnejši inhibitorji encima PGHS2.

V študijah [30, 81, 87] so preučili vpliv selektivnega NSAR, celecoxiba, na pojav aspirinske intolerance pri aspirinsko-intolerantnih astmatičnih bolnikih. Poročajo, da so doze celecoxiba med 200 mg in 400 mg za aspirinsko-intolerantne astmatike še varne. Po drugi strani pa poročajo [4, 73, 74], da so selektivni inhibitorji varni le pri nizkih terapevtskih dozah. V literaturi [4] je naveden celo redek primer osebe, ki ni tolerirala niti 15 mg celecoxiba in 10 mg aspirina. Tudi v naši raziskavi [25] (Priloga 3) z modelom podrobneje preučimo vrednosti mejnih doz za celecoxib. Izračunane mejne doze v posameznih modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ so: 115 mg v $AIA^{(1)}$, 223 mg v $AIA^{(2)}$ in 1300 mg v $AIA^{(3)}$ [25]. Ti rezultati kažejo na podobnost z izračuni mejnih doz za aspirin in ibuprofen, in sicer da je populacija $AIA^{(1)}$ najbolj ogrožena populacija ob doziranju NSAR, saj kaže najnižjo toleranco tako na selektivne kakor tudi neselektivne NSAR. Pri tej populaciji bi se, sodeč po naših izračunih, bronhokonstrikcija pojavila tudi pri nizkih dozah celecoxiba. Pri populaciji $AIA^{(2)}$ je vrednost mejne doze za celecoxib nekoliko nad 200 mg, vendar znotraj intervala, ki ga kot varnega navajajo v študijah [30, 81, 87]. Doziranje 400 mg celecoxiba, kar je maksimalna doza, ki so jo uporabljali v študijah [30, 81, 87], ne bi sprožilo bronhokonstrikcije le pri populaciji $AIA^{(3)}$, saj je izračunana mejna doza za celecoxib pri tej populaciji 1300 mg.

V raziskavi [25] podrobneje preučimo tudi redek primer osebe, ki ni tolerirala 15 mg celecoxiba in 10 mg aspirina [4]. S simulacijami določimo, kolikšne bi morale biti ekspresije encimov PGHS1, PGHS2 in LTC_4S glede na referenčno modelno stanje ATA, da bi bili mejni dozi za celecoxib in aspirin hkrati približno enaki 15 mg oz. 10 mg. Modelna rešitev, ki se nam zdi najverjetnejša, je, da bi ta oseba utegnila imeti 6-krat nižjo ekspresijo encima PGHS1 v primerjavi z ATA, kar je v primerjavi z $AIA^{(1)}$ še 2-krat nižje. Zelo malo verjetni se nam zdita modelni rešitvi, ki ju dobimo s spreminjanjem ekspresij encimov LTC_4S in PGHS2, saj jih moramo za dosego želenih rezultatov spremeniti za faktor 100 ali več glede na modelno stanje ATA. V znanstveni literaturi ne poročajo, da bi se ekspresiji LTC_4S in PGHS2 pri aspirinsko-intolerantnih astmatikih tako zelo razlikovali od ekspresij teh dveh encimov pri aspirinsko-tolerantnih astmatikih. Ti dve modelni rešitvi se nam tako ne zdita fiziološko smiselni.

Tretja možnost, ki omogoča varno doziranje NSAR aspirinsko-intolerantnim astmatikom, je doziranje NSAR skupaj z zdravili, ki učinkujejo kot inhibitorji encima 5-LOX [16, 26, 45, 65]. V naših študijah [19, 25] (prilogi 2 in 3) podrobneje preučimo možnosti

uporabe dveh inhibitorjev encima 5-LOX, in sicer sintetičnega analoga PGE_2 − nocloprosta in ABT-761, v kombinaciji z aspirinom in ibuprofenom. Osnovna ideja te strategije je, da z doziranjem inhibitorja 5-LOX preprečimo povišano produkcijo LTC_4 med učinkovanjem NSAR in se tako izognemo bronhokonstrikciji ter pojavu drugih simptomov aspirinske intolerance.

Nocloprost se v klinični praksi že uporablja kot učinkovina, ki bolnike pri zdravljenju z NSAR ščiti pred nastankom rane na želodcu [76]**.** Avtorji v literaturi [65] pa ga predlagajo tudi kot možno zdravilo, ki bi v kombinaciji z NSAR lahko preprečilo pojav bronhokonstrikcije in drugih simptomov aspirinske intolerance. Eksperimentalno je potrjeno, da PGE_2 inhibira encim 5-LOX [32], relaksira celice gladkih mišic dihalnih poti in učinkuje protivnetno pri astmatičnih napadih, ki so posledica zaužitja NSAR [45, 68] ali pa so posledica učinkovanja drugih alergenov [26, 33] in telesnega napora [50]. V navedenih eksperimentalnih študijah so astmatični bolniki vdihnili PGE_2 v obliki razpršila. V naši študiji [19] predpostavljamo, da bolnik zaužije nocloprost v obliki tablete. V modelnih simulacijah je tako poleg produkcije aiPGs v celici potrebno upoštevati še absorpcijo in eliminacijo zaužitega sintetičnega analoga PGE_2 v krvni plazmi. Časovni potek totalne koncentracije aiPGs ($[aiPGs]_{TOT}$) v krvni plazmi je pri tem podan z enačbo:

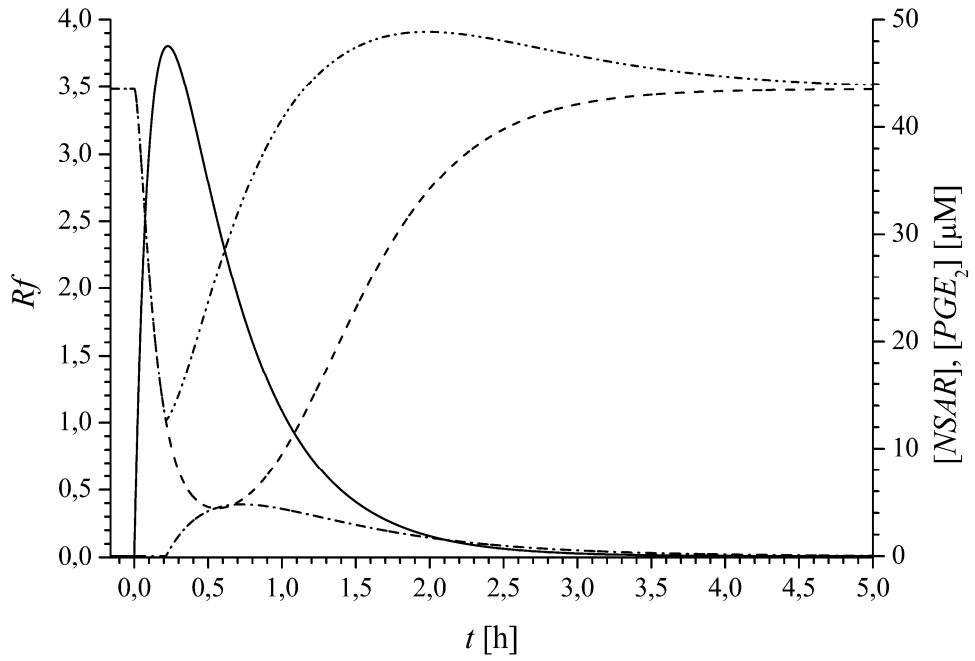
$$[aiPGs]_{TOT} = [aiPGs] + \frac{D^{(PGE_2)} k_a{}^{(PGE_2)}}{(V/F)^{(PGE_2)} (k_a{}^{(PGE2)} - k^{(PGE2)})} (e^{-k^{(PGE2)} t} - e^{-k_a{}^{(PGE2)} t}) . \qquad (21)$$

Vrednost prvega člena v enačbi (21) je določena z osnovno modelno enačbo (3), drugi člen pa opisuje časovni potek absorpcije in eliminacije nocloprosta, ki ga bolnik zaužije v obliki tablete. Totalno koncentracijo aiPGs v celici $[aiPGs]_{TOT}$, ki je določena z enačbo (21), upoštevamo v enačbah (14) in (20). Predpostavljamo tudi, da imata zaužiti nocloprost in naravni PGE_2, ki se tvori s presnovo AA v celici, enak inhibitorni učinek na encim 5-LOX.

S simulacijami preučimo, kako dozirati nocloprost v kombinaciji s 1000 mg aspirina in 200 mg ter 400 mg ibuprofena, da bi se izognili pojavu bronhokonstrikcije. Za aspirin študiramo strategijo le v modelnem stanju $AIA^{(1)}$, saj je le v tem modelnem stanju mejna doza aspirina manjša od 1000 mg. Za obe dozi ibuprofena, 200 mg in 400 mg, pa to strategijo študiramo v vseh treh modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, saj so mejne doze ibuprofena v vseh teh stanjih manjše od 200 mg. Kot osrednji kriterij za napoved bronhokonstrikcije uporabimo pogoj $Rf = 1$. Predpostavimo, da aspirinsko-intoleranten astmatik najprej zaužije

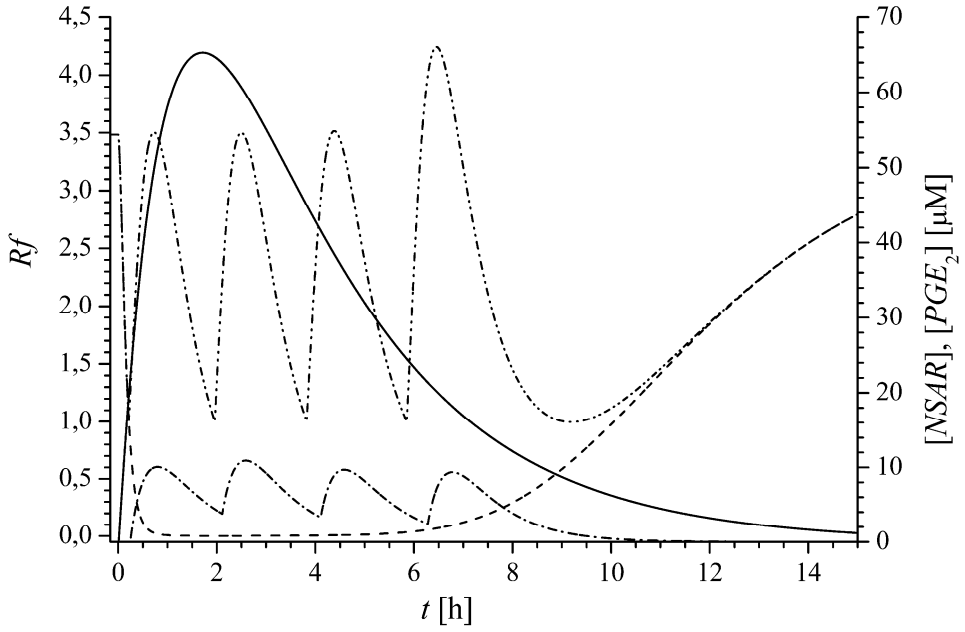
NSAR, nato pa sledi doziranje ene ali več zaporednih doz nocloprosta. Z modelom določimo čas doziranja nocloprosta in število ter vrednosti doz nocloprosta tako, da se vrednost *Rf* med učinkovanjem NSAR ne zniža pod vrednost ena. Študija temelji na analizi časovnih potekov *Rf* za vsako posamezno modelno stanje $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. Najprej analiziramo časovne poteke *Rf* samo ob prisotnosti NSAR. Po doziranju NSAR se *Rf* iz bazalne vrednosti zniža na minimalno vrednost, ki je za dozo, višjo od mejne, pod vrednostjo ena. Pri tem določimo čas od doziranja NSAR pa do trenutka, ko je $Rf = 1$ ($t_{Rf=1}$). V naslednjem koraku simuliramo še časovne poteke *Rf* ob prisotnosti NSAR in nocloprosta. Ob času $t_{Rf=1}$ doziramo nocloprost in določimo dozo nocloprosta, za katero se vrednost *Rf* ne zniža pod 1, hkrati pa naraste nazaj na bazalno vrednost. Če se po doziranju nocloprosta vrednost *Rf* ponovno spusti na kritično vrednost 1, tedaj ob naslednjem času $t_{Rf=1}$ ponovno doziramo naslednjo dozo nocloprosta. Postopek lahko tako večkrat ponovimo in dosežemo, da je vrednost *Rf* ves čas učinkovanja NSAR večja od 1. Ob zadnjem doziranju nocloprosta izjemoma dopustimo tudi višje doze, za katere se je vrednost *Rf* dvignila nekoliko nad bazalno vrednost, vendar le, če se s tem izognemo nadaljnjemu doziranju nocloprosta. V nadaljevanju bomo pokazali, da se ta strategija v podrobnostih bistveno razlikuje, kadar uporabljamo različne kombinacije zdravil (npr. aspirin in nocloprost ali ibuprofen in nocloprost), kar je posledica razlik v farmakokinetičnih lastnosti nocloprosta in uporabljenih NSAR.

Slika 8 prikazuje časovne poteke *Rf* v stanju $AIA^{(1)}$, ki so izračunani v simulacijah z dozo 1000 mg aspirina. Prikazani so: časovni potek *Rf* po doziranju aspirina (prekinjena krivulja: črta-črta), časovni potek *Rf* po doziranju aspirina in nocloprosta (prekinjena krivulja: črta-pika-pika-črta) ter časovna poteka koncentracije aspirina (neprekinjena krivulja) in nocloprosta (prekinjena krivulja: črta-pika-črta), ki smo ju uporabili v simulacijah. Po zgoraj opisanem postopku izvedemo najprej simulacijo z aspirinom. Pred doziranjem aspirina, ob času $t < 0$, zavzame *Rf* bazalno vrednost 3,5. Ob času $t = 0$ doziramo aspirin in vrednost *Rf* prične padati. Približno 0,22 h po doziranju aspirina je $Rf = 1$. Od tega trenutka naprej je možen pojav bronhokonstrikcije. Vrednost *Rf* je manjša od ena še približno 0,89 h, nakar počasi naraste nazaj na bazalno vrednost. V drugi simulaciji ob času $t_{Rf=1} = 0{,}22$ h doziramo nocloprost. V simulaciji določimo, da se za dozo 46 mg nocloprosta vrednost *Rf* dvigne nazaj − približno do bazalne vrednosti. Po doziranju nocloprosta se vrednost *Rf* ne zniža več pod vrednost ena. V primeru 1000 mg doze aspirina bi torej zadostovala le ena 46 mg doza nocloprosta.



Slika 8. Strategija doziranja nocloprosta v kombinaciji s 1000 mg aspirina v modelnem stanju AIA$^{(1)}$ [19]. *Neprekinjena krivulja*: časovni potek koncentracije aspirina v krvni plazmi. *Prekinjena krivulja črta-črta*: časovni potek *Rf* po doziranju 1000 mg aspirina. *Prekinjena krivulja črta-pika-črta*: časovni potek nocloprosta za dozo 46 mg. *Prekinjena krivulja črta-pika-pika-črta*: časovni potek *Rf* po doziranju 1000 mg aspirina in dozi 46 mg nocloprosta.

Enako strategijo študiramo še za 200 mg in 400 mg ibuprofena v modelnih stanjih AIA$^{(1)}$, AIA$^{(2)}$ in AIA$^{(3)}$. Simulacije so podrobneje razložene le za stanje AIA$^{(1)}$ pri dozi 200 mg ibuprofena, saj so v ostalih obravnavanih primerih podobne. Modelne simulacije doziranja nocloprosta v kombinaciji z 200 mg ibuprofena v modelnem stanju AIA$^{(1)}$ so prikazane na sliki 9. Simulacije se bistveno razlikujejo od primera z aspirinom, saj ima ibuprofen v primerjavi z nocloprostom precej počasnejšo farmakokinetiko, kar je razvidno iz primerjave časovnih skal na slikah 8 in 9. V tem primeru je zato potrebno dozirati več zaporednih doz nocloprosta. Ker je ibuprofen tudi močnejši inhibitor encimov PGHS1 in PGHS2 v primerjavi z aspirinom, so vrednosti doz nocloprosta v tem primeru višje. Potek simulacij je podoben tistemu, ki smo ga opisali na primeru aspirina. Najprej izvedemo simulacijo časovnega poteka *Rf* za dozo 200 mg ibuprofena. Pred doziranjem ibuprofena $t < 0$ je vrednost $Rf = 3{,}5$, po doziranju ibuprofena pa prične vrednost *Rf* padati (prekinjena krivulja: črta-črta). Vrednost $Rf = 1$ po času 0,22 h od doziranja ibuprofena, pod ena pa ostane še nadaljnjih 9,8 h. Ob času 0,22 h doziramo tudi prvo dozo 97 mg nocloprostra (prekinjena krivulja: črta-pika-pika-črta). Vrednost *Rf* naraste sprva nazaj na 3,5, vendar prične ponovno padati in je po času 1,95 h od doziranja ibuprofena ponovno enaka ena. Takrat doziramo drugo dozo 84 mg nocloprosta.



Slika 9. Strategija doziranja nocloprosta v kombinaciji z 200 mg ibuprofena v modelnem stanju $AIA^{(1)}$ [19]. *Neprekinjena krivulja*: časovni potek koncentracije ibuprofena v krvni plazmi. *Prekinjena krivulja črta-črta:* časovni potek *Rf* po doziranju 200 mg aspirina. *Prekinjena krivulja črta-pika-črta*: časovni potek nocloprosta. *Prekinjena krivulja črta-pika-pika-črta*: časovni potek *Rf* po doziranju 200 mg ibuprofena in večih zaporednih dozah nocloprosta.

Postopek nato ponovimo še 2-krat, in sicer ob času 3,81 h po doziranju ibuprofena, ko doziramo 75 mg nocloprosta, in ob času 5,8 h po doziranju ibuprofena, ko doziramo še 84 mg nocloprosta. Zadnja doza nocloprosta je namenoma nekoliko višja. Vrednost *Rf* se dvigne nekoliko nad bazalno vrednost, vendar se s tem izognemo še najmanj eni nadaljnji dozi nocloprosta. V simulacijah določeni časi doziranja nocloprosta in njegove ustrezne doze so za vse izbrane doze aspirina in ibuprofena v modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ podani v tabeli 10.

Rezultati v tabeli 10 prikazujejo možne načine doziranja nocloprosta, s katerimi bi se pri doziranju NSAR aspirinsko-intolerantnim astmatikom lahko izognili pojavu bronhokonstrikcije. Rezultati v tabeli so izračunani kot rešitve modelnih enačb, torej kot eksaktne napovedi matematičnega modela. V klinični praksi bi bil postopek doziranja nocloprosta po vsej verjetnosti poenostavljen v smislu odmerjenih doz in časa doziranja. Tudi s simulacijami smo ugotovili, da bi doziranje nocloprosta v kombinaciji z aspirinom in ibuprofenom lahko bilo bolj enostavno. Denimo v primeru, ko nocloprost doziramo v kombinaciji s 1000 mg aspirina, lahko obe zdravili doziramo hkrati. V tem primeru bi zadostovala celo manjša doza nocloprosta, približno 15 mg. Možen je tudi splošnejši in enostavnejši način doziranja ibuprofena. Ta je naslednji: prvo dozo nocloprosta doziramo

skupaj z izbrano dozo ibuprofena, nadaljnje doze nocloprosta pa na vsaki dve uri. Možni enostavnejši načini doziranja nocloprosta v kombinaciji z aspirinom in ibuprofenom v stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ so prikazani v tabeli 11.

Omeniti je tudi potrebno, da so načini doziranja nocloprosta, ki so prikazani v tabelah 10 in 11, določeni le za tiste populacije aspirinsko-intolerantnih astmatikov, za katere smo v literaturi dobili eksperimentalne podatke o ekspresijah encimov PGHS1, PGHS2 in LTC_4S. Ker ekspresije teh encimov v modelu nastopajo kot modelni parametri, katerih vrednosti lahko spremenimo tudi za več redov velikosti, je z modelom mogoče določiti ustrezen način doziranja nocloprosta praktično za vsakega aspirinsko-intolerantnega astmatika, za katerega bi na razpolago imeli ustrezne podatke o ekspresijah encimov PGHS1, PGHS2 in LTC_4S. Z modelom lahko tako način doziranja NSAR simuliramo za vsakega bolnika posebej, v kolikor bi se izkazalo, da je to potrebno.

Predstavljeni rezultati kažejo, da je način doziranja nocloprosta odvisen predvsem od farmakokinetičnih lastnosti nocloprosta in uporabljenega NSAR. Nocloprost in aspirin imata podobne farmakokinetične lastnosti in tako se učinki obeh zdravil na presnovo AA pokažejo ob približno enakem času. To je tudi razlog, da je v kombinaciji z aspirinom potrebno dozirati le eno dozo nocloprosta. Ibuprofen ima precej počasnejšo farmakokinetiko, zato je v tem primeru nocloprost potrebno dozirati večkrat zaporedoma. S podrobnejšo analizo rezultatov v tabeli 10 še ugotovimo, da so vrednosti in število zaporednih doz nocloprosta, ki jih doziramo v posameznih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, odvisne tudi od doze ibuprofena. Ker je pri večji dozi ibuprofena vrednost *Rf* dalj časa pod vrednostjo ena (glej vrednosti $\Delta t_{Rf\leq 1}$ v tabeli 9), je nocloprost potrebno dozirati večkrat zaporedoma. Ker se vrednost *Rf* pri višji dozi ibuprofena zniža do nižje minimalne vrednosti, je posledično potrebno dozirati tudi višje doze nocloprosta, da je med učinkovanjem ibuprofena vrednost *Rf* višja od ena. V splošnem lahko zaključimo, da bi bila ta strategija uspešna. V primeru uporabe NSAR, ki ima počasnejšo farmakokinetiko, pa je nocloprost potrebno dozirati večkrat zaporedoma.

Zelo previdno bi morali načrtovati strategijo doziranja nocloprosta v kombinaciji z dozo 300 mg ibuprofena za populacijo $AIA^{(3)}$. Senzitivnostna analiza namreč kaže, da je sistem v stanju $AIA^{(3)}$ pri dozi 300 mg ibuprofena zelo občutljiv na spremembe v ekspresiji encima PGHS1, ki lahko nastopijo kot posledica interindividualnih razlik. Le-te v tem primeru v modelu opišemo z majhnimi spremembami vrednosti parametra v_{maks3}. Že majhne razlike v vrednosti tega parametra bi pri dozi okrog 300 mg ibuprofena lahko zelo vplivale na

Tabela 10. Tabelarični prikaz doziranja nocloprosta v kombinaciji s 1000 mg aspirina v modelnem stanju $AIA^{(1)}$ in v kombinaciji z 200 mg in 400 mg ibuprofena v modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ [19]. Od drugega stolpca naprej so podani časi doziranja (zgoraj) in ustrezne doze nocloprosta (spodaj).

Modelno stanje	t_1 [h] $D_1^{(PGE_2)}$ [mg]	t_2 [h] $D_2^{(PGE_2)}$ [mg]	t_3 [h] $D_3^{(PGE_2)}$ [mg]	t_4 [h] $D_4^{(PGE_2)}$ [mg]	t_5 [h] $D_5^{(PGE_2)}$ [mg]
1000 mg aspirina					
$AIA^{(1)}$	0,22 46	/ /	/ /	/ /	/ /
200 mg ibuprofena					
$AIA^{(1)}$	0,22 97	1,95 84	3,81 75	5,80 84	/ /
$AIA^{(2)}$	0,32 374	2,52 343	5,20 31	/ /	/ /
$AIA^{(3)}$	1,18 15	/ /	/ /	/ /	/ /
400 mg ibuprofena					
$AIA^{(1)}$	0,16 112	1,90 99	3,70 88	5,50 77	7,50 114
$AIA^{(2)}$	0,23 439	2,30 417	4,60 346	7,30 23	/ /
$AIA^{(3)}$	0,55 213	3,00 22	/ /	/ /	/ /

Tabela 11. Tabelarični prikaz poenostavljenih načinov doziranja nocloprosta v kombinaciji s 1000 mg aspirina in 200 ter 400 mg ibuprofena v modelnih stanjih $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ [19].

Modelno stanje	$t_1 = 0$ $D_1^{(PGE_2)}$ [mg]	$t_2 = 2h$ $D_2^{(PGE_2)}$ [mg]	$t_3 = 4h$ $D_3^{(PGE_2)}$ [mg]	$t_4 = 6h$ $D_4^{(PGE_2)}$ [mg]	$t_5 = 8h$ $D_5^{(PGE_2)}$ [mg]
1000 mg aspirina					
$AIA^{(1)}$	15	/	/	/	/
200 mg ibuprofena					
$AIA^{(1)}$	130	100	70	70	/
$AIA^{(2)}$	300	210	150	/	/
$AIA^{(3)}$	30	/	/	/	/
400 mg ibuprofena					
$AIA^{(1)}$	160	120	100	70	60
$AIA^{(2)}$	420	300	210	150	/
$AIA^{(3)}$	160	70	/	/	/

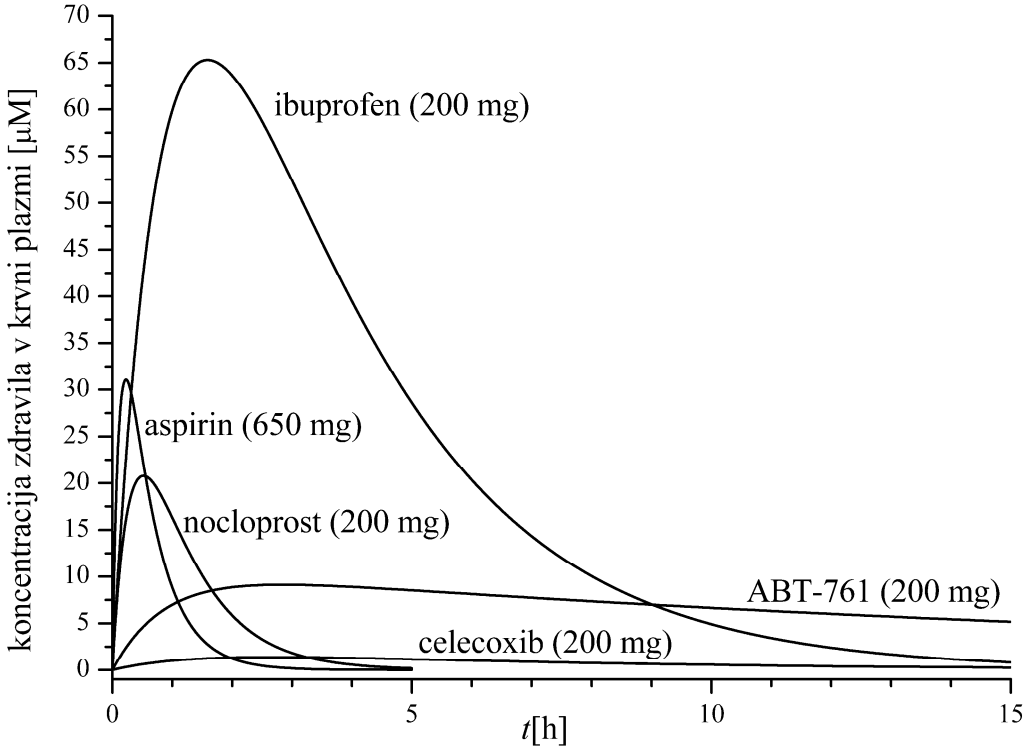
napovedane doze nocloprosta $D^{(PGE_2)}$. V študiji [19] smo podrobneje preučili ta primer za dozo 200 mg ibuprufena, pri čemer smo predpostavili, da interindividualne razlike v ekspresiji PGHS1 znašajo 10 %. Če je modelno stanje AIA$^{(3)}$ definirano z vrednostmi v_{maks1} = 0,11 μMs^{-1}, v_{maks3} = 0,096 μMs^{-1} in v_{maks6} = 0,23 μMs^{-1}, potem je po poenostavljenem načinu doziranja nocloprosta, ki je predstavljen v tabeli 11, hkrati z 200 mg ibuprofena potrebno dozirati 30 mg nocloprosta, da se izognemo bronhokonstrikciji. Za 10 % nižja vrednost parametra v_{maks3} zviša dozo nocloprosta na 70 mg. Enaki spremembi v parametrih v_{maks1} ali v_{maks6} doze nocloprosta ne spremenita bistveno. Pri načrtovanju oz. napovedovanju te strategije za populacijo AIA$^{(3)}$ pri dozah blizu 300 mg ibuprofena bi torej morali biti še posebej previdni in bi morali predvideti morebitne večje razlike v napovedanih dozah nocloprosta, ki bi utegnile nastopiti kot posledica interindividualnih razlik. Iz tega stališča bi ta strategija pod določenimi okoliščinami lahko postala precej zapletena, ko bi bilo potrebno dozirati več doz nocloprosta zaporedoma. Druga alternativa tej strategiji je uporaba inhibitorja 5-LOX, ABT-761. Ta ima počasnejšo farmakokinetiko, predvsem na račun počasnejše faze eliminacije. Zraven ABT-761 je tipičen inhibitor 5-LOX, ki ga uporabljajo v kliničnih študijah, še zileuton.

V študiji [16] so kot inhibitor 5-LOX uporabili zileuton, ki so ga aspirinsko-intolerantnim astmatikom dozirali 4-krat dnevno po 600 mg. Poročajo, da se je s takšno terapijo zdravstveno stanje bolnikom izjemno izboljšalo. Zileuton inhibira produkcijo cisteinil levkotrienov in s tem zmanjša možnosti pojava bronhokonstrikcije ter drugih kliničnih znakov aspirinske intolerance [16]. Poročajo celo, da se je bolnikom povrnil vonj [16]. V naši študiji [25] kot drug možen inhibitor encima 5-LOX uporabimo učinkovino ABT-761 oz. atreleuton, ki spada v zadnjo generacijo inhibitorjev 5-LOX. Tipične doze ABT-761, ki so jih uporabljali v različnih študijah, so med 10 mg do 300 mg [59, 83, 84, 85]. ABT-761 je 150-krat močnejši inhibitor 5-LOX v primerjavi s zileutonom [59] in ima precej počasnejšo fazo eliminacije v primerjavi z aspirinom, ibuprofenom, celecoxibom in nocloprostom. Primerjava farmakokinetičnih lastnosti omenjenih zdravil je prikazana na sliki 10.

V simulacijah opišemo časovni potek ABT-761 z enačbo (19), v enačbi (14), ki opisuje inhibicijo encima 5-LOX z aiPGs, pa upoštevamo še dodaten člen, ki opiše inhibicijo encima 5-LOX z ABT-761. Ustrezna enačba za α_5, ki upošteva inhibicijo 5-LOX z aiPGs in z ABT-761, je:

$$\alpha_5 = 1 + \frac{[aiPGs]}{K_{I5}} + \frac{[5-LOXIB]}{K_{I5}^{(ABT)}}, \qquad (22)$$

kjer je $K_{I5}^{(ABT)}$ inhibitorna konstanta encima 5-LOX z ABT-761, [*5-LOXIB*] pa koncentracija ABT-761.



Slika 10. Primerjava farmakokinetičnih lastnosti aspirina, ibuprofena, celecoxiba, nocloprosta in ABT-761 [25].

V simulacijah ugotovimo, da je ibuprofen v kombinaciji z ABT-761 smiselno dozirati tako, da najprej doziramo ABT-761, nato pa po približno 2,5 h, ko je koncentracija ABT-761 v krvni plazmi maksimalna, doziramo še ibuprofen. Tako je doza ABT-761 manjša kot v primeru, če bi ibuprofen in ABT-761 dozirali hkrati. Za posamezna modelna stanja $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ pri dozi 200 mg ibuprofena določimo doze ABT-761 tako, da je ves čas učinkovanja ibuprofena vrednost *Rf* večja od ena. Izračunane doze so: 510 mg za stanje $AIA^{(1)}$, 700 mg za stanje $AIA^{(2)}$ in 36 mg za stanje $AIA^{(3)}$. Čeprav ima ibuprofen precej počasno farmakokinetiko, v vseh primerih zadostuje le ena doza ABT-761.

4 Povzetek ugotovitev in zaključki

V pričujočem doktorskem delu smo predstavili raziskave, v katerih preučujemo vpliv NSAR na razvoj aspirinske intolerance pri astmatičnih bolnikih. Temelj raziskav predstavlja matematični model, ki je rezultat našega raziskovalnega dela [18]. Model je zasnovan na podlagi rezultatov iz številnih eksperimentalnih študij [1, 13, 28, 29, 31, 42, 49, 52, 53, 55, 71], v katerih so podrobno preučili kinetiko posameznih encimskih reakcij presnove AA.

Model smo verificirali z eksperimentalnimi rezultati iz študij [15, 57, 58, 74] o spremenjenih ekspresijah encimov PGHS1, PGHS2 in LTC_4S pri neastmatikih, aspirinsko-tolerantnih in aspirinsko-intolerantnih astmatikih. S tremi prostimi modelnimi parametri, v_{maks3}, v_{maks1} in v_{maks6}, ki v modelu opisujejo maksimalne aktivnosti encimov PGHS1, PGHS2 in LTC_4S, smo definirali pet različnih modelnih stanj, ki opišejo neastmatike (NA), aspirinsko-tolerantne astmatike (ATA) in tri različe populacije aspirinsko-intolerantnih astmatikov ($AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$). Kot osrednji kriterij za izračun modelnih napovedi smo uporabili razmerje med koncentracijama aiPGs in LTC_4 – *Rf* [65]. Modelne napovedi mejnih doz, napovedani časi pojava bronhokonstrikcije in napovedani časovni intervali trajanja bronhokonstrikcije v različnih modelnih stanjih so v večini primerov blizu eksperimentalno izmerjenim rezultatom [69, 78]. Majhne interindividualne razlike, ki znašajo nekaj odstotkov v ekspresiji encimov PGHS1, PGHS2 in LTC_4S med populacijami $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$, ne vodijo do bistveno drugačnega kvalitativnega obnašanja modela. To bi se lahko zgodilo v določenih primerih ob večjih interindividualnih razlikah.

Iz napovedanih vrednosti mejnih doz lahko izpeljemo naslednje zaključke.

1. Modelno stanje $AIA^{(1)}$, ki je definirano kot stanje z znižano ekspresijo encima PGHS1, kaže najbolj buren odziv na učinkovanje NSAR. Ta modelna populacija aspirinsko-intolerantnih astmatikov kaže najnižjo toleranco na aspirin, ibuprofen in celecoxib, možnost pojava bronhokonstrikcije pa je povečana že pri nizkih dozah NSAR. Znižana ekspresija encima PGHS1 bi lahko bila verjeten vzrok za pojav bronhokonstrikcije pri aspirinski intoleranci.
2. Modelna populacija aspirinsko-intolerantnih astmatikov s povišano ekspresijo encima LTC_4S, ki jo opišemo z modelnim stanjem $AIA^{(2)}$, je v smislu bronhokonstrikcije občutljiva na nekoliko višje doze aspirina, nizke doze ibuprofena in terapevtske doze celecoxiba. Ugotavljamo, da je znižana ekspresija encima LTC_4S možen vzrok za pojav bronhokonstrikcije pri nizkih dozah ibuprofena, terapevtskih dozah celecoxiba in visokih terapevtskih dozah aspirina.

3. Modelno stanje $AIA^{(3)}$, s katerim opišemo modelno populacijo aspirinsko-intolerantnih astmatikov z znižano ekspresijo encima PGHS2, kaže zelo šibak odziv na učinkovanje NSAR. Izračunane mejne doze različnih NSAR v tem modelnem stanju so precej višje kot v modelnih stanjih $AIA^{(1)}$ in $AIA^{(2)}$. Iz različnih kriterijev, ki smo jih podrobneje opisali v tem delu, ocenjujemo, da bi bolniki iz te populacije pri nizkih dozah aspirina in celecoxiba najverjetneje kazali simptome aspirinske intolerance, ki so povezani z vnetjem zgornjih dihalnih poti. Pojav bronhokonstrikcije pri tej populaciji pa je možen že pri nizkih dozah ibuprofena.

Napovedali smo strategijo, s katero bi aspirinsko-intolerantnim astmatikom lahko omogočili varno doziranje NSAR, brez tveganja bronhokonstrikcije. Preučimo možnosti uporabe dveh inhibitorjev 5-LOX v kombinaciji z aspirinom in ibuprofenom in sicer sintetičnega analoga PGE_2 – nocloprosta in učinkovine ABT-761. Ko nocloprost doziramo skupaj z aspirinom, zadostuje že ena doza nocloprosta, da se izognemo bronhokonstrikciji. V primeru, ko nocloprost doziramo v kombinaciji z ibuprofenom, moramo nocloprost dozirati večkrat zaporedoma, v različnih dozah. Pri tem je število doz nocloprosta − kakor tudi njihove vrednosti − odvisno od doze ibuprofena. To strategijo moramo previdno načrtovati v stanju $AIA^{(3)}$ pri dozi 300 mg ibuprofena. Pri tej dozi iboprufena je model v stanju $AIA^{(3)}$ zelo občutljiv na interindividualne razlike v ekspresiji encima PGHS1. Majhne razlike v ekspresiji tega encima lahko vodijo do precejšnih razlik v napovedanih dozah nocloprosta $D^{(PGE_2)}$. Iz teh ugotovitev predvidevamo, da bi bilo to strategijo v praksi enostavno izvesti le za kombinacijo aspirina in nocloprosta. Strategija se bistveno poenostavi, če v kombinaciji z iboprufenom uporabimo inhibitor 5-LOX, ABT-761, ki ima počasno fazo eliminacije iz krvne plazme in je zelo učinkovit inhibitor 5-LOX.

V raziskavah smo izhajali iz naslednjih štirih tez (prepisano iz vloge za prijavo doktorskega dela).

1. Razvili bomo izvirni matematični model, s katerim bomo opisali glavne značilnosti presnove arahidonske kisline.

2. Na osnovi eksperimentalnih podatkov iz literature o ekspresijah encimov prostaglandin H sintaz 1 in 2 ter levkotrien C_4 sintaze pri aspirinsko-intolerantnih astmatikih bomo napovedali različne populacije aspirinsko-intolerantnih astmatikov in za te populacije pokazali različno obnašanje sistema ob doziranju NSAR. S tem bomo napovedali različna možna stanja aspirinske intolerance.

3. Model bo uspešno opisal razmerja koncentracij protivnetnih prostaglandinov in cisteinil levkotrienov v krvi neastmatikov, aspirinsko-tolerantnih astmatikov in aspirinsko-intolerantnih astmatikov.

4. Z modelom bomo proučevali strategijo doziranja NSAR, ki bi v kombinaciji z aplikacijo sintetičnih analogov protivnetnih prostaglandinov aspirinsko-intolerantnim astmatikom omogočila varno in učinkovito zdravljenje z NSAR brez tveganja bronhokonstrikcije.

Raziskave, predstavljene v tem doktorskem delu, so dale naslednje izvirne znanstvene prispevke in odgovore na zastavljene teze.

- Izviren matematični model, s katerim preučujemo aspirinsko intoleranco na nivoju molekul, celic in organov [18, 19, 25]. Gre za že uveljavljen, toda razmeroma nov pristop sistemske biologije (ang. Systems Biology), za katero je značilen t. i. "multiscale approach". Pri tem težimo k razumevanju bioloških sistemov na osnovi povezovanja različnih delov sistema na različnih nivojih.
- Pokazali smo, da ekspresije encimov PGHS1, PGHS2 in LTC_4S bistveno vplivajo na produkcijo LTC_4 in PGE_2 že v odsotnosti NSAR [18].
- Pokazali smo, da sklopitev osnovnega modela presnove AA s farmakokinetičnim modelom absorpcije in eliminacije NSAR v krvni plazmi omogoča napoved časovnega poteka razmerja *Rf* v odvisnosti od časovno odvisne koncentracije NSAR. Iz tega časovnega poteka pa je možno napovedati mejno dozo NSAR, čas pojava bronhokonstrikcije in čas trajanja le-te za različne populacije aspirinsko-intolerantnih astmatikov [19].
- S podrobno teoretično analizo smo pokazali, da sta čas doziranja inhibitorja 5-LOX in njegova doza pomembna klinična parametra pri strategiji, s katero bi aspirinsko-intolerantnim astmatikom omogočili varno doziranje NSAR brez tveganja bronhokonstrikcije [25].

Ugotavljamo, da smo potrdili vse zastavljene teze. Opisani izvirni znanstveni prispevki prispevajo k napredku raziskav aspirinske intolerance in so bili objavljeni v obliki dveh izvirnih znastvenih člankov (prilogi 1 in 2) v kvalitetnih mednarodnih znanstvenih revijah s faktorjem vpliva ter v obliki samostojnega poglavja v znanstveni monografiji (priloga 3).

5 Možnosti za nadaljnje znanstveno-raziskovalno delo

V pričujočem doktorskem delu smo pokazali, da matematični model, ki smo ga razvili v raziskavah, omogoča dokaj širok pristop k preučevanju biokemijskih procesov, vpletenih v razvoj aspirinske intolerance. Model je možno uporabiti tudi za simulacije v okviru raziskav drugih obolenj, pri katerih so osrednjega pomena produkti AA. Nekatere raziskave kažejo, da bi LTC_4 in PGE_2 lahko bila osrednjega pomena v diagnosticiranju aspirinske intolerance in drugih bolezni, kot sta rana na želodcu in črevesni rak [66, 67]. Avtorja v [66, 67] sta nakazala možnosti razvoja diagnostičnih testov na osnovi merjenja koncentracij LTC_4 in PGE_2 v levkocitih zdravih ljudi in bolnikov. V izmerjenih podatkih sta poskušala najti vzorce v produkciji LTC_4 in PGE_2, ki bi bili značilni za bolnike z zgoraj omenjenimi boleznimi. Naš matematični model bi lahko služil tudi kot teoretično orodje, s katerim bi bilo možno pojasniti različne opažene vzorce v takšnih eksperimentalnih podatkih. Iz tega vidika je model uporaben tudi v namene razvoja diagnostičnih testov za različne bolezni, katerih izvor je v neobičajni regulaciji ali hitrosti nastajanja produktov AA.

V zadnjih nekaj letih smo se s sodelavci aktivno posvečali tudi matematičnemu modeliranju kalcijeve signalne poti, ki vodi do razvoja sile v gladkih mišičnih celicah dihalnih poti. Razvoj sile v le-teh omogoča interakcija miozinskih prečnih mostičkov z aktivnimi mesti na aktinskih vlaknih. Produkcija sile je odvisna od deleža na aktinska vlakna vezanih in fosforiliranih prečnih mostičkov. Proces fosforilacije prečnih mostičkov regulira encim kinaza lahkih verig miozina (MLCK). Ker je aktivnost encima MLCK odvisna od koncentracije kalcijevih ionov Ca^{2+} v citoplazmi gladkih mišičnih celic dihalnih poti, je od koncentracije ionov Ca^{2+} odvisen tudi razvoj sile v teh celicah. Proces defosforilacije prečnih mostičkov regulira encim fosfataza lahkih verig miozina (MLCP). Defosforilirani prečni mostički se sprostijo iz aktinskih vlaken, pri čemer se sila zmanjša, lahko pa ostanejo vezani na aktinska vlakna in vzdržujejo silo v celicah tudi ob znižani koncentraciji Ca^{2+} ionov v citoplazmi. S sodelavci smo objavili več študij, v katerih smo podrobno preučili nekatere bistvene elemente kalcijeve signalne poti od nastanka kalcijevega signala do procesov fosforilacije in defosforilacije miozinski prečnih mostičkov ter interakcijo med miozinskimi prečnimi mostički in aktinskimi vlakni [12, 17, 20 - 24].

Kljub temu da je zgoraj opisana kalcijeva signalna pot, ki vodi do razvoja sile v gladkih mišičnih celicah dihalnih poti, razmeroma dobro raziskana, pa ostaja odprto vprašanje, kako na krčenje gladkih mišičnih celic dihalnih poti vpliva stimulacija z vnetnim

mediatorjem LTC_4. Znano je, da se LTC_4 veže na receptorja Cys-LT_1 in Cys-LT_2 na celični membrani [70, 72, 73, 74] ter da to sproži razvoj sile v celicah, vendar pa signalna pot od stimulacije celic z LTC_4 do razvoja sile ni povsem natančno poznana. Kot osrednji problem za prihodnje raziskovalno delo bi zato lahko izpostavili raziskave signalne poti, ki vodi do razvoja sile v gladkih mišičnih celicah dihalnih poti preko stimulacije z LTC_4. Tako bi povezali model iz pričujočega doktorskega dela z že obstoječimi modeli od kalcija odvisnega razvoja sile. S takšno združitvijo modelov bi si lahko postavili širše zastavljena raziskovalna vprašanja. Tako bi lahko celoviteje in bolj poglobljeno raziskovali, kako na razvoj bronhokonstrikcije pri astmi vplivajo cisteinil levkotrieni, ter mnogo bolje razumeli, katera populacija aspirinsko-intolerantnih astmatikov, ki jih obravnavamo v tem doktorskem delu, je po zaužitju NSAR najbolj izpostavljena bronhokonstrikciji.

V tem doktorskem delu smo pokazali, kako lahko z modelom napovemo nekatere parametre, ki bi utegnili biti tudi klinično uporabni, kot so npr. mejna doza, čas pojava bronhokonstrikcije in čas trajanja bronhokonstrikcije. Vse te modelne napovedi smo izračunali iz pogoja $Rf = 1$ [65]. Kot možne raziskave v prihodnosti vidimo tudi teoretično preučevanje različnih kriterijev, kot sta *Rf* ali absolutna vrednost koncentracije LTC_4 v celici, ki bi lahko bili relevantni v napovedih bronhokonstrikcije in morda tudi drugih simptomov aspirinske intolerance.

Literatura

1. Aharony D, Stein R (1986) Kinetic mechanism of guinea pig neutrophil 5-lipoxygenase. J Biol Chem 261: 11512−11519

2. Babu KS, Salvi SS (2000) Aspirin and asthma. Chest 118: 1470−1476

3. Bachert C, van Cauwenberge P, Khaltaev N, Bousquet J (2002) Allergic rhinitis and its impact on asthma. Executive summary of the workshop report. Allergy 57: 841–855

4. Baldassarre S, Schandene L, Choufani G, Michils A (2006) Asthma attacks induced by low doses of celecoxib, aspirin, and acetaminophen. J Allergy Clin Immunol 117: 215−217

5. Barnes NC, Smith LJ (1999) Biochemistry and physiology of the leukotrienes. Clin Rew Allerg Immu 17: 27−42

6. Barnes JP, Chung KF, Page CP (1998) Inflammatory mediators of asthma: an update. Pharmacological reviews 50: 515−596

7. Bell RL, Harris RR, Malo PE, Bouska JB, Shaughnessy TK, Hulkower KI, Brooks CD, Carter GW (1997) ABT-761 attenuates bronchoconstriction and pulmonary inflammation in rodents. J Pharmacol Exp Ther 280: 1366−1373

8. Bochenek G, Bánska K, Szabó Z, Nizankovska E, Szczeklik A (2002) Diagnosis, prevention and treatment of aspirin-induced asthma and rhinitis. Current Drug Targets-Inflammation & Allergy 1: 1−11

9. Bogatcheva N, Sergeeva M, Dudek S, Verin A (2005) Arachidonic acid cascade in endothelial pathobiology. Microvasc Res 69: 107−127

10. Boršoš I, Jenko K, Iglič Č (2006) Priprava bolnikov s sindromom aspirinske intolerance na kirurški poseg. V: Košnik M (urednik), Zbornik sestanka: Aspirinska intoleranca in rinitis. Bolnišnica Golnik – Klinični oddelek za pljučne bolezni in alergijo, Medicinska fakulteta Univerze v Ljubljani

11. Bousquet J, Ansotegui IJ, van Ree R, Burney PG, Zuberbier T, van Cauwenberge P (2004) European union meets the challenge of the growing importance of allergy and asthma in Europe. Allergy 59: 1−4

12. Brumen M, Fajmut A, Dobovišek A, Roux E (2005) Mathematical modelling of Ca^{2+} oscillations in airway smooth muscle cells. J Biol Phys 31: 515−524

13. Callan OH, So O-Y, Swinney DC (1996) The kinetic factors that determine the affinity and selectivity for slow binding inhibition of human prostaglandin H synthase 1 and 2 by indomethacin and flurbiprofen. J Biol Chem 271: 3548−3554

14. Chen H, Zhang J, Liu J (2007) Structural-diversity-enhanced cellular ability to detect subthreshold extracellular signals. Phys. Rev. E 75: 041910

15. Cowburn AS, Sladek K, Soja A, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose J, Austen KF, Holgate ST, Sampson AP (1998) Over-expression of leukotriene C_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest 101: 834−846

16. Dahlén B, Nizankowska E, Szczeklik A, Zetterström O, Bochenek G, Kumlin M, Mastalerz L, Pinis G, Swanson LJ, Boodhoo TI, Wright S, Dubé LM, Dahlén SE (1998) Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics. Am J Respir Crit Care Med 157: 1187−1194

17. Dobovišek A (2008) Matematično modeliranje vpliva fosforilacije regulatornih verig lahkega miozina na mehansko napetost v gladkih mišicah dihalnih poti: magistrsko delo. Fakulteta za naravoslovje in matematiko, Maribor.

18. Dobovišek A, Fajmut A, Brumen M (2011) Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study. J Pharmacokinet Pharmacodyn 38: 261−278

19. Dobovišek A, Fajmut A, Brumen M (2012) Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE_2 analogue: a theoretical approach.
Med Biol Eng Comput 50: 33−42

20. Fajmut A, Brumen M, Schuster S (2005) Mathematical modelling of the interactions between Ca^{2+}, calmodulin and myosin light chain kinase. FEBS lett 579: 4361−4366

21. Fajmut A, Jagodič M, Brumen M (2005) Mathematical modeling of the myosin light chain kinase activation. J Chem Inf Mod 45: 1605−1609

22. Fajmut A, Dobovišek A, Brumen M (2005) Mathematical modeling of the relation between myosin phosphorylation and stress development in smooth muscles. J Chem Inf Mod 45: 1610−1615

23. Fajmut A (2006) Modeliranje biokemijskih procesov kot sestavnih elementov kalcijeve signalizacije v procesu skrčitve gladkih mišičnih celic dihalnih poti: doktorska disertacija. Pedagoška fakulteta, Maribor

24. Fajmut A, Brumen M (2008) MLC-kinase/phosphatase control of Ca^{2+} signal transduction in airway smooth muscles. J Theor Biol 3: 474−481

25. Fajmut A, Dobovišek A, Brumen M (2012) Mathematical modeling in aspirin-induced asthma: theory and clinical applications, Nova Publisher, New York (v tisku)

26. Gauvreau GM, Watson RM, O'Byrne PM (1999) Protective effects of inhaled PGE_2 on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med 159: 31–36

27. Goltsov A, Lebedeva G, Humphery-Smith I, Goltsov G, Demin O, Goryanin I (2010)
In silico screening of nonsteroidal anti-inflammatory drugs and their combined action
on prostaglandin H synthase-1. Pharmaceuticals 3: 2059−2081

28. Gonchar M, Sergeeva M, Mevkh A, Varfolomeyev S (1999) Kinetics of prostanoid synthesis by macrophages is regulated by arachidonic acid sources. Eur J Biochem 265: 779–787

29. Gupta N, Gresser M, Ford-Hutchinson A (1998) Kinetic mechanism of glutathione conjugation to leukotriene A_4 by leukotriene C_4 synthase. Biochim Biophys Acta 1391: 157−168

30. Gyllfors P, Bochenek G, Overholt J, Drupka D, Kumlin M, Sheller J, Nizankowska E, Isakson PC, Mejza F, Lefkowith JB, Dahlén SE, Szczeklik A, Murray JJ, Dahlén B (2003) Biochemical and clinical evidence that aspirin-intolerant asthmatic subjects tolerate the cyclooxygenase 2-selective analgetic drug celecoxib. J Allergy Clin Immunol 111: 1116−1121

31. Haeggström J, Bergman T, Jörnvall H, Rådmark O (1988) Guinea-pig liver leukotriene A_4 hydrolase. Purification, characterization and structural properties. Eur J Biochem 174: 717−724

32. Harizi H, Juzan M, Moreau J-F, Gualde N (2003) Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B_4 production from dendritic cells via an IL-10-dependent mechanism. J Immunol 170: 139−146

33. Hartert TV, Dworski RT, Mellen BG, Oates JA, Murray JJ, Sheller JR (2000) Prostaglandin E_2 decreases allergen-stimulated release of prostaglandin D_2 in airways of subjects with asthma. Am J Respir Crit Care Med 162: 637−640

34. Häusser M, Spruston N, Stuart GJ (2000) Diversity and dynamics of dendritic signaling. Science 290: 739–744

35. Heinrich R, Schuster S (1996) The regulation of cellular systems. Chapman & Hall, New York

36. Hull MA, Stanley CW Ko, Hawcroft G (2004) Prostaglandin EP receptors: Targets for treatment and prevention of colorectal cancer. Mol Cancer Ter 3: 1031−1039

37. Jambekhar SS, Breen PJ (2009) Basic pharmacokinetics. Pharmaceutical Press, London

38. Kanamoto H, Takemura M, Ohyama K (2009) Detection of 5-lipoxygenase activity in the Liwerwort Marchantia polymorpha L. Biosci Biotechnol Biochem 73: 2549−2551

39. Klipp E, Herwig R, Kowald A, Wierling C, Lehrach H (2005) Systems biology in practice. Concepts, implementation and application. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

40. Klueglich M, Ring A, Scheuerer S, Trommeshauser D, Schuijt C, Liepold B, Berndl G (2005) Ibuprofen extrudate, a novel, rapidly dissolving ibuprofen formulation: relative bioavailability compared to ibuprofen lysinate and regular ibuprofen, and food effect on all formulations. J Clin Pharmacol 45: 1055−1061

41. Košnik M, Mušič E, Matjaž F, Šuškovič S (1998) Relative safety of meloxicam in NSAID-intolerant patients. Allergy 53: 1231−1233

42. Lam BK, Gagnon L, Austen KF, Soberman RJ (1990) The mechanism of leukotriene B_4 export from human polymorphonuclear leukocytes. J Biol Chem 265: 13438−13441

43. Macey R, Oster G (2000) Berkeley madonna 8.0.1. University of California at Berkeley, Berkeley

44. Marhl M, Gosak M, Perc M, Roux E (2010) Importance of cell variability for calcium signaling in rat airway myocytes. Biophys Chem 148: 42–50

45. Mastalerz L, Nizankowska E, Sladek K, Szczeklik A (1994) Protective effects of prostaglandin E_2 on airway obstruction induced by aspirin in aspirin-intolerant asthmatics. Eur Respir J 7: 434S

46. Maurya MR, Subramaniam S (2007) A kinetic model for calcium dynamics in RAW 264.7 cells: 1. Mechanisms, parameters, and subpopulational variability. Biophys J 93: 709–728

47. Mazzetti L, Franchi-Micheli S, Nistri S, Quattrone S, Simone R, Ciu M, Zilletti L, Failli P (2003) The ACh-induced contraction in rat aortas is mediated by the Cys Lt1 receptor via intracellular calcium mobilization in smooth muscle cells. BJP: 707−715

48. McKay RD (1998) The origins of cellular diversity in the mammalian central nervous system. Cell 58: 815–821

49. Meade E, Smith W, DeWitt D (1993) Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268: 6610−6614

50. Melillo E, Woolley KL, Manning PJ, Watson RM, O'Byrne PM (1994) Effect of inhaled PGE_2 on exercise-induced bronchoconstriction in asthmatic subjects. Am J Respir Crit Care Med 149: 1138–1141

51. Microcal Software Inc. (1997) Microcal origin 5.0, Northampton

52. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1994) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 90: 11693−11697

53. Noguchi M, Miyano M, Kuhara S, Matsumoto T, Noma M (1994) Interfacial kinetic reaction of human 5-lipoxygenase. Eur J Biochem 222: 285−292

54. Obase Y, Shimoda T, Tomari S, Mitsuta K, Kawano T, Matsuse H, Kohno S (2002) Effects of Pranlukast on chemical mediators in induced sputum on provocation tests in atopic and aspirin-intolerant asthmatic patients. Chest 121: 143−150

55. Owen Jr WF, Soberman RJ, Yoshimoto T, Sheffer AL, Lewis RA, Austen KF (1987) Synthesis and release of leukotriene C_4 by human eosinophils. J Immunol 138: 532−538

56. Paulson SK, Vaughn MB, Jessen SM, Lawal Y, Gresk CJ, Yan B, Maziasz TJ, Cook CS, Karim A (2001) Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption. J Pharmacol Exp Ther 297: 638−645

57. Picado C, Fernandez-Morata JC, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, Mullol J (1999) Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. Am J Respir Crit Care Med 160: 291−296

58. Pierzchalska M, Szabo Z, Sanak M, Soja J, Szczeklik A (2003) Deficient prostaglandin E_2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin induced asthma. J Allergy Clin Immunol 111: 1041−1048

59. Reid JJ (2001) ABT-761 (Abbott). Curr Opin Investig Drugs 2: 68−71

60. Rolin S, Masereel B, Dogné JM (2005) Prostanoids as pharmacological targets in COPD and asthma. Eur J Pharmacol 533: 89−100

61. Rosado A, Vives R, González R, Poza P, Rodríguez J (2000) Intolerance to non-steroidal antiinflammatory drugs with respiratory reaction: clinical and diagnostic features. Alergol Inmunol Clin 15: 153−159

62. Rowland M, Riegelman S, Harris PA, Sholkof SD (1972) Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J Pharm Sci 61: 379−385

63. Sánchez-Borges M (2010) NSAID hypersensitivity (Respiratory, cutaneous, and generalized anaphylactic symptoms). Med Clin N Am 94: 853−864

64. Sánchez-Borges M, Caballero-Fonseca F, Capriles-Hullet A, González-Aveledo L (2010) Hypersensitivity reactions to nonsteroidal anti-inflammatory drugs: an update. Pharmaceuticals 3: 10−18

65. Schäfer D, Schmid M, Göde UC, Baenkler HW (1999) Dynamics of eicosanoids in peripheral blood cells during provocation in aspirin-intolerant asthmatics. Eur Respir J 13: 638−646

66. Schäfer D, Baenkler HW (2005) Functional eicosanoid test and typing (FET) of peripheral blood cells in eicosanoid related diseases. J Physiol Pharmacol 56: 103−118

67. Schäfer D (2006) Testing and typing of eicosanoid-patterns. J Physiol Pharmacol 57: 47−64

68. Sestini P, Armetti L, Gambaro G, Pieroni M, Refini RM, Sala A, Vaghi A, Folco GC, Bianco S, Robuschi M (1996) Inhaled PGE_2 prevents aspirin-induced bronchoconstriction and urinary LTE_4 excretion in aspirin-sensitive asthma. Am J Respir Crit Care Med 153: 572–575

69. Setipane RA, Schrank PJ, Simon RA, Mathison DA, Christiansen SC, Stevenson DD (1995) Prevalence of cross-sensitivity with acetaminophen in aspirin-sensitive asthmatic subjects. J Allergy Clin Immunol 96: 480−485

70. Setoguchi H, Nishimura J, Hirano K, Takahashi S, Kanaide H (2001) Leukotriene C_4 enhances the contraction of porcine tracheal smooth muscle trough the activation of Y-27632, a rho kinase inhibitor, sensitive pathway. Br J Pharmacol 132: 111−118

71. So O-Y, Scarafia LE, Mak AY, Callan OH, Swinney DC (1998) The dynamics of prostaglandin H synthases. Study with prostaglandin H-synthase 2 Y355F unmask mechanisms of time-dependent inhibition and allosteric activation. J Biol Chem 273: 5801−5807

72. Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH (2002) Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. N Engl J Med 347:1493−1499

73. Stevenson D, Szczeklik A (2006) Clinical and pathologic perspectives on aspirin sensitivity and asthma. J Allergy Clin Immunol 118: 773−786

74. Szczeklik A, Stevenson D (1999) Aspirin-induced asthma: advances in pathogenesis and management. J Allergy Clin Immunol 104: 5−13

75. Szczeklik A, Nizankowska E (2000) Clinical features and diagnosis of aspirin-induced asthma. Thorax 55: S42−S44

76. Täuber U, Brudny–Klöppel M, Jakobs U, Madetzki C, Mahler M (1993) Pharmacokinetics of nocloprost in human volunteers and its relation to dose. Eur J Clin Pharmacol 44: 497−500

77. Vane J, Botting R (1987) Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J 1: 89−96

78. Varghese M, Lockey RF (2008) Aspirin-exacerbated asthma. Allergy, Asthma and clinical immunology 4: 75−83

79. Voet D, Voet JG (1995) Biochemistry. John Willey & Sons. Inc, New York

80. Widal MF, Abrami P, Lenmoyez J (1922) Anaphylaxie et idiosyncrasie. Presse Med 30:189−192

81. Woessner KM, Simon RA, Stevenson DD (2002) The safety of celecoxib in patients with aspirin-sensitive asthma. Arthritis Rheum 46: 2201−2206

82. Wolfram Research, inc. (2003) Mathematica 5.0.0.0, Oxofordshire

83. Wong SL, Drajesk J, Chang MS, Witt G, Awni WM (1998) Dose-proportional pharmacokinetics of a new 5-lipoxygenase inhibitor, ABT-761, in healthy volunteers. Biopharm Drug Dispos 19: 159−162

84. Wong SL, Drajesk J, Chang M, Lanni C, Witt G, Hansen R, Awni WM (1998) Pharmacokinetics and pharmacodynamics of single and multiple oral doses of a novel 5-lipoxygenase inhibitor (ABT-761) in healthy volunteers. Clin Pharmacol Ther 63: 324−331

85. Wong SL, O'Dea RF, Dube LM, Awni WM (1998) Effects of ABT-761, a novel 5-lipoxygenase inhibitor, on the pharmacokinetics of a single dose of ethinyl estradiol and levonorgestrel in healthy female volunteers. J Clin Pharmacol 38: 642−648

86. Ying S, Meng Q, Scadding G, Parikh A, Corrigan CJ, Lee TH (2006) Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. J Allergy Clin Immunol 117: 312−318

87. Yoshida S, Ishizaki Y, Onuma K, Shoji T, Nakagawa H, Amayasu H (2000) Selective cyclooxygenase 2 inhibitor in patients with aspirin induced asthma. J Allergy Clin Imunol 106: 1201−1202

Dodatek A

V tem dodatku bomo opisali osnovne principe modeliranja encimskih reakcij. Dodatek je razdeljen na tri dele. V prvem delu obravnavamo ireverzibilno dvostanjsko kinetično shemo encimske reakcije in izpeljemo izraz za hitrost reakcije v odvisnosti od koncentracije substrata, ki jo opišemo s t. i. Michaelis-Mentenino kinetiko. V drugem delu nato obravnavamo enako encimsko reakcijo ob prisotnosti inhibitorja in izpeljemo hitrost reakcije v odvisnosti od koncentracije substrata in inhibitorja. V tretjem delu dodatka izpeljemo Cheng-Prussoevo enačbo, ki omogoča izračun inhibitornih ravnotežnih konstant K_I iz eksperimentalno določenih koncentracij IC_{50}. Obsežnejšo obravnavo encimskih reakcij najde bralec v literaturi [79].

A1 Michaelis-Mentenina kinetika

Obravnavajmo ireverzibilno dvostanjsko encimsko reakcijo. Ustrezna kinetična shema za reakcijo je prikazana na sliki D1. Na kinetični shemi je s simbolom E označen prost encim, ES je kompleks encima in substrata, S je substrat, P je produkt, k_1^+, k_1^- in k_2^+ pa so hitrostne konstante.

$$\mathrm{E+S} \underset{k_1^-}{\overset{k_1^+}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_2^+} \mathrm{E+P}$$

Slika D1. Ireverzibilna, dvostanjska kinetična shema encimske reakcije.

Hitrost reakcije je definirana kot odvod koncentracije produkta [*P*] po času *t* in je za kinetično shemo, prikazano na sliki D1, podana kot:

$$v \equiv \frac{d[P]}{dt} = k_2^+ [ES]. \qquad \text{(D1)}$$

Zanima nas, kako se hitrost reakcije spreminja v odvisnosti od koncentracije substrata [*S*]. Koncentraciji [*E*] in [*ES*] sta spremenljivki, katerih časovno spreminjanje v splošnem opišemo z dvema diferencialnima enačbama prvega reda. Zadostuje, če zapišemo samo diferencialno enačbo za [*ES*]:

$$\frac{d[ES]}{dt} = k_1^+ [E][S] - k_1^- [ES] - k_2^+ [ES], \tag{D2}$$

saj predpostavljamo, da se količina encima v celici ohranja in lahko [*E*] določimo iz enačbe za totalno koncentracijo encima $[E]_{\mathrm{TOT}}$, ki je konstantna:

$$[E]_{\mathrm{TOT}} = [E] + [ES]. \tag{D3}$$

Dodatno predpostavljamo, da je totalna koncentracija encima v celici $[E]_{\mathrm{TOT}}$ mnogo manjša kot koncentracija substrata [*S*]. Takšni pogoji vodijo do t. i. kvazi stacionarnega stanja, v katerem velja $\frac{d[ES]}{dt} \approx 0$. Iz tega sledi, da je časovni odvod na desni strani enačbe (D2) enak nič:

$$0 = k_1^+ [E][S] - k_1^- [ES] - k_2^+ [ES]. \tag{D4}$$

Iz enačbe (D3) izrazimo [*E*], dobljeno enačbo vstavimo v (D4) in izrazimo [*ES*]:

$$[ES] = \frac{[E]_{\mathrm{TOT}} [S] k_1^+}{k_1^- + k_2^+ + k_1^+ [S]}. \tag{D5}$$

Sedaj enačbo (D5) vstavimo v (D1) in jo preuredimo v naslednjo obliko:

$$v = \frac{k_2^+ [E]_{\mathrm{TOT}} [S]}{\frac{k_1^- + k_2^+}{k_1^+} + [S]}. \tag{D6}$$

Vpeljemo maksimalno hitrost reakcije v_{maks} in Michaelis-Mentenino konstanto *K*:

$$v_{\mathrm{maks}} = k_2^+ [E]_{\mathrm{TOT}}, \tag{D7}$$

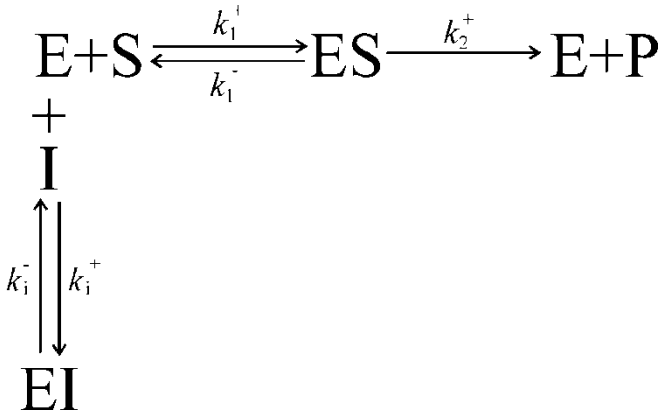
$$K = \frac{k_1^- + k_2^+}{k_1^+}. \qquad \text{(D8)}$$

Upoštevajoč enačbi (D7) in (D8) sledi iz (D6) splošno znan izraz za hitrost encimske reakcije, ki ga v literaturi pogosto imenujejo kar Michaelis-Mentenina kinetika:

$$v = \frac{v_{\text{maks}}\,[S]}{K + [S]}. \qquad \text{(D10)}$$

A2 Michaelis-Mentenina kinetika ob prisotnosti inhibitorja

Sedaj obravnavajmo ireverzibilno dvostanjsko encimsko reakcijo ob prisotnosti inhibitorja, kot je prikazano na sliki D2. Na shemi smo s simbolom I označili inhibitor, z EI kompleks encima in inhibitorja, s k_i^+ in k_i^- pa hitrostni konstanti vezave inhibitorja na encim in disociacije inhibitorja z encima. Pomen ostalih simbolov je enak kot na sliki D1. Obravnavamo reverzibilno kompetitivno inhibicijo encima. Inhibitor s substratom "tekmuje" za vezavno mesto na encimu. Inhibicija je reverzibilna, kar pomeni, da inhibitor lahko disociira iz encima, encim pa je neaktiven le takrat, ko je nanj vezan inhibitor.



Slika D2. Kinetična shema reverzibilne kompetitivne inhibicije encimske reakcije.

Hitrost reakcije je določena z enačbo (D1), časovno spreminjanje spremenljivk [*ES*] in [*EI*] pa v splošnem opišeta diferencialni enačbi:

$$\frac{d[ES]}{dt} = k_1^+ [E][S] - k_1^- [ES] - k_2^+ [ES], \qquad \text{(D11)}$$

$$\frac{d[EI]}{dt} = k_{\mathrm{i}}^{+}[E][I] - k_{\mathrm{i}}^{-}[EI]. \tag{D12}$$

Koncentracijo [*E*] ponovno določimo iz enačbe za totalno koncentracijo encima v celici, ki je za ta primer podana kot:

$$[E]_{\mathrm{TOT}} = [E] + [ES] + [EI]. \tag{D13}$$

Podobno kot v prejšnjem primeru predpostavljamo, da je totalna koncentracija encima $[E]_{\mathrm{TOT}}$ mnogo manjša, kot sta koncentraciji substrata [*S*] in inhibitorja [*I*]. To vodi do kvazi stacionarnega stanja, v katerem velja $\frac{d[ES]}{dt} \approx 0$ in $\frac{d[EI]}{dt} \approx 0$. Tako sta odvoda na levi strani enačb (D.11) in (D.12) enaka nič:

$$0 = k_1^{+}[E][S] - k_1^{-}[ES] - k_2^{+}[ES], \tag{D14}$$

$$0 = k_{\mathrm{i}}^{+}[E][I] - k_{\mathrm{i}}^{-}[EI]. \tag{D15}$$

Iz enačbe (D15) izrazimo [*EI*], rezultat vstavimo v enačbo (D13) in dobimo enačbo:

$$[E]_{\mathrm{TOT}} = [E] + [ES] + \frac{k_{\mathrm{i}}^{+}}{k_{\mathrm{i}}^{-}}[E][I]. \tag{D16}$$

Iz enačbe (D16) izrazimo [*E*], rezultat vstavimo v enačbo (D14) in izrazimo [*ES*]. Sledi enačba:

$$[ES] = \frac{[E]_{\mathrm{TOT}}[S]}{\frac{k_1^{-} + k_2^{+}}{k_1^{+}}\left(1 + \frac{[I]}{K_{\mathrm{I}}}\right) + [S]}, \tag{D17}$$

kjer je K_I disociacijska ravnotežna konstanta za kompleks encima in inhibitorja, podana kot količnik hitrostnih konstant k_i^-/k_i^+. Vpeljemo še brezdimenzijsko količino α, ki je določena z enačbo:

$$\alpha = (1 + \frac{[I]}{K_I}) \ . \tag{D19}$$

Enačbo (D19) upoštevamo v (D17), upoštevamo pa tudi enačbi za maksimalno hitrost reakcije (D7) in za Michaelis-Mentenino konstanto (D8). Upoštevajoč definicijo za hitrost encimske reackcije (D1) dobimo:

$$v = \frac{v_{maks}[S]}{K\alpha + [S]} . \tag{D.20}$$

A3 Cheng-Prussoeva enačba

Pri eksperimentu sta vrednosti [*S*] in [*I*] konstantni vrednosti oz. parametra, ki ju lahko kontrolirajo eksperimentatorji. Reakcijo sprožijo ali na celični kulturi ali v pripravljeni raztopini. Denimo, da opazujemo tak eksperiment. Reakcija naj na začetku poteka brez inhibitorja. Naj bo poznana koncentracija substrata $[S]_0$. Pri vrednosti $[S]_0$ je − ob odsotnosti inhibitorja − hitrost reakcije določena z enačbo (D10). Enak rezultat dobimo tudi po enačbi (D20), pri čemer vstavimo [*I*] = 0. Ko eksperimentalec doda inhibitor, se bo hitrost reakcije po enačbi (D20) zmanjšala. Pri eksperimentu običajno določijo koncentracijo inhibitorja, ki hitrost reakcije zavre za 50 %, t. j. IC_{50}.

S podatkom za IC_{50} lahko določimo disociacijsko ravnotežno konstanto K_I za kompleks encima in inhibitorja. Izračun K_I iz IC_{50} omogoča Cheng-Prussoeva enačba. Po definiciji IC_{50} lahko zapišemo:

$$v_I([S]_0, IC_{50}) = \frac{1}{2} v([S]_0) \ . \tag{D21}$$

V enačbi (D21) smo z v_I označili hitrost reakcije, ki poteka ob prisotnosti inhibitorja, z v pa hitrost reakcije, ki poteka brez prisotnosti inhibitorja. Prva je podana z enačbo (D20), druga pa z enačbo (D10). Ti dve enačbi vstavimo v pogoj (D21) in dobimo:

$$\frac{v_{\text{maks}}[S]_0}{K(1+\frac{IC_{50}}{K_i})+[S]_0} = \frac{v_{\text{maks}}[S]_0}{2K+2[S]_0} \ . \qquad \text{(D22)}$$

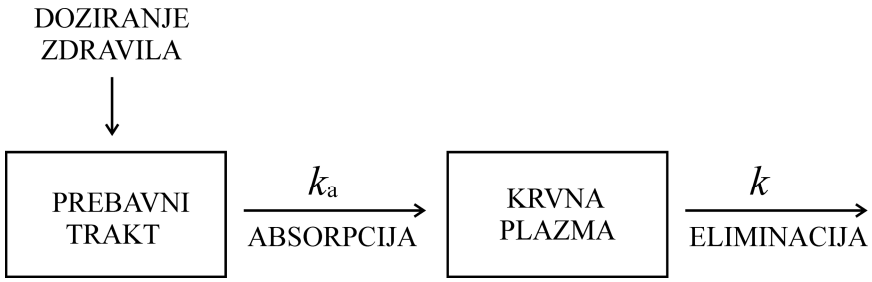
V enačbi (D22) krajšamo produkt $v_{\text{maks}}[S]_0$ in izrazimo K_I. Sledi Cheng-Prussoeva enačba:

$$K_I = \frac{IC_{50}}{1+\frac{[S]_0}{K}} \ . \qquad \text{(D23)}$$

Dodatek B

Farmakokinetični model

V tem dodatku bomo opisali farmakokinetični model, ki ga v naših študijah uporabimo za opis časovnih potekov različnih zdravil [37]. Zdravilo se dozira v prebavni trakt, nato se absorbira v krvno plazmo, nato pa iz nje izloči. S simboloma k_a in k smo označili hitrostni konstanti absorpcije in eliminacije zdravila. Shema modela je prikazana na sliki D3.



Slika D3. Shema farmakokinetičnega modela. Zdravilo se dozira v prebavni trakt, nato se absorbira v krvno plazmo in nato iz nje izloči. S simboloma k_a in k smo označili hitrostni konstanti absorpcije in eliminacije zdravila.

Naj bo $[C]_1$ koncentracija zdravila v prebavnem traktu in $[C]_2$ koncentracija zdravila v krvni plazmi. Časovno spreminjanje koncentracije zdravila v prebavnem traktu in krvni plazmi opiše sistem dveh diferencialnih enačb:

$$\frac{d[C]_1}{dt} = -k_a [C]_1, \tag{D24}$$

$$\frac{d[C]_2}{dt} = k_a [C]_1 - k[C]_2. \tag{D25}$$

Sistem enačb (D24) in (D25) rešimo analitično v programu Mathematica 5 [82]. Rešitvi sta podani s funkcijama:

$$[C]_1(t) = Ae^{-k_a t}, \tag{D26}$$

$$[C]_2(t) = A\frac{k_a}{k_a - k}(e^{-kt} - e^{-k_a t}) + Be^{-kt}, \tag{D27}$$

kjer sta A in B konstanti, ki ju določimo iz začetnih pogojev. Ta se glasita:

$$[C]_1(0) = [C]_0, \tag{D28}$$

$$[C]_2(0) = 0. \tag{D29}$$

Upoštevajoč začetna pogoja (D28) in (D29) v enačbah (D26) ter (D27) ugotovimo, da je $A = [C]_0$ in $B = 0$. Koncentracijo $[C]_0$ lahko povežemo z dozo zdravila po enačbi:

$$[C]_0 = \frac{DF}{V}, \tag{D30}$$

kjer je D doza, F je delež v kri absorbiranega zdravila in V je navidezni volumen porazdelitve. Če v enačbi (D27) upoštevamo enačbo (D30) in $B = 0$, sledi:

$$[C]_2(t) = \frac{DFk_a}{V(k_a - k)}(e^{-kt} - e^{-k_a t}). \tag{D31}$$

Priloge

Priloga 1

Objavljeni izvirni znanstveni članek

Dobovišek A, Fajmut A, Brumen M (2011) Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study. J Pharmacokinet Pharmacodyn 38: 261−278

Priloga 2

Objavljeni izvirni znanstveni članek

Dobovišek A, Fajmut A, Brumen M (2012) Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE_2 analogue: a theoretical approach.
Med Biol Eng Comput 50: 33−42

Priloga 3

Samostojni prispevek v znanstveni monografiji (v tisku)

Fajmut A, Dobovišek A, Brumen M (2012) Mathematical modeling in aspirin-induced asthma: theory and clinical applications, Nova Publisher, New York

Priloga 1

Objavljeni izvirni znanstveni članek

Dobovišek A, Fajmut A, Brumen M (2011) Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study. J Pharmacokinet Pharmacodyn 38: 261−278

J Pharmacokinet Pharmacodyn (2011) 38:261–278
DOI 10.1007/s10928-011-9192-6

Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study

A. Dobovišek · A. Fajmut · M. Brumen

Received: 18 July 2010 / Accepted: 7 February 2011 / Published online: 18 February 2011

Abstract Altered expressions of the key enzymes in arachidonic acid (AA) metabolism, prostaglandin synthase 1 and 2 and cysteinyl leukotriene C_4 synthase, are of importance in understanding aspirin-induced asthma. We propose a mathematical model of AA metabolism and its interaction with non-steroidal anti-inflammatory drugs (NSAIDs). Model simulations depict the impact of modified expressions of the above enzymes on the time dependent synthesis of cysteinyl leukotrienes and anti-inflammatory prostaglandins before and during NSAID exposure in different model states describing healthy humans as well as aspirin-tolerant and -intolerant asthmatics. The results are compared and evaluated with experimental data taken from the literature. Our results identify the decreased expression of prostaglandin H synthase 1 and increased expression of leukotriene C_4 synthase as the key elements in AA metabolism that contribute to increased leukotriene C_4 and decreased anti-inflammatory prostaglandins after NSAID dosing in aspirin-intolerant patients. On the other hand, the decreased expression of prostaglandin H synthase 2 implies permanently increased leukotriene C_4 and lowers the sensitivity to increased drug doses. The model is used for identification of susceptible patient populations for aspirin and ibuprofen, and for identification of critical aspirin doses that might induce bronchoconstriction.

Electronic supplementary material The online version of this article (doi:10.1007/s10928-011-9192-6) contains supplementary material, which is available to authorized users.

A. Dobovišek (✉) · A. Fajmut · M. Brumen
Faculty of Natural Sciences and Mathematics, Faculty of Medicine, Faculty of Health Sciences, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia
e-mail: andrej.dobovisek@uni-mb.si

M. Brumen
Institute Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia

Keywords Mathematical model · Arachidonic acid metabolism · Leukotrienes · Prostaglandins · Non-steroidal anti-inflammatory drug

Introduction

In some asthmatic patients, attacks of asthma can be induced by ingestion of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs). This clinical syndrome is known as aspirin-induced asthma or aspirin-intolerant asthma (AIA) and is relevant to asthmatic patients who are affected by the aggressive inflammation of upper and lower respiratory tract after ingestion of NSAIDs [1, 2]. Typical symptoms associated with AIA are nasal polyps, rhino-conjunctivitis and asthma attacks [1–3]. The latter usually occur within 30–60 min after ingestion of full therapeutic dose of NSAID [3]. Statistical data show that AIA affects about 10–20% of adult asthmatic patients [1, 2]. Several Refs. [1–6] report that inflammatory reactions of AIA are induced during the action of NSAID by increased synthesis of cysteinyl leukotrienes, a product of lipoxygenase pathway of arachidonic acid (AA) metabolism. Leukotriene C_4 (LTC_4), a cysteinyl leukotriene, is the most potent pro-inflammatory mediator and causes bronchoconstriction in asthmatic patients as well as in healthy humans [7].

It is generally accepted that the target of NSAID action is metabolism of AA in inflammatory and epithelial cells. Hypersensitivity to NSAIDs is mediated by their action on two AA metabolic pathways, the first towards enhanced production of cysteinyl leukotrienes, and the second towards inhibited production of pro-(piPGs) and anti-inflammatory prostaglandins (aiPGs). These are the lipoxygenase and cyclooxygenase pathway of AA metabolism [1, 2, 8]. Figure 1 schematically represents AA metabolism and its interaction with NSAID. Via the cyclooxygenase pathway, AA is transformed into aiPGs and piPGs by the enzymes prostaglandin H synthase 1 (PGHS1) and prostaglandin H synthase 2 (PGHS2). PGHS1 is constitutively expressed in the cells and mainly produces aiPGs, whereas the enzyme PGHS2 is induced during inflammation and enhances synthesis of piPGs [2, 9–11]. In the first step of the lipoxygenase pathway, AA is converted into leukotriene A_4 (LTA_4) by the enzyme 5-lipoxygenase (5-LOX). A direct conversion of AA into LTA_4 is considered, since the intermediate product of this reaction, 5-hydroperoxyeicosatetraenoic acid, is a very unstable compound [12] and could be considered to be in equilibrium. Then LTA_4 is either converted into leukotriene B_4 (LTB_4) by leukotriene A_4 hydrolase (LTA_4H) or conjugated with glutathione into the cysteinyl leukotriene, LTC_4, by leukotriene C_4 synthase (LTC_4S). The enzyme LTC_4S is an important link in the metabolic chain of the lipoxygenase pathway. In this enzymatic reaction, LTA_4 is involved as a substrate and LTC_4 as a product, whereby LTA_4 alone affects the velocity of its own degradation [13].

An important product of the cyclooxygenase pathway is prostaglandin E_2 (PGE_2). This metabolite is one of the aiPGs. In healthy individuals and in patients with asthma, PGE_2 inhibits the enzyme 5-LOX, and thus limits the production of cysteinyl leukotrienes [1, 2, 4, 14]. On the other hand, it is known that NSAIDs inhibit both enzymes of the cyclooxygenase pathway, PGHS1 and PGHS2, thereby

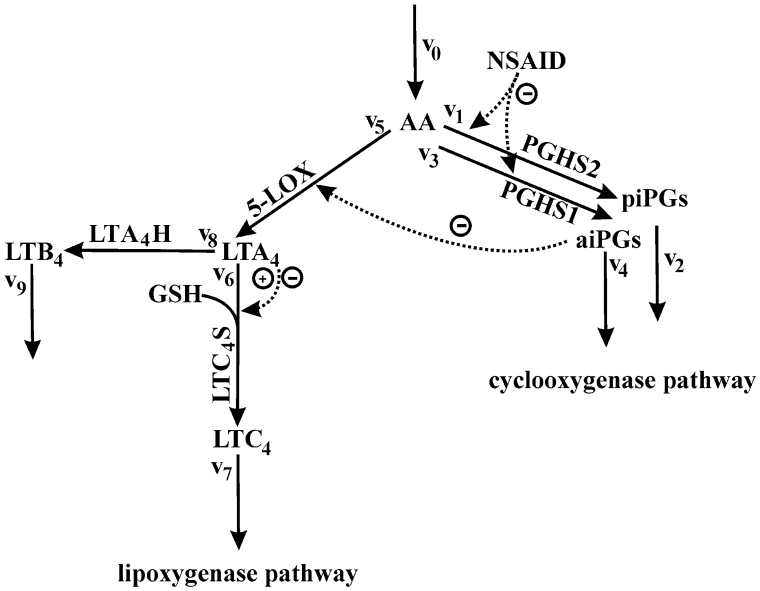


Fig. 1 Kinetic scheme of arachidonic acid metabolism. *Abbreviations* used: arachidonic acid (AA), pro-inflammatory prostaglandins (piPGs), anti-inflammatory prostaglandins (aiPGs), leukotriene A_4 (LTA_4), leukotriene B_4 (LTB_4), leukotriene C_4 (LTC_4), glutathione (GSH), prostaglandin H synthase 1 (PGHS1), prostaglandin H synthase 2 (PGHS2), 5-lipoxygenase (5-LOX), leukotriene A_4 hydrolase (LTA_4H), leukotriene C_4 synthase (LTC_4S), non-steroidal anti-inflammatory drug (NSAID)

inhibiting total prostaglandin formation [4, 9, 15]. The inhibition of PGHS1 by NSAID results in a rapid decrease in the aiPGs synthesis, which consequently also involves PGE_2. This reduces the inhibitory effect of PGE_2 on 5-LOX and tilts degradation of AA towards cysteinyl leukotriene production [1–3]. Thus, the action of NSAID increases the synthesis of critical metabolites cysteinyl leukotrienes stemming from the inhibitory effect of NSAID on the enzyme PGHS1, since it reduces synthesis of aiPGs and accelerates synthesis of cysteinyl leukotrienes. However, why the interruption of aiPGs synthesis by NSAIDs does not induce inflammatory reactions in all humans, or at least in all asthmatic patients, still remains an unsolved question in the research of aspirin-induced asthma [1]. Several authors [1–3, 16–19] try to resolve this question by: (1) analyzing expressions and activities of the key enzymes of AA metabolism PGHS1, PGHS2 and LTC_4S and (2) by comparing the data of prostaglandin and leukotriene production for aspirin-intolerant (AIA) and aspirin-tolerant (ATA) asthmatic patients and normal subjects (NS) [18].

Our approach to this problem is theoretical: we propose a mathematical model of interaction between AA metabolism and NSAID. The model incorporates all the main pathways in AA metabolism and relies on theoretical and experimental biochemical, as well as clinical, data in terms of model parameters and variables. The model predictions of aspirin-tolerant and -intolerant asthmatic cases are compared with biochemical [18] and clinical [3] experimental data.

Materials and methods

Mathematical model

The kinetic scheme of AA metabolism and its interaction with NSAID is given in Fig. 1 as a set of six first-order ordinary differential equations, describing the time evolution of system variables, which are concentrations of metabolites $[AA]$, $[piPGs]$, $[aiPGs]$, $[LTA_4]$, $[LTB_4]$ and $[LTC_4]$,

$$\frac{d[AA]}{dt} = v_0 - v_1 - v_3 - v_5, \tag{1}$$

$$\frac{d[piPGs]}{dt} = v_1 - v_2, \tag{2}$$

$$\frac{d[aiPGs]}{dt} = v_3 - v_4, \tag{3}$$

$$\frac{d[LTA_4]}{dt} = v_5 - v_6 - v_8, \tag{4}$$

$$\frac{d[LTB_4]}{dt} = v_8 - v_9, \tag{5}$$

$$\frac{d[LTC_4]}{dt} = v_6 - v_7, \tag{6}$$

where v_j, $j = 1\ldots9$ are the reaction velocities.

For the model simulations we use two NSAIDs, aspirin and ibuprofen, as well as a combination of aspirin and PGE_2-analogue, nocloprost. Different parameter values are considered. The complete set of model parameters, their values used in model simulations, and references consulted, are given in Table 1.

Reaction velocity v_0

The influx v_0 of AA is taken to be constant in all simulations, $v_0 = 0.7$ μM s^{-1}. The model variables and fluxes achieve stationary states for physiologically relevant values of free model parameters, and, furthermore, the level of $[AA]$ is about 10 μM, which is a typical value [9, 10, 14].

Reaction velocity v_1

Reaction of the enzyme PGHS2 is described with the steady-state Michaelis–Menten model, describing interactions of the enzyme with substrate AA and inhibitor NSAID [11, 15]:

$$v_1 = \frac{v_{\max\,1}\,[AA]}{K_1\alpha_1 + [AA]} \tag{7}$$

Table 1 Model parameters and their values used for model simulations

Parameter	Parameter value and its units	Description of parameter	References
Reaction 1			
v_{max1}	Free parameter	Maximal velocity of the reaction	[11]
K_1	2.5 μM	Michaelis–Menten constant	[9]
$K_{I1}^{(ASA)}$	85 μM	Equilibrium dissociation constant of inhibitory action of aspirin on PGHS2	[20]
$K_{I1}^{(IBU)}$	60 μM	Equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS2	[20]
Reaction 2			
k_2	0.0028 s^{-1}	Rate constant of piPGs efflux	[10]
Reaction 3			
v_{max3}	Free parameter	Maximal velocity of the reaction	–
K_3	3.0 μM	Michaelis–Menten constant	[9]
$K_{I3}^{(ASA)}$	4.0 μM	Equilibrium dissociation constant of inhibitory action of aspirin on PGHS1	[20]
$K_{I3}^{(IBU)}$	1.3 μM	Equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS1	[20]
Reaction 4			
k_4	0.0028 s^{-1}	Rate constant of aiPGs efflux	[10]
Reaction 5			
v_{max5}	86 μM s^{-1}	Maximal velocity of the reaction	[22]
K_5	25.4 μM	5-LOX-AA equilibrium dissociation constant	[21]
K_{I5}	0.03 μM	Equilibrium dissociation constant of inhibitory action of aiPGs on 5-LOX	[14]
Reaction 6			
v_{max6}	Free parameter	Maximal velocity of the reaction	[13]
A	56 μM	Constant	[13]
B	1.4	Constant	[13]
C	0.17 μM^{-1}	Constant	[13]
Reaction 7			
k_7	0.0015 s^{-1}	Rate constant of LTC_4 efflux	[23]
Reaction 8			
v_{max8}	3.7 μM s^{-1}	Maximal velocity of the reaction	[24]
K_8	27 μM	Michaelis–Menten constant	[24]
Reaction 9			
v_{max9}	5.74 μM s^{-1}	Maximal velocity of the reaction	[25]
K_9	239 μM	Half-saturation constant	[25]
Other parameters			
v_0	0.7 μM s^{-1}	AA influx	–

where v_{max1} is the maximal velocity of the reaction. It is considered to be a free model parameter; K_1 is the Michaelis–Menten constant. Inhibitory action of NSAID on the enzyme PGHS2 is given by α_1 as:

$$\alpha_1 = 1 + \frac{[NSAID]}{K_{I1}} \tag{8}$$

where K_{I1} is the equilibrium dissociation constant of inhibitory action of NSAID on PGHS2. The value of K_1 is 2.5 μM [9]. Parameter K_{I1}, for aspirin denoted as $K_{I1}^{(ASA)}$, is calculated with the Cheng-Prussof equation using $IC_{50} = 1110$ μM for inhibition of PGHS2 by aspirin [20], and by considering substrate concentrations from experimental procedures $[AA] = 30$ μM [20], and the value $K_1 = 2.5$ μM [9]. Parameter $K_{I1}^{(ASA)}$ obtained in this way is 85 μM. The K_{I1} for ibuprofen, denoted as $K_{I1}^{(IBU)}$, is calculated using $IC_{50} = 775$ μM [20] and with $[AA]$ and K_1 as above. Parameter $K_{I1}^{(IBU)}$ is 60 μM.

Reaction velocity v_2

The piPGs efflux is described as [10]:

$$v_2 = k_2[piPGs] \tag{9}$$

where $k_2 = 0.0028$ s^{-1} [10].

Reaction velocity v_3

Reaction of the enzyme PGHS1 is described as [11, 15]:

$$v_3 = \frac{v_{\max\,3}\,[AA]}{K_3\alpha_3 + [AA]} \tag{10}$$

where v_{max3} is the maximal velocity of the reaction and is considered to be a free model parameter; K_3 is the Michaelis–Menten constant. Inhibitory action of NSAID on the enzyme PGHS1 is described by α_3:

$$\alpha_3 = 1 + \frac{[NSAID]}{K_{I3}} \tag{11}$$

where K_{I3} is the equilibrium dissociation constant of inhibitory action of NSAID on PGHS1. The value of parameter K_3 is 3.0 μM [9]. Values of other model parameters are calculated as described for reaction velocity v_1. They are $K_{I3}^{(ASA)} = 4.0$ μM, $K_{I3}^{(IBU)} = 1.3$ μM.

Reaction velocity v_4

Velocity for this reaction is [10]:

$$v_4 = k_4[aiPGs] \tag{12}$$

where the rate constant k_4 for aiPGs efflux is 0.0028 s^{-1} [10].

Reaction velocity v_5

Degradation of AA by the enzyme 5-LOX is described with Michaelis–Menten kinetics [21]:

$$v_5 = \frac{v_{\max 5}[AA]}{K_5\alpha_5 + [AA]} \tag{13}$$

where α_5 describes the inhibitory action of aiPGs on 5-LOX:

$$\alpha_5 = 1 + \frac{[aiPGs]}{K_{I5}} \tag{14}$$

Parameter $K_{I5} = 0.03$ μM is calculated with the Cheng-Prussof equation by using $IC_{50} = 0.04$ μM for inhibition of 5-LOX by PGE_2 [14], and by considering the substrate concentration $[AA] = 10$ μM [14] and $K_5 = 25.4$ μM [21]. Parameter $v_{\max 5}$ = 25.76 μmol min^{-1} mg^{-1} [22] is recalculated into the units used in our model, $v_{\max 5}$ = 86 μM s^{-1}, where the enzyme concentration is 0.2 g L^{-1} [22].

Reaction velocity v_6

The expression of reaction velocity for the mechanism of LTA_4 and glutathione conjugation with LTC_4S results in the production of LTC_4, and corresponding parameters are taken from [13]:

$$v_6 = \frac{v_{\max 6}[LTA_4]}{A + B[LTA_4] + C[LTA_4]^2} \tag{15}$$

Parameters A, B and C are expressed as $A = (K_4K_1/[GSH]) + K_3$, $B = 1 + K_4/[GSH]$, $C = K_4/(K_5[GSH])$, where $K_1 = 40$ μM, $K_3 = 40$ μM, $K_4 = 400$ μM, $K_5 = 2.3$ μM and $[GSH] = 1000$ μM [13]. Model parameter $v_{\max 6}$ is a free model parameter.

Reaction velocity v_7

The efflux of LTC_4 is:

$$v_7 = k_7[LTC_4] \tag{16}$$

This kinetics is in accordance with published data [23], where $k_7 = 0.0015$ s^{-1} is obtained.

Reaction velocity v_8

Conversion of LTA_4 into LTB_4 by the enzyme LTA_4H is [24]:

$$v_8 = \frac{v_{\max 8}[LTA_4]}{K_8 + [LTA_4]} \tag{17}$$

Parameters $K_8 = 27$ μM and $v_{\max 8} = 68$ μmol min^{-1} mg^{-1} are from [24], where the enzyme concentration 0.33×10^{-2} g L^{-1} [24] yields $v_{\max 8} =$ 3.7 μM s^{-1}.

Reaction velocity v_9

Efflux of LTB_4 from cells is described by [25]:

$$v_9 = \frac{v_{\max 9}[LTB_4]}{K_9 + [LTB_4]} \tag{18}$$

where $K_9 = 798$ pmol/10^7cell and v_{max9} = 383 pmol/(10^7cell 20s). Considering an average radius of inflammatory cells of 7 μm, the values $K_9 = 239$ μM and $v_{max9} = 5.7$ μM s^{-1} are obtained.

The system of ordinary differential Eqs. 1–6 is numerically integrated from the initial stationary state that is determined for each model state in the absence of NSAID. Software used in simulations is Berkeley Madonna 8.0.1 (R. Macey and G. Oster, University of California at Berkeley).

Simulation of the time course of NSAID concentration

Time course of [*NSAID*] is described with:

$$[NSAID](t) = \frac{DFk_a}{V(k_a - k)}\left(e^{-kt} - e^{-k_a t}\right), \tag{19}$$

where k and k_a are the first-order elimination and absorption rate constants, D is the drug dose, V is the apparent volume of distribution, and F is the fraction of the drug absorbed. The values of pharmacokinetic (PK) parameters were determined by nonlinear least-squares fitting of the Eq. 19 to the measured time courses of plasma concentration in patients following an oral 650 mg dose of aspirin [26] and 400 mg dose of ibuprofen [27]. The fitting was performed with the software Origin 6.1 (OriginLab Corporation). Digitized experimental data [26, 27] and the corresponding fitted functions are shown in Fig. 2. The PK parameters for nocloprost were taken from the Ref. [28]. All PK parameters are listed in Table 2.

Throughout the paper our results are compared with experimental data [18] of leukotriene and PGE_2 release from peripheral blood cells in normal subjects (NS), aspirin-tolerant asthmatics (ATA) and aspirin-intolerant asthmatics (AIA) before and after aspirin dosing. The authors of this study introduced the relation factor defined as the ratio between concentrations of PGE_2 and leukotrienes [18]. They considered it to be an appropriate quantitative measure for distinguishing between NS, ATA and AIA. Their results show that the relation factor before and after aspirin for AIA is less than one and for NS and ATA it is more than one. In our model the relation factor is defined as the ratio between [*aiPGs*] and [LTC_4]. Results of leukotriene and PGE_2 release measured before and after aspirin [18] are compared with our model predictions of [LTC_4] and [*aiPGs*]. Measured results [18] are recalculated into different ratios and relation factors for NS, ATA and AIA and presented within the intervals of measuring errors. If measured concentrations of PGE_2 and leukotrienes for AIA before aspirin are 20.0 ± 9.7 pg mL^{-1} [18] and 55.5 ± 8.5 pg mL^{-1} [18], respectively, then the minimal value of the relation factor for AIA before aspirin is calculated as

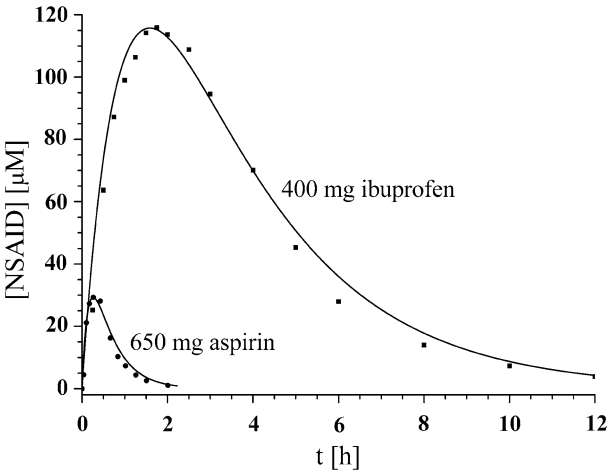


Fig. 2 Time courses of plasma drug concentration in patients following an oral 650 mg dose of aspirin and 400 mg dose of ibuprofen. *Full circles*: digitized experimental data for aspirin [26]; *full squares*: digitized experimental data for ibuprofen [27]. *Solid lines*: Eq. 19 fitted to the experimental data [26, 27]

Table 2 Values of PK parameters for aspirin, ibuprofen and nocloprost used in the model simulations

Parameter	Parameter value and its unit	Description of parameter	References
Aspirin			
k	5.5×10^{-4} s^{-1}	Elimination rate constant	[26]
k_a	23×10^{-4} s^{-1}	Absorption rate constant	[26]
V/F	74 L	Ratio between apparent volume of distribution and fraction of aspirin absorbed	[26]
Ibuprofen			
k	1.0×10^{-4} s^{-1}	Elimination rate constant	[27]
k_a	2.8×10^{-4} s^{-1}	Absorption rate constant	[27]
V/F	8.4 L	Ratio between apparent volume of distribution and fraction of ibuprofen absorbed	[27]
Nocloprost			
k	3.3×10^{-4} s^{-1}	Elimination rate constant	[28]
k_a	8.1×10^{-4} s^{-1}	Absorption rate constant	[28]
V/F	13 L	Ratio between apparent volume of distribution and fraction of nocloprost absorbed	[28]

10.3/64.0 = 0.16 and the maximal as 29.7/47.0 = 0.63. Therefore, considering the experimental errors, in this case the relation factor occupies values between 0.16 and 0.63. All experimental results presented in this paper are recalculated in the same manner.

Model states and free model parameters

Three basic model states were utilized: normal subject (NS), aspirin-tolerant asthmatic (ATA) and aspirin-intolerant asthmatic (AIA). Reports of differences in AIA are ambiguous [1, 16–19], thus we propose three different AIA model states. The analysis thus consists of five different model states NS, ATA and three different AIAs that are characterized with different values of three free model parameters v_{max1}, v_{max3} and v_{max6}, reflecting maximal activities of enzymes PGHS2, PGHS1 and LTC_4S respectively.

The reference values of parameters v_{max1} and v_{max6} originate directly from the literature [11, 13]. Parameter v_{max1} is calculated using the equation $v_{max1} = k_1[PGHS2]_{TOT}$, where $k_1 = 13\ s^{-1}$ and $[PGHS2]_{TOT} = 50$ nM [11] giving $v_{max1} = 0.65\ \mu M\ s^{-1}$. Parameter $v_{max6} = 0.23\ \mu M\ s^{-1}$ is taken directly from [13]. Parameter value v_{max3} is first determined specifically for ATA by obtaining the best agreement between the model and the experiment [18]. The relation factor is calculated with the model before and after aspirin and compared to that obtained in the experiment referring explicitly to ATA patients, to whom a typical dose of aspirin was given [18]. The parameter value $v_{max3} = 0{,}096\ \mu M\ s^{-1}$ gives the best agreement. The values of relation factor 26, predicted by the model before aspirin, and 7.1, predicted after aspirin, fall closest to the interval of the experimentally determined relation factors for ATA patients, which are 13–26 before aspirin dosing and 2.3–8.7 after aspirin dosing [18]. The input NSAID time course used in the model simulation is for a 650 mg dose of aspirin. Since the parameter value v_{max3} is determined specifically for ATA patients and all other parameters are determined nonspecifically (the experimental studies do not report any pathology), the whole set of parameter values, including $v_{max1} = 0.65\ \mu M\ s^{-1}$ and $v_{max6} = 0.23\ \mu M\ s^{-1}$, defines the model state ATA. Parameter values v_{max1}, v_{max3} and v_{max6} are in simulations later modified according to the experimental differences in expressions of the enzymes PGHS1, PGHS2 and LTC_4S between NS and ATA as well as between AIA and ATA patients.

In our description of the model state NS, we refer to studies [1, 16, 18]. The finding that expression of the enzyme LTC_4S is 4-fold lower in healthy humans compared to ATA patients [1, 16] is taken into account by 4-fold lower value of v_{max6} in NS compared to ATA, since v_{max6} is, according to Michaelis–Menten kinetics, proportional to $[LTC_4S]_{TOT}$.

The following observations are used as a basis for introducing three different AIA model states:

$AIA^{(1)}$: Reference [18] reports that activity of PGHS1 in AIA patients might be reduced in comparison to ATA patients. This is consistent with [17], which reports that the expression of PGHS1 in AIA patients is 3-fold lower in comparison to ATA patients.

$AIA^{(2)}$: References [1, 16] report 5-fold higher expression of LTC_4S in AIA compared to ATA patients.

$AIA^{(3)}$: Reference [19] reports 6-fold lower expression of PGHS2 in AIA compared to ATA patients.

Table 3 Values of free model parameters v_{max1}, v_{max3} and v_{max6} in model state NS, ATA and three different AIAs

Parameter	Model state				
	NS	ATA (Ref.)	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
v_{max1} ($\mu M\ s^{-1}$)	0.65	0.65 [11, 15]	0.65	0.65	0.11
v_{max3} ($\mu M\ s^{-1}$)	0.096	0.096	0.032	0.096	0.096
v_{max6} ($\mu M\ s^{-1}$)	0.057	0.23 [13]	0.23	1.15	0.23

The model state $AIA^{(1)}$ describes an aspirin-intolerant asthmatic with decreased basal activity of the enzyme PGHS1. This state is simulated with $v_{max3} = 0.032\ \mu M\ s^{-1}$, which is 3-fold lower than in the model state ATA. The model state $AIA^{(2)}$ describes an aspirin-intolerant asthmatic with 5-fold higher expression of the enzyme LTC_4S in comparison to ATA. It is simulated with 5-fold higher value of $v_{max6} = 1.15\ \mu M\ s^{-1}$ in comparison to ATA. The model state $AIA^{(3)}$ describes an aspirin-intolerant asthmatic with 6-fold lower expression of the enzyme PGHS2. This state is simulated with a 6-fold lower value of $v_{max1} = 0.11\ \mu M\ s^{-1}$ in comparison to ATA. The values of free model parameters are given in Table 3.

Results

Our model describes the production of AA metabolites and takes into account inhibitory actions of NSAIDs on the enzymes PGHS1 and PGHS2 in the cyclooxygenase pathway. This inhibition lowers aiPGs and piPGs production and tilts the degradation of AA in favor of the lipoxygenase pathway, thus increasing the production of LTC_4. This is a consequence of the decreased inhibitory action of aiPGs on the enzyme 5-LOX in the lipoxygenase pathway. The kinetic scheme proposed should be considered as a unique entity, retaining a complex behavior of NSAID action within the interplay between the two AA degradation pathways. The model considers all interrelations between metabolites of AA.

Analysis of time courses of LTC_4 and aiPGs after aspirin dosing and calculations of relation factors

Time courses of system variables $[LTC_4]$ and $[aiPGs]$ before aspirin at $t \leq 0$ and after aspirin for $t > 0$ are calculated. Occurrence of $[LTC_4]$ maxima and $[aiPGs]$ minima after aspirin is analyzed. In all model states except for $AIA^{(3)}$, $[LTC_4]$ maxima occur after $[aiPGs]$ minima. Minimal values of $[aiPGs]$ are achieved at different times after aspirin, ranging from 23 min in $AIA^{(3)}$ to 29 min in $AIA^{(1)}$. Maximal values of $[LTC_4]$ are achieved from 21 min for $AIA^{(3)}$, to 37 min for $AIA^{(1)}$. The times of $[aiPGs]$ minima and $[LTC_4]$ maxima after aspirin are listed in Table 4 for all model states. In comparison, the maximum in aspirin time course is at $t = 15$ min. The model results show that the initial reduction of inhibitory effect of aiPGs on the enzyme 5-LOX after aspirin dosing is indeed a crucial event in AA

Table 4 Time analysis of events in aiPGs and LTC_4 production after aspirin

Event	Model predictions: t (min)					Exp. data: *t* (min)	Reference
	NS	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$		
Minima in [*aiPGs*]	25	25	29	26	23	–	–
Maxima in [LTC_4]	33	33	37	33	21	20	[18]

metabolism that causes an increase in [LTC_4]. However, minimal [*aiPGs*] and maximal [LTC_4] do not occur at the same time after aspirin, but are delayed for approximately 2–8 min, depending on the model state. Only in $AIA^{(3)}$ minimal [*aiPGs*] comes after maximal [LTC_4]; however, both extremes occur earlier than in other states. This is a consequence of 6-fold lower PGHS2 activity in $AIA^{(3)}$.

Reference [18] shows that in an experimental study of cysteinyl leukotriene C_4 and aiPGs kinetic measurements on peripheral blood cells taken from NS, ATA and AIA patients, the optimal release of AA metabolites from cells was detected after 20 min, regardless of whether the blood was taken from normal or asthmatic subjects and before or after inhalative provocation with aspirin. Our model results are in agreement with this finding (see Table 4).

Shunting of AA metabolism toward the lipoxygenase pathway leads to increased [LTC_4] and eventually to clinical signs of aspirin-induced asthma, including bronchoconstriction. Reference [3] reports that clinical signs such as rhinitis, conjunctivitis and asthma attack in AIA usually occur within 30–60 min after ingestion of aspirin or other NSAIDs. On the other hand, it can be estimated from experimental data [7] that smooth muscle strips contract approximately 15 min after stimulation with LTC_4. If we consider the rough assumption that stimulation of smooth muscles with LTC_4 in humans is most extensive at maximal LTC_4 release from the cells, we can estimate the time interval between aspirin ingestion and bronchoconstriction in our model to be 36–52 min. This value is the sum of our theoretically determined time interval between aspirin dosing and the occurrence of maximal [LTC_4] (21–37 min for AIA) and the abovementioned time value of 15 min [7].

In our simulations we calculated the relation factors at time 0 (before aspirin) and at their extreme value after aspirin for all model states and for three different aspirin doses: 650, 65 and 6500 mg. Calculated relation factors are summarized in Table 5 and compared to Ref. [18].

The relation factors for all AIA states before aspirin are much lower and differ significantly with respect to corresponding relation factors for ATA and NS. This could be explained by the fact that LTC_4 in AIA states is higher in comparison to ATA and NS even before aspirin dosing. This result is qualitatively in accordance with a clinical report [1] that in AIA patients cysteinyl leukotrienes are, in comparison to ATA patients and healthy humans, continuously and aggressively synthesized even before any exposure to aspirin or other NSAIDs [1]. Our theoretically predicted relation factors for ATA and NS fall within the measured intervals of Ref. [18], whereas those for all AIA states are slightly overestimated.

Table 5 Comparison of relation factors predicted by the mathematical model with those calculated from experimental data [18]

Relation factor	$D^{(ASA)}$ [mg]	Model predictions					Exp. data [18]		
		NS	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$	NS	ATA	AIA
$([aiPGs]/[LTC_4])_B$	–	104	26	3.5	5.9	3.5	104–249	13–26	0.16–0.63
$([aiPGs]/[LTC_4])_A$	65	85	21	2.8	4.8	3.4	–	–	–
$([aiPGs]/[LTC_4])_A$	650	28	7.1	0.66	1.6	2.9	3.2–8.7	2.3–8.7	0.029–0.070
$([aiPGs]/[LTC_4])_A$	6500	0.88	0.22	0.0075	0.050	0.71	–	–	–

Relation factors are calculated for three different aspirin doses: 65, 650 and 6500 mg for NS, ATA, $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$ before and after aspirin. Subscripts *B* and *A* have the following meaning: *B*-before aspirin, *A*-after aspirin. Experimental data are given in intervals of measuring errors

Relation factors for 65 mg dose of aspirin in all AIA states are higher than one. For 650 mg dose of aspirin, the relation factor is less than one only in $AIA^{(1)}$. For 6500 mg, relation factors fall below one in all model states. These results illustrate the fact that aspirin dose has a major impact on the balance between synthesis of aiPGs and LTC_4 in all states and indicate that the onset of inflammatory reactions in AIA patients may be dose dependent. This is consistent with a report that severity of inflammatory reaction in aspirin-induced asthma is aspirin-dose dependent [3].

Application of the model

Table 5 shows that the relation factor for aspirin is dose dependent. Since the value one of the relation factor is the critical value that distinguishes AIA patients from ATA patients and NS [18], it is possible to determine a limiting dose of aspirin, $D^{(ASA)}_{(limit)}$, in each particular model state, for which the relation factor equals one. Value $D^{(ASA)}_{(limit)}$ is then a measure for maximal dose of aspirin that can be administered to aspirin-intolerant patients. Values of $D^{(ASA)}_{(limit)}$ obtained for all model states are presented in Table 6.

The results indicate that the decreased expression of PGHS1 in $AIA^{(1)}$ and the increased expression of LTC_4S in $AIA^{(2)}$ can be seen as important factors in AA metabolism for occurrence of aspirin-induced asthma for clinically reasonable doses of aspirin. The state $AIA^{(1)}$ is identified as the most susceptible among all the states for a 650 mg dose of aspirin. On the other hand, $D^{(ASA)}_{(limit)}$ for $AIA^{(3)}$ indicates that decreased expression of the enzyme PGHS2 cannot account for the appearance of aspirin-induced asthma according to the relation factor criterion.

Table 6 Limiting doses of aspirin $D^{(ASA)}_{(limit)}$ for which relation factor after aspirin dosing equals one. $D^{(ASA)}_{(limit)}$ is presented also as relative to 650 mg dose of aspirin

Model state	NS	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
$D^{(ASA)}_{(limit)}$ (mg)	6110	2925	455	1040	5135
$D^{(ASA)}_{(limit)}$/650 mg	9.4	4.5	0.70	1.6	7.9

In the second case, we compare the effects of aspirin and ibuprofen on the production of AA metabolites. In these simulations we again use an aspirin time course that corresponds to a 650 mg dose of aspirin. The effect of ibuprofen is simulated with Eq. 19 for a 100 mg dose, giving the same peak plasma concentration as with aspirin. Other parameters for ibuprofen are given in Table 1 and are denoted with superscript (IBU). In Table 7, relation factors for both time courses in all model states are presented.

Model predictions show that relation factors for ibuprofen are significantly lower than those for aspirin. This indicates that for the same plasma concentration, ibuprofen is a more potent inhibitor of cyclooxygenases. In $AIA^{(1)}$ for ibuprofen, the relation factor is less than one and again minimal. This result shows that $AIA^{(1)}$ is the most sensitive state, which is in agreement with previous results for aspirin, and identifies aspirin-intolerant asthmatics with decreased PGHS1 expression as the most susceptible patient population for both drugs. In $AIA^{(2)}$, the relation factor for aspirin is more than one and for ibuprofen it is less than one. This identifies aspirin-intolerant asthmatics with increased LTC_4S expression as a susceptible patient population, but only for higher doses of aspirin and for 100 mg dose of ibuprofen. In ATA, the relation factor for aspirin is more than one and for ibuprofen, it is slightly below one. The comparison of the results shows that the relation factor for ibuprofen is approximately 9-fold lower for NS, ATA and $AIA^{(2)}$, 1.5-fold lower for $AIA^{(3)}$ and 41-fold lower for $AIA^{(1)}$, compared with aspirin. This again shows that $AIA^{(1)}$ is the most sensitive state and that it is affected by ibuprofen much more than the other states.

The differences in simulations of aspirin and ibuprofen do not occur only in the values of relation factors but also in the time courses of $[LTC_4]$ and $[aiPGs]$. This is a consequence of different pharmacokinetic properties of aspirin and ibuprofen. Minima in $[aiPGs]$ and maxima in $[LTC_4]$ were observed approximately 1.9 h after ibuprofen, which is almost 1.6 h later than that of aspirin. In the analysis for aspirin, we estimated the time between the NSAID dosing and bronchoconstriction as the sum of the theoretically predicted time between aspirin dosing and maximal $[LTC_4]$ and the measured time between stimulation of smooth muscles with LTC_4 and bronchoconstriction (15 min) [7]. Clinical observations show that the asthmatic attack in AIA patients occurs 30–60 min after the ingestion of NSAID irrespective of its type [3]. Since the same approach of calculation is not applicable in the case of ibuprofen, we propose another criterion for prediction of bronchoconstriction after the drug dosing. It is considered as a sum of the time interval between drug dosing and the moment in which the relation factor falls below one, and the experimentally

Table 7 Comparison of relation factors calculated for 650 mg dose of aspirin and 100 mg dose of ibuprofen

Relation factor	Model state				
	NS	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
$([aiPGs]/[LTC_4])$ for aspirin	28	7.1	0.66	1.6	2.9
$([aiPGs]/[LTC_4])$ for ibuprofen	3.0	0.75	0.016	0.17	2.0

predicted 15 min. With this approach, the predicted time between NSAID dosing and bronchoconstriction is estimated to be 33 min in $AIA^{(1)}$ and 44 min in $AIA^{(2)}$ after 100 mg of ibuprofen and 32 min in $AIA^{(1)}$ after 650 mg of aspirin.

Schäfer et al. [18] propose a strategy by which the overproduction of cysteinyl leukotrienes is compensated by ingestion of PGE_2 or its analogue. They propose the development of a drug that would combine pure NSAID and either PGE_2, its analogue, other possible 5-LOX inhibitor or leukotriene receptor antagonist [18]. Our model enables the simulation of the action of a drug composed of NSAID and PGE_2, e.g. of aspirin and PGE_2-analogue- nocloprost, for which pharmacokinetic data were available [28]. Time courses of both components of this combined drug are described with the Eq. 19 but with different values of PK parameters that are given in Table 2. In the simulation, the total aiPGs concentration in the cell is considered to be the sum of aiPGs synthesized from AA and additional aiPGs entering the cell with the drug. The latter is in the model described with the time course for PGE_2-analogue. Furthermore, we assumed that in inhibition of 5-LOX, an added PGE_2-analogue has the same kinetic properties as the native one. Expression for inhibitory effect of aiPGs on 5-LOX (Eq. 14) thus stands also for PGE_2-analogue. Since in the time course for PGE_2-analogue elimination is already taken into account, we did not consider its transport from the cell by the reaction velocity v_4 (Eq. 12). The model enables the estimation of the dose of PGE_2-analogue $\left(D^{(PGE_2)}\right)$, for which the relation factors in AIA states achieve the same values as in ATA, after dosing 650 mg of aspirin. The predicted value of $D^{(PGE_2)}$ for $AIA^{(1)}$ (the only state in which the relation factor after 650 mg dose of aspirin drops below one) is 690 mg.

Discussion

Our mathematical model of NSAID action on AA metabolism describes the kinetics of aiPGs and LTC_4 that play a major role in occurrence of aspirin-induced asthma. The aim of this study is to evaluate theoretically experimental observations of Refs. [1–3, 16–19] about different expressions of the enzymes PGHS1, PGHS2 and LTC_4S in AA metabolism that might distinguish aspirin-intolerant asthmatics from aspirin-tolerant asthmatics and healthy humans. Differences between the model states, NS, ATA and three AIAs, are simulated with free model parameters v_{max3}, v_{max1} and v_{max6} that reflect maximal activities of PGHS1, PGHS2 and LTC_4S, respectively. We consider a proportional relationship between the enzyme expressions and their activities. The evaluation of the model is supported by the relation factor used as a quantitative criterion for considering which of the proposed aspirin-intolerant model states $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$ show characteristic behaviour of aspirin-induced asthma.

Our results point to differences between ATA and different AIA states as follows:

i) 3-fold lower PGHS1 activity in $AIA^{(1)}$ in comparison to ATA results before NSAID in a decrease of $[aiPGs]$ and in an increase of $[LTC_4]$. This system

property decreases the relation factor. After NSAID, relation factor in $AIA^{(1)}$ falls below one for 455 mg dose of aspirin. For a 100 mg dose of ibuprofen, it is far below one and is the lowest among all the states. Thus, $AIA^{(1)}$ is recognized as being the most susceptible for aspirin and ibuprofen and represents a prime candidate for explaining aspirin-induced asthma.

ii) 5-fold higher LTC_4S activity in $AIA^{(2)}$ in comparison to ATA results before NSAID in a sole increase of $[LTC_4]$. This system property decreases the relation factor but less than in $AIA^{(1)}$. After NSAID, the relation factor in $AIA^{(2)}$ falls below one for a 1040 mg dose of aspirin. For a 100 mg dose of ibuprofen, it is lower than one. $AIA^{(2)}$ is thus recognized as being susceptible for 100 mg dose of ibuprofen and is also a candidate for explaining aspirin-induced asthma.

iii) 6-fold lower PGHS2 activity in $AIA^{(3)}$ in comparison to ATA results before NSAID in an increase of $[aiPGs]$ and $[LTC_4]$. This system property does not contribute to a larger decrease in relation factor. After NSAID, the relation factor in $AIA^{(3)}$ for a 100 mg dose of ibuprofen is more than one and it falls below one for a high dose of aspirin (approx. 5000 mg). At such high doses, even the relation factors for NS and ATA are close to one. $AIA^{(3)}$ is thus not recognized as being susceptible for aspirin or ibuprofen. However, the highest $[LTC_4]$ in $AIA^{(3)}$ relative to ATA before and after NSAID, as well as very small changes in $[LTC_4]$ and $[aiPGs]$ during the action of NSAID, could indicate a different system behaviour compared to $AIA^{(1)}$ and $AIA^{(2)}$. This might lead to other clinical signs of aspirin-induced asthma than bronchoconstriction.

The experiments in Ref. [18] were performed on aspirin-intolerant patients who were selected according to spirometric testing of FEV_1 by which bronchoconstriction after the inhalative aspirin administration was proven. In the same experiment it was shown that the relation factor for aspirin-intolerant patients is lower than one [18]. Expressions of key enzymes were not determined in this study. Our model simulations show that $AIA^{(1)}$ and $AIA^{(2)}$ reach relation factors lower than one for both simulated doses, i.e. a 650 mg dose of aspirin and a 100 mg dose of ibuprofen. On the other hand, it is known that bronchoconstriction is not the only sign of aspirin-induced asthma. Other include rhinitis, conjunctivitis, anosmia, rhinorrhea and formation of nasal polyps [1, 3]. The study, according to which we defined $AIA^{(3)}$ and in which a decreased expression of PGHS2 was detected, included only patients with nasal polyps [19]. For this patient population, the model predicts relation factors for a 650 mg dose of aspirin and a 100 mg dose of ibuprofen above one. However, for $AIA^{(3)}$ the model predicts very high levels of LTC_4 already in the basal state before NSAID dosing and very small relative increase in LTC_4 after NSAID. In this context it is reasonable to raise the question whether the absolute value of $[LTC_4]$ or its relative value with respect to $[aiPGs]$, i.e. relation factor, is important as a quantitative criterion for predictions of clinical signs typical of aspirin-induced asthma. Our model simulations combined with the experimental findings [18] suggest that aspirin-intolerant patients with decreased PGHS1 expression and increased LTC_4S expression, in which the relation factor drops

below one, have a tendency towards bronchoconstriction. The tendency of aspirin-intolerant patients with decreased PGHS2 in which [LTC_4] is constitutively high and the response to NSAID is not as abrupt as in the other two populations, tend towards the occurrence of rhinitis and formation of nasal polyps.

Acknowledgements The authors acknowledge financial support from the state budget by the Slovenian Research Agency (Program No. P1-0055).

References

1. Szczeklik A, Stevenson D (1999) Aspirin-induced asthma: advances in pathogenesis and management. J Allergy Clin Immunol 104:5–13
2. Babu KS, Salvi SS (2000) Aspirin and asthma. Chest 118:1470–1476
3. Stevenson D, Szczeklik A (2006) Clinical and pathologic perspectives on aspirin sensitivity and asthma. J Allergy Clin Immunol 118:773–786
4. Vane J, Botting R (1987) Inflammation and the mechanism of action of anti-inflammatory drugs. FASEB J 1:89–96
5. Drazen JM (1998) Leukotrienes as mediators of airway obstruction. Am J Respir Crit Care Med 158:193–200
6. Barnes NC, Smith LJ (1999) Biochemistry and physiology of the leukotrienes. Clin Rev Allergy Immunol 17:27–42
7. Setoguchi H, Nishimura J, Hirano K, Takahashi S, Kanaide H (2001) Leukotriene C_4 enhances the contraction of porcine tracheal smooth muscle trough the activation of Y-27632, a rho kinase inhibitor, sensitive pathway. Br J Pharmacol 132:111–118
8. Bogatcheva N, Sergeeva M, Dudek S, Verin A (2005) Arachidonic acid cascade in endothelial pathobiology. Microvasc Res 69:107–127
9. Meade E, Smith W, DeWitt D (1993) Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268:6610–6614
10. Gonchar M, Sergeeva M, Mevkh A, Varfolomeyev S (1999) Kinetics of prostanoid synthesis by macrophages is regulated by arachidonic acid sources. Eur J Biochem 265:779–787
11. So O-Y, Scarafia LE, Mak AY, Callan OH, Swinney DC (1998) The dynamics of prostaglandin H synthases. Study with prostaglandin H-synthase 2 Y355F unmask mechanisms of time-dependent inhibition and allosteric activation. J Biol Chem 273:5801–5807
12. Kanamoto H, Takemura M, Ohyama K (2009) Detection of 5-lipoxygenase activity in the liwerwort *Marchantia polymorpha L.* Biosci Biotechnol Biochem 73:2549–2551
13. Gupta N, Gresser M, Ford-Hutchinson A (1998) Kinetic mechanism of glutathione conjugation to leukotriene A_4 by leukotriene C_4 synthase. Biochim Biophys Acta 1391:157–168
14. Harizi H, Juzan M, Moreau J-F, Gualde N (2003) Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B_4 production from dendritic cells via an IL-10-dependent mechanism. J Immunol 170:139–146
15. Callan OH, So O-Y, Swinney DC (1996) The kinetic factors that determine the affinity and selectivity for slow binding inhibition of human prostaglandin H synthase 1 and 2 by indomethacin and flurbiprofen. J Biol Chem 271:3548–3554
16. Cowburn AS, Sladek K, Soja A, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose J, Austen KF, Holgate ST, Sampson AP (1998) Over-expression of leukotriene C_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest 101:834–846
17. Pierzchalska M, Szabo Z, Sanak M, Soja J, Szczeklik A (2003) Deficient prostaglandin E_2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin induced asthma. J Allergy Clin Immunol 111:1041–1048
18. Schäfer D, Schmid M, Göde UC, Baenkler H-W (1999) Dynamics of eicosanoids in peripheral blood cells during provocation in aspirin-intolerant asthmatics. Eur Respir J 13:638–646

19. Picado C, Fernandez-Morata JC, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, Mullol J (1999) Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. Am J Respir Crit Care Med 160:291–296
20. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1994) Selectivity of non-steroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 90:11693–11697
21. Aharony D, Stein R (1986) Kinetic mechanism of guinea pig neutrophil 5-lipoxygenase. J Biol Chem 261:11512–11519
22. Noguchi M, Miyano M, Kuhara S, Matsumoto T, Noma M (1994) Interfacial kinetic reaction of human 5-lipoxygenase. Eur J Biochem 222:285–292
23. Owen WF Jr, Soberman RJ, Yoshimoto T, Sheffer AL, Lewis RA, Austen KF (1987) Synthesis and release of leukotriene C_4 by human eosinophils. J Immunol 138:532–538
24. Haeggström J, Bergman T, Jörnvall H, Rådmark O (1988) Guinea-pig liver leukotriene A_4 hydrolase. Purification, characterization and structural properties. Eur J Biochem 174:717–724
25. Lam BK, Gagnon L, Austen KF, Soberman RJ (1990) The mechanism of leukotriene B_4 export from human polymorphonuclear leukocytes. J Biol Chem 265:13438–13441
26. Rowland M, Riegelman S, Harris PA, Sholkof SD (1972) Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J Pharm Sci 61:379–385
27. Klueglich M, Ring A, Scheuerer S, Trommeshauser D, Schuijt C, Liepold B, Berndl G (2005) Ibuprofen extrudate, a novel, rapidly dissolving ibuprofen formulation: relative bioavailability compared to ibuprofen lysinate and regular ibuprofen, and food effect on all formulations. J Clin Pharmacol 45:1055–1061
28. Täuber U, Brudny–Klöppel M, Jakobs U, Madetzki C, Mahler M (1993) Pharmacokinetics of nocloprost in human volunteers and its relation to dose. Eur J Clin Pharmacol 44:497–500

Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin intolerant asthma – a theoretical study

Andrej Dobovišek · Aleš Fajmut · Milan Brumen

Supplementary material

Analysis of time courses of LTC_4 and aiPGs before and after aspirin dosing

We analyze and compare concentrations of crucial metabolites, LTC_4 and aiPGs, in model states NS, ATA, $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$. The basis for this comparison are predicted time courses of [LTC_4] and [*aiPGs*] before and after aspirin dosing, which are presented in Supplementary Figs. 1 and 2. These time courses show the temporal evolution of the model variables [LTC_4] and [*aiPGs*] from its basal state before aspirin at $t \leq 0$ and after aspirin for $t > 0$. The values of variables [LTC_4] and [*aiPGs*] are expressed as relative to the absolute values in the model state ATA before aspirin.

Supplementary Fig. 1 shows that the model states NS, ATA and $AIA^{(2)}$ have the same basal levels of aiPGs and that aiPGs undergoes the most significant drop following aspirin. This is due to the fact that maximal activities of PGHS1 and PGHS2 are the same in all these states. This result also indicates that modified activity of LTC_4S in these states has no impact on aiPGs before and after aspirin. On the other hand, $AIA^{(1)}$ has a significantly lower basal [*aiPGs*] value in comparison to NS, ATA and $AIA^{(2)}$ due to its 3-fold lower activity of PGHS1. Model state $AIA^{(3)}$ has a somewhat higher basal [*aiPGs*] value, which is due to its 6-fold lower activity of PGHS2.

Supplementary Fig. 2 shows that levels of LTC_4 before and during aspirin differ significantly among the states. States NS and ATA have low basal levels of LTC_4, whereas the basal levels of LTC_4 in all AIA states are significantly increased. Although [*aiPGs*] in NS undergoes a large decrease after aspirin, the increase of [LTC_4] after aspirin is very low due to 4-fold lower activity of LTC_4S in comparison to ATA, $AIA^{(1)}$ and $AIA^{(3)}$ and 20-fold lower in comparison to $AIA^{(2)}$. Basal LTC_4 in ATA is approximately 5-fold higher than in NS but approximately 2- and 4-fold lower than in $AIA^{(1)}$ and $AIA^{(2)}$. In $AIA^{(3)}$ the basal LTC_4 is approximately 2-fold higher than in $AIA^{(2)}$. In $AIA^{(1)}$ larger basal production of LTC_4 is due to lower basal aiPGs, which is a consequence of reduced inhibitory effect of aiPGs on 5-LOX. In $AIA^{(2)}$ this is due to increased LTC_4S activity and in $AIA^{(3)}$ this is a consequence of the reduced PGHS2 activity in the cyclooxygenase pathway, which leads to a larger production of AA metabolites through the lipoxygenase pathway. After aspirin dosing, in all cases except in $AIA^{(3)}$, LTC_4 increases approximately 2-fold above the basal levels. In $AIA^{(3)}$, sensitivity of LTC_4 production to aspirin is diminished, probably due to relatively small impact of aspirin on aiPGs production.

In Supplementary Tables 1 and 2 different ratios of [LTC_4] and [*aiPGs*] obtained with the model simulations are presented. The ratios are calculated for the basal values before aspirin as well as for the maxima of [LTC_4] and minima of [*aiPGs*] that occur after aspirin. The ratios are calculated for all model states and are compared with corresponding experimental values [18] which are given within the intervals of measuring errors. For example, if measured [LTC_4] for ATA and NS before aspirin are (40.2±9.6) $pg \cdot mL^{-1}$ [18] and (1.5±0.4) $pg \cdot mL^{-1}$ [18], respectively, then, by considering the experimental errors the ratio between [LTC_4] measured for ATA and NS before aspirin, occupy the values between 30.6 /1.9 ≅ 16 and

48.9 /1.1 ≅ 45. Experimental results presented in Supplementary Tables 1 and 2 are recalculated in the same manner.

Comparison of the model predictions and experimental data in Table 1 shows that the ratios of [LTC_4] and [*aiPGs*] for ATA and NS predicted by the model are generally in the same order of magnitude as the corresponding values calculated with measured data [18]. Larger differences can be seen in the ratios $[LTC_4]_B^{ATA} / [LTC_4]_B^{NS}$, $[aiPGs]_B^{ATA} / [aiPGs]_B^{NS}$ and $[aiPGs]_A^{ATA} / [aiPGs]_A^{NS}$. In the first case, differences occur because the model predicts higher [LTC_4] in NS than in the experiment. It would be possible to achieve better agreement with experimental data for all [LTC_4] ratios, if total concentration of the enzyme LTC_4S in NS was lower by several-fold; however, this would be in contrast with experimental results [1, 16], which state that the expression of LTC_4S in ATA patients is 4-fold higher than in NS. This ratio is also considered in our model simulations. In the case of $[aiPGs]_B^{ATA} / [aiPGs]_B^{NS}$, differences occur because the values of [*aiPGs*] in both basal states are equal. This derives from the fact that the expressions and thus also total concentrations and maximal activities of PGHS1 are equal in both ATA and NS. There is no experimental evidence which would indicate that maximal activities of PGHS1 are different in these two states. Moreover, experimental results [17] show statistically irrelevant differences in the expression of PGHS1 for ATA patients and NS. Since ATA and NS differ from each other only in the activity of LTC_4S, which does not influence the level of aiPGs, the ratio $[aiPGs]^{ATA} / [aiPGs]^{NS}$ equals one before and after aspirin.

The general conclusions derived from the above analysis are:

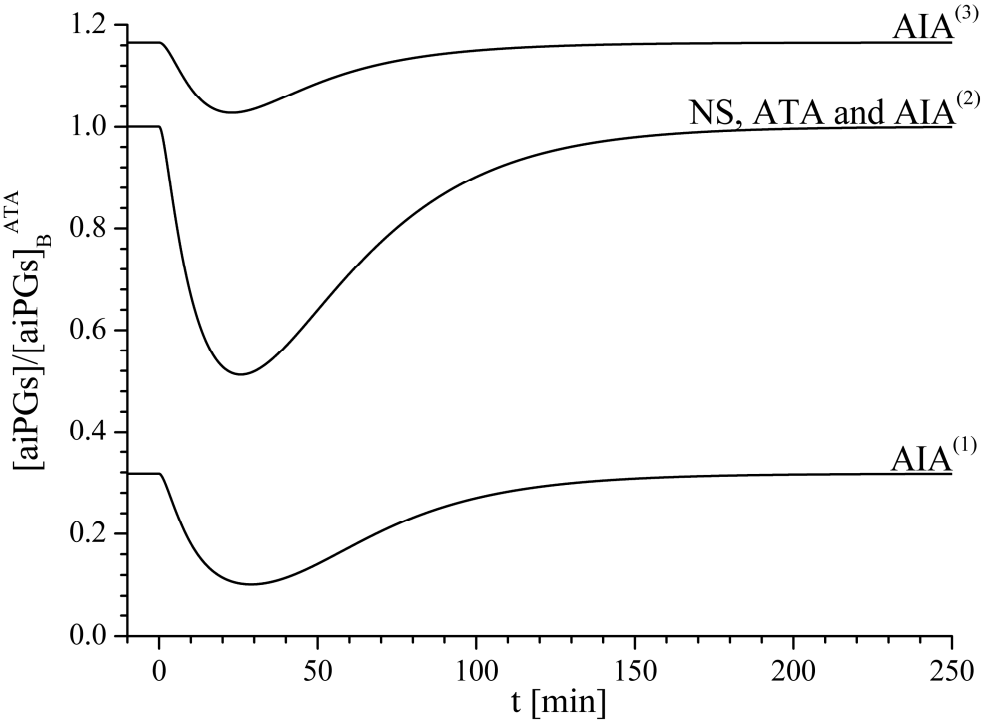
i) The ratios of [LTC_4] and [*aiPGs*] for ATA and NS predicted with the model are generally in the same order of magnitude as the corresponding ratios calculated with measured data [18].

ii) All theoretically predicted ratios of [LTC_4] for AIA$^{(1)}$, AIA$^{(2)}$ and AIA$^{(3)}$ are in the same order of magnitude as the ratios calculated with measured data [18].

iii) In contrast to the results for [LTC_4], only the ratios for [*aiPGs*] predicted in AIA$^{(1)}$ agree with experimental data [18] within the same order of magnitude. Ratios in AIA$^{(2)}$ and AIA$^{(3)}$ do not differ much from each other but differ from the results of reference [18] and the results in AIA$^{(1)}$.

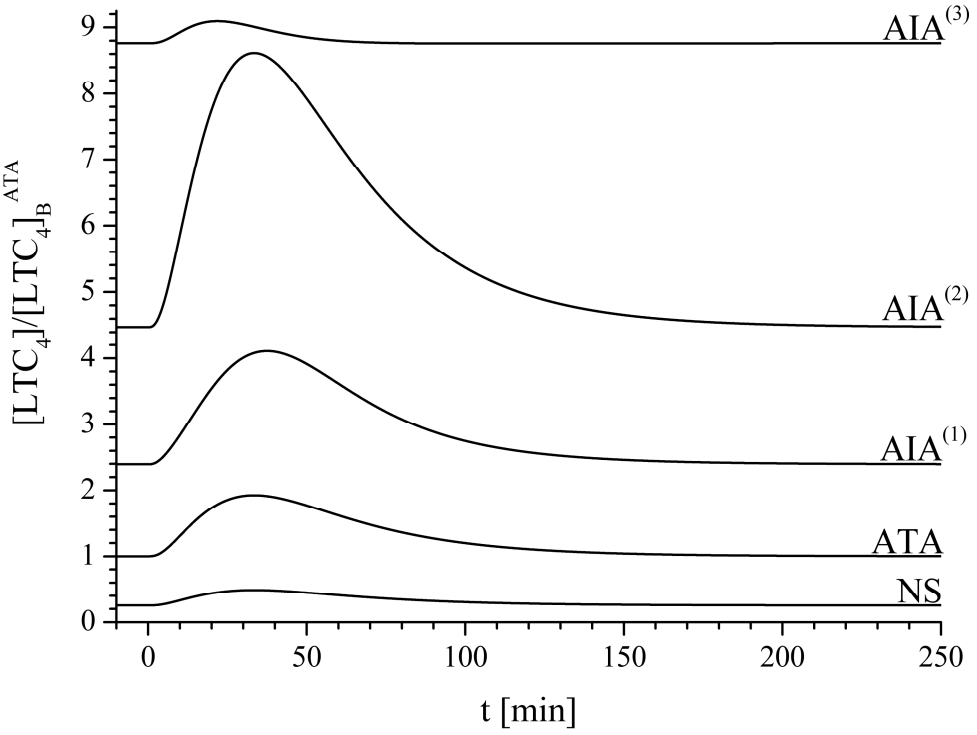
Our results show that all three model AIA states satisfactorily explain occurrence of markedly increased LTC_4 in comparison to NS and ATA; however, only AIA$^{(1)}$ theoretically predicts that the synthesis of aiPGs in AIA is lower than in NS and ATA. This is in accordance with reference [18].

(Supplementary Figure 1)



Supplementary Fig. 1. Time courses of model variable [*aiPGs*] in model states NS, ATA, AIA$^{(1)}$, AIA$^{(2)}$ and AIA$^{(3)}$ during the action of aspirin ($t > 0$) with respect to the basal value in model state ATA before application of aspirin $\left[aiPGs\right]_{\mathrm{B}}^{\mathrm{ATA}}$ ($t \leq 0$). Results are calculated for 650 mg dose of aspirin.

(Supplementary Figure 2)



Supplementary Fig. 2. Time courses of model variable [LTC_4] in model states NS, ATA, AIA$^{(1)}$, AIA$^{(2)}$ and AIA$^{(3)}$ during the action of aspirin ($t > 0$) with respect to the basal value in model state ATA before application of aspirin $\left[LTC_4\right]_{\mathrm{B}}^{\mathrm{ATA}}$ ($t \le 0$). Results are calculated for 650 mg dose of aspirin.

Supplementary Table 1. Comparison of LTC_4 and aiPGs concentrations in the model states NS and ATA before (subscript B, basal value) and after (subscript A, maximal value for LTC_4 or minimal value for aiPGs) 650 mg dose of aspirin. Model results are obtained from time courses presented in Supplementary Figs. 1 and 2.

Ratio	**Model predictions**	**Exp. data [18]**
$[LTC_4]_B^{ATA} / [LTC_4]_B^{NS}$	3.9	16–45
$[LTC_4]_A^{NS} / [LTC_4]_B^{NS}$	1.9	3.1–8.3
$[LTC_4]_A^{ATA} / [LTC_4]_B^{ATA}$	1.9	0.80–2.9
$[LTC_4]_A^{ATA} / [LTC_4]_A^{NS}$	3.9	4.3–15
$[aiPGs]_B^{ATA} / [aiPGs]_B^{NS}$	1.0	2.4–4.0
$[aiPGs]_A^{NS} / [aiPGs]_B^{NS}$	0.51	0.10–0.26
$[aiPGs]_A^{ATA} / [aiPGs]_B^{ATA}$	0.51	0.26–0.50
$[aiPGs]_A^{ATA} / [aiPGs]_A^{NS}$	1.0	4.1–11

Supplementary Table 2. Comparison of LTC_4 and aiPGs concentrations in three AIA model states with respect to their values in NS and ATA states before (subscript B, basal value) and after (subscript A, maximal value for LTC_4 or minimal value for aiPGs) 650 mg dose of aspirin. Model results are obtained from time courses presented in Supplementary Figs. 1 and 2.

Ratio	AIA$^{(1)}$	AIA$^{(2)}$	AIA$^{(3)}$	Exp. data [18]
$[LTC_4]_B^{AIA^{(i)}} / [LTC_4]_B^{NS}$	9.5	8	35	25–58
$[LTC_4]_B^{AIA^{(i)}} / [LTC_4]_B^{ATA}$	2.4	4.5	8.8	0.94–2.1
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_B^{AIA^{(i)}}$	1.7	1.9	1.0	3.2–5.8
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_A^{NS}$	8.4	18	19	22–46
$[LTC_4]_A^{AIA^{(i)}} / [LTC_4]_A^{ATA}$	2.1	4.5	4.7	2.2–6.9
$[aiPGs]_B^{AIA^{(i)}} / [aiPGs]_B^{NS}$	0.32	1.0	1.2	0.037–0.15
$[aiPGs]_B^{AIA^{(i)}} / [aiPGs]_B^{ATA}$	0.32	1.0	1.2	0.013–0.045
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_B^{AIA^{(i)}}$	0.32	0.51	0.88	0.27–2.5
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_A^{NS}$	0.20	1.0	2.0	0.16–0.90
$[aiPGs]_A^{AIA^{(i)}} / [aiPGs]_A^{ATA}$	0.20	1.0	2.0	0.025–0.12

Priloga 2

Objavljeni izvirni znanstveni članek

Dobovišek A, Fajmut A, Brumen M (2012) Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE_2 analogue: a theoretical approach. Med Biol Eng Comput 50: 33–42

Med Biol Eng Comput (2012) 50:33–42
DOI 10.1007/s11517-011-0844-x

ORIGINAL ARTICLE

Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE_2 analogue: a theoretical approach

A. Dobovišek · A. Fajmut · M. Brumen

Received: 4 June 2011 / Accepted: 7 November 2011 / Published online: 26 November 2011

Abstract Aspirin-induced asthma (AIA) is a severe inflammatory disease, which affects aspirin-intolerant patients after ingestion of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs). In this article, a mathematical model describing arachidonic acid metabolism and its interaction with NSAIDs, is used to study the strategy for safe managing of NSAIDs to AIA patients. Three different AIA patient populations are taken into consideration. First, the values of aspirin and ibuprofen limiting doses that might induce symptoms of AIA are calculated and compared to experimentally observed threshold doses to enlighten which AIA patient population is susceptible to aspirin and ibuprofen. Second, the methodology of NSAID administration is studied on AIA populations susceptible to aspirin and ibuprofen by using 1,000 mg dose of aspirin and 200 or 400 mg dose of ibuprofen followed by PGE_2 analogue dosing. Our model results show that successive doses of PGE_2 analogue applied at appropriate time after aspirin or ibuprofen ingestion would enable administration of both NSAIDs to AIA patients. PGE_2 analogue doses and the corresponding times of their applications are calculated. The model is also used to estimate the duration of symptoms of AIA for different aspirin and ibuprofen doses.

A. Dobovišek (✉) · A. Fajmut · M. Brumen
Faculty of Natural Sciences and Mathematics, Faculty of Medicine, Faculty of Health Sciences, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia
e-mail: andrej.dobovisek@uni-mb.si

M. Brumen
Institute Jožef Stefan, Jamova cesta 39, 1000 Ljubljana, Slovenia

Keywords Mathematical model · Aspirin-induced asthma · Non-steroidal anti-inflammatory drug · Leukotrienes · Prostaglandins

1 Introduction

Around 10–20% of adult asthmatic patients are susceptible to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) [21, 26, 27]. In these patients, typical therapeutic doses of NSAIDs induce aggressive inflammation of upper and lower airways, accompanied by bronchoconstriction, nasal congestion, loss of smell, swollen eyes as well as flushing head and neck [26, 27]. This clinical syndrome is known as aspirin-induced asthma (AIA). The symptoms usually occur 30–60 min after NSAID ingestion [26] and can last up to 9 h [23]. The main role in occurrence of AIA is attributed to cysteinyl leukotriene C_4 (LTC_4), a product of arachidonic acid (AA) metabolism [26, 27]. LTC_4 is a potent pro-inflammatory mediator that causes bronchoconstriction in asthmatic patients and healthy humans (HH) [24].

It is generally known that the metabolism of AA is the target of NSAID action in epithelial and inflammatory cells. Figure 1 shows the kinetic scheme of AA degradation, which we already published [3] and is also considered in the present article. Two main metabolic pathways are taken into account; cyclooxygenase and lipoxygenase pathway [1, 26]. In the cyclooxygenase pathway, AA is transformed into anti-(aiPGs) and pro-(piPGs) inflammatory prostaglandins by the enzymes prostaglandin H synthase 1 (PGHS1) and prostaglandin H synthase 2 (PGHS2) [5, 14, 27]. Along the lipoxygenase pathway, AA is first transformed into leukotriene A_4 (LTA_4) by the enzyme 5-lipoxygenase (5-LOX). Then LTA_4 is further

transformed into leukotriene B_4 (LTB_4) by the enzyme LTA_4 hydrolase (LTA_4H) or it is conjugated with glutathione (GSH) into LTC_4 by the enzyme leukotriene C_4 synthase (LTC_4S).

The balance between synthesis of prostaglandins and leukotrienes depends on the production of prostaglandin E_2 (PGE_2), which is one of aiPGs. PGE_2 inhibits the enzyme 5-LOX and decreases the synthesis of leukotrienes [7, 22]. The target of inhibitory action of PGE_2 is 5-LOX activating protein (FLAP) via the autocrine mechanism; however, the entire mechanism is not completely revealed [7, 13, 26]. Majority of NSAIDs inhibit both enzymes of cyclooxygenase pathway, PGHS1 and PGHS2 [14, 25], thus they reduce the total prostaglandin production and consequently also lower the synthesis of PGE_2 [30]. The decrease in PGE_2 synthesis reduces the inhibitory effect on 5-LOX, which leads to enhanced synthesis of leukotrienes [7, 26, 27, 30]. This seems to be the critical event that tilts AA metabolism in favour of the lipoxygenase pathway and leads to overproduction of LTC_4.

In the study [21], of which the long-term goal was to check the possibility of a simple and cheap in vitro whole blood tests for routine diagnosis of aspirin intolerance, authors introduced the concept of relation factor (Rf). It is defined as the ratio between PGE_2 and cysteinyl leukotriene concentrations in peripheral blood cells [21]. They showed that the value of Rf of selected AIA patients who had bronchoconstrictive reactions after aspirin inhalation, is always, before and after NSAID exposure, lower than one [21]. For ATA patients and HH it is always more than one [21]. These findings are of special importance, since they enable quantitative distinction between AIA and ATA patients and HH.

There are several experimental findings showing that AIA patients may be susceptible to NSAIDs due to altered expressions of the enzymes PGHS1, PGHS2 and LTC_4S in comparison to ATA patients and HH: (i) PGHS1 expression in AIA patients is threefold lower in comparison to ATA patients and there are no significant differences in PGHS1 expression between ATA and HH [17], (ii) LTC_4S expression is fivefold higher in AIA patients compared to ATA patients and 20-fold higher compared to HH [2, 27] and (iii) PGHS2 expression is sixfold lower in AIA patients compared to ATA patients [16]. These experimental data suggest that there exist different populations of aspirin-intolerant asthmatics.

In our previous paper [3], we established the model of AA metabolism, defined different model states, describing HH, aspirin-tolerant and aspirin-intolerant asthmatics, and investigated the role of altered expressions of the enzymes PGHS1, PGHS2 and LTC_4S on the production of aiPGs and LTC_4. In the present work, we theoretically investigate a possible strategy for administration of NSAIDs to AIA patients, in which one NSAID dose is followed by one or multiple doses of PGE_2 analogue. We test and evaluate the strategy, suggested by Ref. [21], according to which NSAID would be combined with oral-stable PGE_2 analogue–nocloprost. We predict the number and the level of PGE_2 analogue dose(s) for 1,000 mg dose of aspirin as well as for 200 and 400 mg doses of ibuprofen. Three different populations of AIA patients are taken into consideration, which differ in expressions of the enzymes PGHS1, PGHS2 and LTC_4S. We discuss the methodology of PGE_2 analogue dosing, because it strongly depends on pharmacokinetic properties of the NSAID type and its dose as well as on pharmacokinetic properties of PGE_2 analogue. The relation factor (Rf) is used in the model as a quantitative criterion in predicting bronchoconstriction. Its value has to retain above one to avoid the condition, in which the probability for bronchoconstriction is increased. Model predictions are discussed and compared to clinical experimental data, taken from the literature.

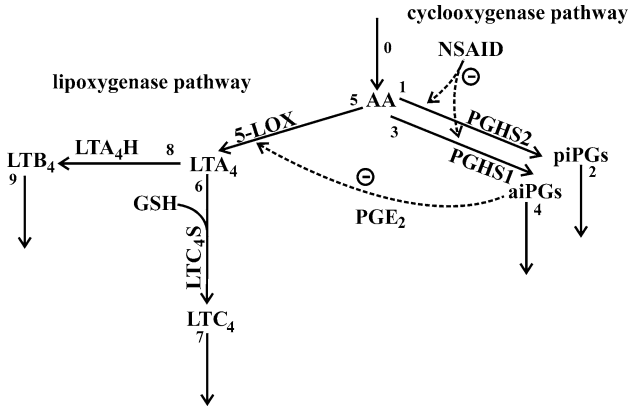


Fig. 1 Kinetic scheme of arachidonic acid metabolism and its interaction with NSAID considered for mathematical modelling [3]. *Numbers* refer to reaction velocities v_i, $i = 0, 1, \ldots, 9$

2 Methods

2.1 Mathematical model

The mathematical model, used in this work, was first presented in our recently published paper [3], where more detailed description could be found. Here, the model equations are presented in a compact form and are briefly discussed.

The basic framework of interaction between NSAIDs and AA metabolism is illustrated by the kinetic scheme in Fig. 1. Mathematical description consists of six first-order differential equations, describing time evolution of system variables, which are concentrations of metabolites [AA], [piPGs], [aiPGs], [LTA_4], [LTB_4] and [LTC_4]:

$$\frac{d[AA]}{dt} = v_0 - v_1 - v_3 - v_5, \quad (1)$$

$$\frac{d[piPGs]}{dt} = v_1 - v_2, \quad (2)$$

$$\frac{d[aiPGs]}{dt} = v_3 - v_4, \quad (3)$$

$$\frac{d[LTA_4]}{dt} = v_5 - v_6 - v_8, \quad (4)$$

$$\frac{d[LTB_4]}{dt} = v_8 - v_9, \quad (5)$$

$$\frac{d[LTC_4]}{dt} = v_6 - v_7, \quad (6)$$

where v_i, $i = 0, 1, \ldots, 9$, are the reaction velocities as indicated in Fig. 1.

In all model simulations constant influx of AA is assumed $v_0 = 0.7$ μM s^{-1}. Effluxes of piPGs, aiPGs and LTC_4 from the cell are taken into account by the reaction velocities v_2, v_4 and v_7, respectively:

$$v_i = k_i[x_i] \quad \text{for } i = 2, 4, 7 \quad (7)$$

where k_i is the appropriate rate constant and x_i is the metabolite as follow: x_2 = piPGs, x_4 = aiPGs and $x_7 = LTC_4$.

Conversion of LTA_4 into LTB_4 by the enzyme LTA_4H as well as the efflux of LTB_4 from the cell is described by the reaction velocities v_8 and v_9:

$$v_i = \frac{v_{\max i}[x_i]}{K_i + [x_i]} \quad \text{for } i = 8, 9 \quad (8)$$

where $v_{\max i}$ is the maximal reaction velocity and K_i is the Michaelis–Menten constant; $x_8 = LTA_4$ and $x_9 = LTB_4$.

Conversion of AA into piPGs, aiPGs and LTA_4 is described by the reaction velocities v_1, v_3 and v_5:

$$v_i = \frac{v_{\max i}[AA]}{K_i(1 + [y_i]/K_{Ii}) + [AA]} \quad \text{for } i = 1, 3, 5 \quad (9)$$

where K_{Ii} is the dissociation equilibrium constant describing either inhibitory action of NSAID on the enzymes PGHS1 and PGHS2, or inhibitory action of aiPGs on the enzyme 5-LOX. $v_{\max i}$ is the maximal reaction velocity. AA is a substrate for all reactions, symbol y_i stands for different inhibitors of the enzymes PGHS1, PGHS2 and 5-LOX as follows: $y_1 = y_3$ = NSAID and $y_5 = aiPGs_{TOT}$. y_1 and y_3 are described by Eq. 11 and y_5 by Eq. 12.

Reaction velocity of LTA_4 conjugation with GSH into LTC_4 by the enzyme LTC_4S is described by the expression:

$$v_6 = \frac{v_{\max 6}[LTA_4]}{A + B[LTA_4] + C[LTA_4]^2} \quad (10)$$

where A, B and C are parameters and $v_{\max 6}$ is the maximal reaction velocity.

Time courses of aspirin and ibuprofen plasma concentrations are described by a standard pharmacokinetic model that takes into account absorption and elimination phases of the drug:

$$[NSAID] = \frac{D^{(j)}k_a^{(j)}}{(V/F)^{(j)}\left(k_a^{(j)} - k_e^{(j)}\right)}\left(e^{-k_e^{(j)}t} - e^{-k_a^{(j)}t}\right), \quad (11)$$

where $D^{(j)}$ is the drug dose, $(V/F)^{(j)}$ is the ratio between apparent volume of drug distribution and fraction of the drug absorbed, $k_a^{(j)}$ and $k_e^{(j)}$ are the first-order absorption and elimination rate constants, respectively. Superscript (j) denotes pharmacokinetic parameters for either aspirin, j = ASA, or ibuprofen, j = IBU.

Total aiPGs concentration $[aiPGs_{TOT}]$ is given by the equation:

$$[aiPGs_{TOT}] = [aiPGs] + \frac{D^{(PGE_2)}k_a^{(PGE_2)}}{(V/F)^{(PGE_2)}\left(k_a^{(PGE_2)} - k_e^{(PGE_2)}\right)} \times \left(e^{-k_e^{(PGE_2)}t} - e^{-k_a^{(PGE_2)}t}\right). \quad (12)$$

The first term in the above equation considers the formation of aiPGs in the cell (see Eq. 3) and the second term describes the level of exogenous PGE_2 analogue, administered orally to the patient, by the absorption and elimination phases. We consider the inhibitory effect of endogenous and exogenous PGE_2 on 5-LOX to be the same since there is strong evidence that PGE_2 exerts the inhibition in an autocrine fashion [7, 8, 13]. Relation factor (Rf) is defined as [21]:

$$Rf = \frac{[aiPGs_{TOT}]}{[LTC_4]}. \quad (13)$$

In accordance with the experimental findings of Ref. [21], bronchoconstriction occurs when $Rf < 1$. Descriptions of the model parameters and their values, used in model simulations, are taken from our previous work [3] and are given in Table 1. Model parameters $v_{\max 1}$, $v_{\max 3}$ and $v_{\max 6}$ are defined and described in the Sect. 2.2. Parameter $D^{(PGE_2)}$ is a result of the model simulations and is given in the Sect. 3.

2.2 Model states

We use two basic model states ATA and AIA, whereby we distinguish three AIA states: $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$, according to experimental findings about the differences in expressions of the key enzymes [2, 16, 17, 27]. All AIA model states differ from each other in the parameter values $v_{\max 1}$, $v_{\max 3}$ and $v_{\max 6}$, reflecting different expressions of the enzymes PGHS2, PGHS1 and LTC_4S, respectively.

Table 1 Values of the model parameters used in model simulations [3]

Parameter	Parameter value and its units	Description of parameter
v_0	0.7 μM s^{-1}	AA influx
K_1	2.5 μM	Michaelis–Menten constant
$K_{I1}^{(ASA)}$	85 μM	Equilibrium dissociation constant of inhibitory action of aspirin on PGHS2
$K_{I1}^{(IBU)}$	60 μM	Equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS2
k_2	0.0028 s^{-1}	Rate constant of piPGs efflux
K_3	3.0 μM	Michaelis–Menten constant
$K_{I3}^{(ASA)}$	4.0 μM	Equilibrium dissociation constant of inhibitory action of aspirin on PGHS1
$K_{I3}^{(IBU)}$	1.3 μM	Equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS1
k_4	0.0028 s^{-1}	Rate constant of aiPGs efflux
v_{max5}	86 μM s^{-1}	Maximal velocity of the reaction
K_5	25.4 μM	5-LOX-AA equilibrium dissociation constant
K_{I5}	0.03 μM	Equilibrium dissociation constant of inhibitory action of aiPGs on 5-LOX
A	56 μM	Constant
B	1.4	Constant
C	0.17 μM^{-1}	Constant
k_7	0.0015 s^{-1}	Rate constant of LTC_4 efflux
v_{max8}	3.7 μM s^{-1}	Maximal velocity of the reaction
K_8	27 μM	Michaelis–Menten constant
v_{max9}	5.74 μM s^{-1}	Maximal velocity of the reaction
K_9	239 μM	Half-saturation constant
$k_e^{(ASA)}$	5.5×10^{-4} s^{-1}	Elimination rate constant for aspirin
$k_a^{(ASA)}$	23×10^{-4} s^{-1}	Absorption rate constant for aspirin
$(V/F)^{(ASA)}$	74 L	Ratio between apparent volume of distribution and fraction of the absorbed aspirin
$D^{(ASA)}$	1,000 mg	Aspirin dose
$k_e^{(IBU)}$	1.0×10^{-4} s^{-1}	Elimination rate constant for ibuprofen
$k_a^{(IBU)}$	2.8×10^{-4} s^{-1}	Absorption rate constant for ibuprofen
$(V/F)^{(IBU)}$	8.4 L	Ratio between apparent volume of distribution and fraction of the absorbed ibuprofen
$D^{(IBU)}$	200 or 400 mg	Ibuprofen dose
$k_e^{(PGE_2)}$	3.3×10^{-4} s^{-1}	Elimination rate constant for PGE_2 analogue
$k_a^{(PGE_2)}$	8.1×10^{-4} s^{-1}	Absorption rate constant for PGE_2 analogue
$(V/F)^{(PGE_2)}$	13 L	Ratio between apparent volume of distribution and fraction of the absorbed PGE_2 analogue

Direct proportionalities between the expressions of the enzymes and their maximal activities are considered.

Model state ATA represents the reference model state, which was already determined in our previous study [3]. In short: the reference values of model parameters $v_{max1} = 0.65$ μM s^{-1} and $v_{max6} = 0.23$ μM s^{-1} were obtained from experimental studies [6, 25], whereas v_{max3} was determined specifically for ATA by obtaining the best agreement between the model and the experiment [21]. The value of Rf was calculated with the model before and after 650 mg aspirin dose and was compared to that, which was obtained in the experiment, referring explicitly to ATA patients, to whom a typical dose of aspirin was given [21]. The parameter value $v_{max3} = 0{,}096$ μM s^{-1} gave the best agreement. Since the parameter value v_{max3} was determined specifically for ATA patients and all other parameters were determined non-specifically (the experimental studies do not report any pathologies), the whole set of parameter values, $v_{max1} = 0.65$ μM s^{-1}, $v_{max3} = 0.096$ μM s^{-1} and $v_{max6} = 0.23$ μM s^{-1}, defines the model state ATA [3].

Different AIA model states are defined with respect to ATA as follows [3]:

(i) $AIA^{(1)}$: with threefold decreased expression of the enzyme PGHS1: $v_{max1} = 0.65\ \mu M\ s^{-1}$, $v_{max3} = 0.032\ \mu M\ s^{-1}$ and $v_{max6} = 0.23\ \mu M\ s^{-1}$.
(ii) $AIA^{(2)}$: with fivefold increased expression of the enzyme LTC_4S: $v_{max1} = 0.65\ \mu M\ s^{-1}$, $v_{max3} = 0.096\ \mu M\ s^{-1}$ and $v_{max6} = 1.15\ \mu M\ s^{-1}$.
(iii) $AIA^{(3)}$: with sixfold decreased expression of the enzyme PGHS2: $v_{max1} = 0.11\ \mu M\ s^{-1}$, $v_{max3} = 0.096\ \mu M\ s^{-1}$ and $v_{max6} = 0.23\ \mu M\ s^{-1}$.

2.3 Numerical tools and calculation procedures

Software used is Berkeley Madonna 8.0.1 (R. Macey and G. Oster, University of California at Berkeley). The system of differential equations (1)–(6) is numerically integrated from the initial stationary state, which is determined for each model variable in the absence of NSAID in each particular model state. In simulations, we used numerical integration method with variable integration step.

The methodology of combined NSAID and PGE_2 analogue dosing to AIA patients is studied. The condition $Rf = 1$ is a quantitative criterion for determining the number and the level of PGE_2 analogue doses. The following calculation procedure is used: (i) After NSAID dosing, the time course of Rf is calculated and the time for $Rf = 1$ is determined. (ii) PGE_2 analogue is added at the exact time when $Rf = 1$. The dose of PGE_2 analogue is such that Rf does not fall below one and increases back closest to its basal value (the value prior to NSAID dosing). (iii) Each next dose of PGE_2 is added when $Rf = 1$ and the procedure is repeated until Rf does not stay above one. (iv) Exceptions in PGE_2 analogue dosing are allowed for the last dose, if a slightly higher last dose of PGE_2 analogue enables leaving out one additional dose.

The sensitivity analysis of model variable Rf with respect to the model parameters v_{max1}, v_{max3} and v_{max6} is performed in all AIA states for different aspirin and ibuprofen doses. The response coefficients $R^{Rf}_{v_{max\,i}}$ are calculated as [9, 10]:

$$R^{Rf}_{v_{max\,i}} = \frac{\Delta Rf/Rf}{\Delta v_{max\,i}/v_{max\,i}} \quad \text{for } i = 1, 3, 6. \tag{14}$$

In Eq. 14, $\Delta Rf/Rf$ is a relative change in Rf, caused by a small change (1%) in the value of model parameter v_{maxi} ($\Delta v_{maxi}/v_{maxi} = 0.01$). For example, the value of $R^{Rf}_{v_{max\,i}} = 2$ means that following 1% increase in parameter value v_{maxi} the value of Rf will increase for 2%. In these calculations, we consider stationary NSAID concentrations, which correspond to the maximal concentrations reached for selected NSAID doses.

3 Results

3.1 Comparison of predicted limiting doses with measured threshold doses for different AIA model states

For the use of any NSAID in each particular AIA model state, it is possible to calculate a limiting dose that corresponds to the condition $Rf = 1$ [3]. Limiting doses could be considered as the theoretical estimates for critical NSAID doses that could induce bronchoconstriction in AIA. In clinical studies such doses are known as threshold doses [19, 23, 27, 31]. Predicted limiting doses and the threshold doses, reported in clinical studies [19, 23, 31], are given in Table 2.

3.2 Strategies for NSAID and PGE_2 analogue dosing

Authors in Ref. [21] propose possible strategy, by which bronchoconstriction after NSAID dosing to AIA patients could be avoided. It considers the use of oral-stable form of PGE_2–nocloprost in combination with NSAID. Nocloprost is mainly used as a protective prostaglandin in treatment of NSAID-induced gastric ulcers [29], but with a different role than here. Here, we test this strategy theoretically. We predict the number and the level of PGE_2 analogue dose(s) for one dose of aspirin and two doses of ibuprofen in all AIA model states.

The methodology of aspirin and PGE_2 analogue dosing is studied only for population $AIA^{(1)}$, since only in this population the predicted value of $D^{(ASA)}_{limit}$ is lower than reported $D^{(ASA)}_{treshold}$. Figure 2 shows the time courses of Rf after 1,000 mg dose of aspirin with and without PGE_2 analogue, as well as the time courses of aspirin and PGE_2 analogue plasma concentrations.

Before aspirin dosing, Rf possesses its basal (stationary) value 3.5. In the first simulation, aspirin is applied at time $t = 0$ and Rf starts to decrease. Rf reaches one approximately 0.22 h after aspirin dosing. From this time on, bronchoconstriction may occur. The value of Rf is maintained below one for approximately 0.89 h and after that, it slowly increases back to its basal value (dashed line). In the second simulation, PGE_2 analogue is applied at time $t = 0.22$ h (when $Rf = 1$). The minimal dose of PGE_2 analogue ($D^{(PGE_2)}$), for which the value of Rf is maintained above one during the action of aspirin and which returns Rf back to approximately its basal value, is 46 mg.

Table 2 Predicted limiting doses for aspirin ($D_{\text{limit}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{limit}}^{(\text{IBU})}$) in AIA model states and threshold doses for aspirin ($D_{\text{treshold}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{treshold}}^{(\text{IBU})}$) for AIA patients

Dose	AIA$^{(1)}$	AIA$^{(2)}$	AIA$^{(3)}$
$D_{\text{limit}}^{(\text{ASA})}$ (mg)	455	1,025	5,150
$D_{\text{limit}}^{(\text{IBU})}$ (mg)	16	35	175
$D_{\text{treshold}}^{(\text{ASA})}$ (mg)	3–600 [19] 325–650 [23]		
$D_{\text{treshold}}^{(\text{IBU})}$ (mg)	$\cong$400 [31]		

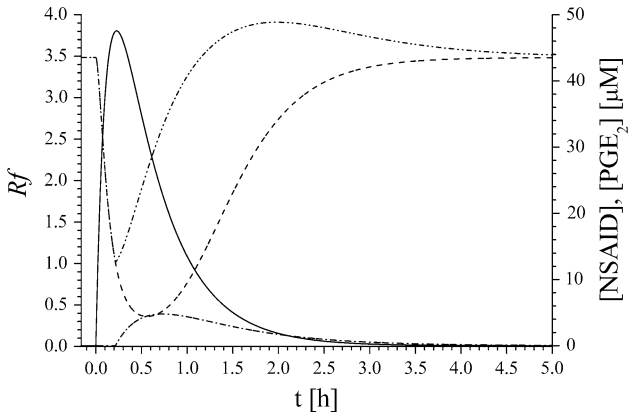


Fig. 2 Model simulations of Rf time courses after 1,000 mg dose of aspirin without and with PGE_2 analogue. Time courses are calculated for AIA$^{(1)}$. *Solid line* time course of aspirin plasma concentration. *Dashed line* time course of Rf after aspirin. *Dash-dot line* time course of PGE_2 analogue plasma concentration. *Dash-dot-dot line* time course of Rf after aspirin followed by PGE_2 analogue

Methodologies for ibuprofen and PGE_2 analogue are studied for all three AIA populations, since predicted values of $D_{\text{limit}}^{(\text{IBU})}$ are in all states lower than $D_{\text{treshold}}^{(\text{IBU})}$. For each AIA population, two cases are studied; for 200 and 400 mg dose of ibuprofen. Simulation of 200 mg dosing to AIA$^{(1)}$ is presented in details and other cases are summarized in Table 3. Figure 3 shows the time course of Rf after 200 mg dose of ibuprofen with and without PGE_2 analogue, as well as the time courses of ibuprofen and PGE_2 analogue plasma concentrations. All time courses are calculated for AIA$^{(1)}$.

Model simulations, presented in Fig. 3, are performed in the same manner as those for aspirin (Fig. 2). Since ibuprofen exhibits slower pharmacokinetics than aspirin, Rf stays below one much longer (9.8 h), compared to aspirin (0.89 h). Thus, successive dosing of PGE_2 analogue is needed to maintain Rf above one during the action of ibuprofen. At time $t = 0.22$ h, the first dose of 97 mg PGE_2 analogue is applied and after that, the procedure is repeated two times: at $t = 3.80$ h with 75 mg, and at $t = 5.83$ h with 89 mg of PGE_2 analogue. Slightly higher last dose of PGE_2 analogue is chosen to avoid next dosing. In Table 3, model results, obtained in simulations, are summarized for all AIA populations and doses.

3.3 Sensitivity analysis

The model system was analysed with the sensitivity analysis in terms of changes in model variable Rf with respect to small variations in model parameters $v_{\max 1}$, $v_{\max 3}$ and $v_{\max 6}$ in the absence or presence of aspirin and ibuprofen. Calculated response coefficients for different aspirin and ibuprofen doses are shown in Figs. 4 and 5, respectively. The response coefficient of Rf with respect to parameter $v_{\max 6}$ is independent of the type and the dose of NSAID. Its value is around -0.9 in all AIA model states before and after NSAID.

4 Discussion

By comparing predicted limiting doses for aspirin ($D_{\text{limit}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{limit}}^{(\text{IBU})}$) with corresponding threshold doses ($D_{\text{treshold}}^{(\text{ASA})}$) and ($D_{\text{treshold}}^{(\text{IBU})}$) (see Table 2), it is possible to identify which of AIA patient populations, considered in this study, is more susceptible to particular NSAID, and at which dose. Our results show that the values $D_{\text{limit}}^{(\text{ASA})}$ are much higher than $D_{\text{limit}}^{(\text{IBU})}$. Ibuprofen is thus recognized as a more potent inhibitor of the enzymes PGHS1 and PGHS2 as aspirin. Predicted value of $D_{\text{limit}}^{(\text{ASA})}$ for AIA$^{(1)}$ falls within reported interval of clinically observed threshold values $D_{\text{treshold}}^{(\text{ASA})}$.

In AIA$^{(2)}$, $D_{\text{limit}}^{(\text{ASA})}$ is slightly above the threshold values and in AIA$^{(3)}$, $D_{\text{limit}}^{(\text{ASA})}$ is much higher than $D_{\text{treshold}}^{(\text{ASA})}$. Thus, patient population AIA$^{(1)}$ could be considered as susceptible to aspirin doses higher than 455 mg of aspirin. In clinical study [18], asthmatic patients who did not tolerate more than 1,000 mg of aspirin were considered as susceptible to aspirin. According to this criterion, patient population AIA$^{(2)}$ could be considered as a boundary case, since $D_{\text{limit}}^{(\text{ASA})}$is $\sim$1,000 mg, and patient population AIA$^{(3)}$ could be considered as insusceptible to aspirin, since $D_{\text{limit}}^{(\text{ASA})}$ is much higher than $D_{\text{treshold}}^{(\text{ASA})}$.

Predicted values of $D_{\text{limit}}^{(\text{IBU})}$ for all three AIA populations are lower than the reported value $D_{\text{treshold}}^{(\text{IBU})}$, which is $\sim$400 mg [31].

These results show important differences in susceptibilities of different AIA patient populations with respect to the type of NSAID. Among three AIA patient populations, AIA$^{(1)}$ and partially AIA$^{(2)}$ could be considered as

Table 3 Model results obtained in simulations with 1,000 mg dose of aspirin followed by PGE_2 analogue in $AIA^{(1)}$ as well as with 200 and 400 mg doses of ibuprofen followed by PGE_2 analogue in $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$

In the second column, the time interval ($\Delta t_{Rf<1}$) in which the value of Rf is lower than one is given. Instants, at which PGE_2 analogue is applied after NSAID dosing and its corresponding dose, are given from the third to the seventh column

Model state	$\Delta t_{Rf<1}$ (h)	t_1 (h) $D_1^{(PGE_2)}$ (mg)	t_2 (h) $D_2^{(PGE_2)}$ (mg)	t_3 (h) $D_3^{(PGE_2)}$ (mg)	t_4 (h) $D_4^{(PGE_2)}$ (mg)	t_5 (h) $D_5^{(PGE_2)}$ (mg)
1,000 mg aspirin						
$AIA^{(1)}$	0.89	0.22	–	–	–	–
		46	–	–	–	–
200 mg ibuprofen						
$AIA^{(1)}$	9.8	0.22	1.95	3.81	5.8	–
		97	84	75	84	–
$AIA^{(2)}$	7.5	0.32	2.52	5.20	–	–
		374	343	31	–	–
$AIA^{(3)}$	1.8	1.18	–	–	–	–
		15	–	–	–	–
400 mg ibuprofen						
$AIA^{(1)}$	11.8	0.16	1.90	3.70	5.50	7.50
		112	99	88	77	114
$AIA^{(2)}$	9.6	0.23	2.30	4.60	7.3	–
		439	417	346	23	–
$AIA^{(3)}$	4.9	0.55	3.00	–	–	–
		213	22	–	–	–

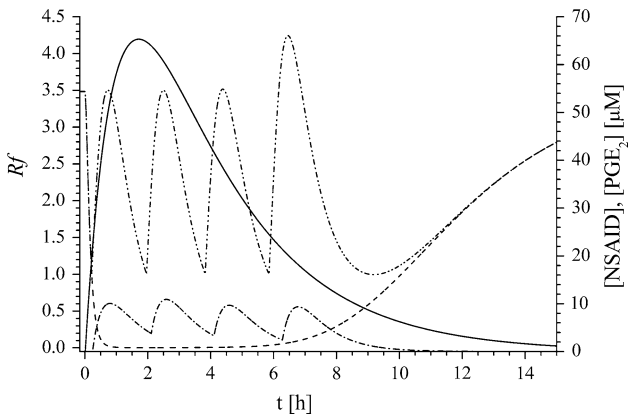


Fig. 3 Model simulations of Rf time courses after 200 mg dose of ibuprofen without and with PGE_2 analogue for model state $AIA^{(1)}$. *Solid line* time course of ibuprofen plasma concentration. *Dashed line* time course of Rf after ibuprofen dosing. *Dash-dot line* time course of PGE_2 analogue plasma concentration after successive dosing. *Dash-dot-dot line* time course of Rf after ibuprofen followed by successive PGE_2 analogue dosing

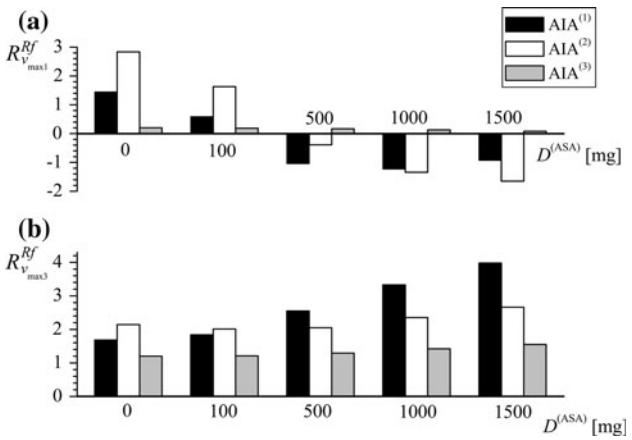


Fig. 4 Response coefficients: **a** $R^{Rf}_{v_{max\,1}}$ and **b** $R^{Rf}_{v_{max\,3}}$ for different aspirin doses

susceptible to aspirin in terms of bronchoconstriction. Thus, decreased expression of PGHS1 and increased expression of LTC_4S could be considered as most important elements responsible for bronchoconstrictive reactions of AIA patients to aspirin. On the other hand, all three populations could be considered as susceptible to ibuprofen. This is in accordance with general observations and reports from clinical practice: ibuprofen is a drug which was most often confirmed as being the cause of NSAID intolerance [18]. Very high predicted value of $D^{(ASA)}_{limit}$ in model state $AIA^{(3)}$ might indicate that decreased expression of PGHS2 in AIA patients may not induce severe bronchoconstriction in case of therapeutically reasonable doses of aspirin. However, it might induce other symptoms related to AIA, since basal LTC_4 levels in this state are several fold higher compared to $AIA^{(1)}$ and $AIA^{(2)}$ (explicitly shown in our previous study [3]).

In in vitro tests it was evidenced that the effect of exogenous PGE_2 is a reduction of leukotriene production in the inflammatory cells from mucosa of human bronchial biopsies [20]. On the other hand, it was shown in in vivo experiments on humans that inhaled PGE_2 prevents aspirin-induced bronchoconstriction and strongly reduces urinary leukotrienes excretion in AIA patients [22].

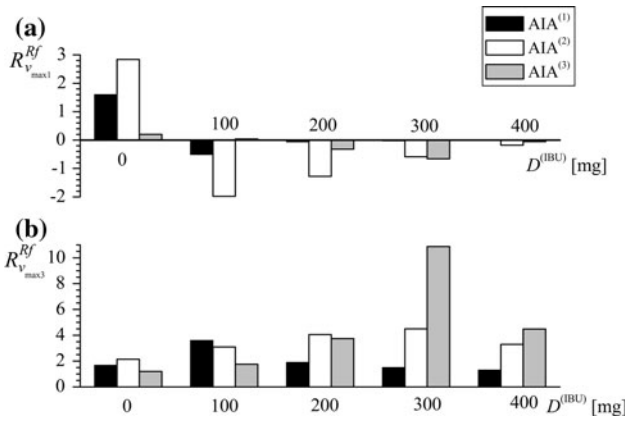


Fig. 5 Response coefficients: **a** $R^{\mathrm{Rf}}_{v_{\max 1}}$ and **b** $R^{\mathrm{Rf}}_{v_{\max 3}}$ for different ibuprofen doses

It was also shown that exogenous PGE_2 results in attenuation of the physiologic early and late asthmatic responses. Its main role, either by exogenous administration or by increase in endogenous production, is the protective anti-inflammatory management of allergic asthma in the early phase of asthmatic response [8]. The protective effects of exogenous PGE_2 were studied and confirmed in asthma, induced by NSAIDs [12, 22], allergens [4, 8] or exercise [15]. In all these cases, PGE_2 was administered by inhalation in form of aerosol. Similarly, misoprostol, the oral-stable analogue of PGE_1, also attenuates bronchoconstriction precipitated by aspirin, although its potency is weaker than that of inhaled PGE_2 [28]. Here we test and evaluate the strategy, suggested by Ref. [21], according to which NSAID would be combined with one or several doses of oral-stable PGE_2 analogue–nocloprost.

The results, presented in Table 3, represent the exact mathematical solution according to the procedure of PGE_2 analogue dosing, described in Sect. 2.3. In practice, the protocols would probably be simpler. Simulations for aspirin and PGE_2 analogue for $AIA^{(1)}$ show that the strategy of NSAID administration, followed by PGE_2 analogue, would be even more effective, if both drugs were taken together. In this case, a lower dose of PGE_2 analogue ($D^{(\mathrm{PGE_2})} = 15$ mg) would be needed to return Rf back to its basal value and to avoid the value 1. This is due to the fact that aspirin and PGE_2 analogue have similar pharmacokinetic properties, and the effect of both drugs on AA metabolism will occur approximately at the same time. General simplified protocol with ibuprofen would be to take ibuprofen and the first dose of PGE_2 analogue together and then every necessary consecutive dose of PGE_2 analogue every 2 h. Doses of PGE_2 for simplified protocols with aspirin and ibuprofen as described above are summarized in Table 4.

Significant differences in methodologies occur for the combination of ibuprofen and PGE_2 analogue, since pharmacokinetics of ibuprofen is much slower than that of PGE_2 analogue. In all AIA states, several consecutive PGE_2 analogue doses are needed to maintain the value of Rf above one after 200 and 400 mg dose of ibuprofen. The number and the level of consecutive PGE_2 analogue doses increase with the increasing ibuprofen dose (see Tables 3, 4). This could be explained with two facts. First, a higher dose of ibuprofen will decrease the value of Rf to a larger extent and thus higher PGE_2 analogue doses are needed to increase Rf back to its basal value. Second, the higher dose of ibuprofen holds the value of Rf below one for a longer period of time. This is visible from our model results, presented in the second column of Table 3, which show that the value $\Delta t_{\mathrm{Rf}<1}$ is higher for the higher ibuprofen dose. If we consider that bronchoconstriction may occur when $\mathrm{Rf} < 1$, the entire time interval $\Delta t_{\mathrm{Rf}<1}$ could be taken as a theoretical estimate for duration of bronchospastic reactions after NSAID exposure. The Ref. [23] reports that the mean clinically observed duration of NSAID-induced bronchospastic reaction in AIA patients is approximately 9.2 h. Our model predictions for 200 and 400 mg dose of ibuprofen in $AIA^{(1)}$ and $AIA^{(2)}$ are within the same range. In cases of 1,000 mg dose of aspirin in $AIA^{(1)}$, as well as of 200 and 400 mg doses of ibuprofen in $AIA^{(3)}$, theoretically predicted duration of bronchospastic reactions is underestimated in comparison to the experimentally observed one.

Generally, the response coefficients, presented in Figs. 4 and 5, do not exceed the value 5. An exception is $R^{\mathrm{Rf}}_{v_{\max 3}}$ in model state $AIA^{(3)}$ for 300 mg dose of ibuprofen, which is higher (see Fig. 5b). The value 5 means that 1% increase in $v_{\max 1}$, $v_{\max 3}$ and $v_{\max 6}$ results in 5% increase of the stationary Rf value. The negative sign of the response coefficient means a decrease in Rf. General conclusions, derived from Figs. 4 and 5 are:

(i) In all cases investigated, model state $AIA^{(2)}$ is the most sensitive and $AIA^{(3)}$ the least sensitive to parameters $v_{\max 1}$ and $v_{\max 3}$ in the absence of NSAID (see Figs. 4a, b and 5a, b for $D^{(\mathrm{ASA})} = D^{(\mathrm{IBU})} = 0$).

(ii) For aspirin doses above 500 mg, the value of $R^{\mathrm{Rf}}_{v_{\max 1}}$ changes its sign in the model states $AIA^{(1)}$ and $AIA^{(2)}$ (see Fig. 4a). In $AIA^{(3)}$, $R^{\mathrm{Rf}}_{v_{\max 1}}$ is positive, close to zero and almost independent of aspirin dose. For ibuprofen doses above 200 mg, $R^{\mathrm{Rf}}_{v_{\max 1}}$ changes its sign in all three AIA states (see Fig. 5a).

(iii) The value of $R^{\mathrm{Rf}}_{v_{\max 3}}$ in $AIA^{(1)}$ increases with aspirin dose. The values of $R^{\mathrm{Rf}}_{v_{\max 3}}$ in $AIA^{(2)}$ and $AIA^{(3)}$ are around 2 and 1, respectively, and almost dose independent (see Fig. 4b).

Table 4 Simplified protocols of PGE_2 dosing in combination with either 1,000 mg of aspirin, 200 or 400 mg of ibuprofen in all AIA model states

Model state	$t_1 = 0$ $D_1^{(PGE_2)}$ (mg)	$t_2 = 2$ h $D_2^{(PGE_2)}$ (mg)	$t_3 = 4$ h $D_3^{(PGE_2)}$ (mg)	$t_4 = 6$ h $D_4^{(PGE_2)}$ (mg)	$t_5 = 8$ h $D_5^{(PGE_2)}$ (mg)
1,000 mg aspirin					
$AIA^{(1)}$	15	–	–	–	–
200 mg ibuprofen					
$AIA^{(1)}$	130	100	70	70	–
$AIA^{(2)}$	300	210	150	–	–
$AIA^{(3)}$	30	–	–	–	–
400 mg ibuprofen					
$AIA^{(1)}$	160	120	100	70	60
$AIA^{(2)}$	420	300	210	150	–
$AIA^{(3)}$	160	70	–	–	–

(iv) The value of $R^{Rf}_{v_{max\,3}}$ in all AIA model states depends on the dose of ibuprofen (see Fig. 5b).

These results show that in most cases investigated, small inter-individual differences among the patients within the same AIA population, which are considered by small changes in parameter values v_{max1}, v_{max3} and v_{max6}, do not strongly affect the stationary values of Rf in particular model state. The model is thus robust enough to maintain its typical behaviour in different AIA model states under small perturbances in v_{max1}, v_{max3} and v_{max6} in the presence or absence of NSAID. According to Ref. [11], this is a desirable feature of the models, describing biological systems. Furthermore, the model also gives stationary solutions for parameter values v_{max1}, v_{max3} and v_{max6}, as well as for NSAID doses within several ranges of magnitude.

The model shows a relatively high sensitivity to parameter value v_{max3} in model state $AIA^{(3)}$ for 300 mg dose of ibuprofen. This means that for ibuprofen doses close to 300 mg, small perturbances in v_{max3} value could lead to significantly different results for $D^{(PGE_2)}$ as presented in Tables 3 and 4. For this reason we additionally investigated the impact of 10% perturbances in v_{max1}, v_{max3} and v_{max6} on $D^{(PGE_2)}$ for 200 mg dose of ibuprofen in the model state $AIA^{(3)}$. If the model state $AIA^{(3)}$ is defined by parameter values $v_{max1} = 0.11\ \mu M\ s^{-1}$, $v_{max3} = 0.096\ \mu M\ s^{-1}$ and $v_{max6} = 0.23\ \mu M\ s^{-1}$, as described in Sect. 2.2, $D^{(PGE_2)} = 15$ mg would be sufficient to maintain Rf above one (see Table 3 for 200 mg dose of ibuprofen). 10% decrease in v_{max3} increases the value of $D^{(PGE_2)}$ to 33 mg. In contrast, 10% increase in v_{max6} and v_{max1} does not change $D^{(PGE_2)}$ significantly. On the other hand, 10% increase in v_{max3}, viewed as an inter-individual variation between AIA patients within $AIA^{(3)}$ population, may lead to 100% increase in $D^{(PGE_2)}$ within 200 to 400 mg dose of ibuprofen. Thus, the strategy for PGE_2 dosing has to be particularly carefully chosen and personalized. In theory, any combination of PGHS1, PGHS2 and LTC_4S expressions could be applied to the model. However, a more detailed selection of AIA patients according to their symptoms, systematically determined relation factors, and expressions of the enzymes is required. Finally, this would enable improvements of the model and would meet the standards of its applicability in the clinical practice.

Presently, AIA is investigated on three different levels, which could be used for distinguishing AIA and ATA patients. These are:

Level 1—gene and molecular level: Analysis of gene expression for the key enzymes PGHS 1 and 2 and LTC_4S [2, 16, 17, 27].

Level 2—cellular level: Measurements of LTC_4 and PGE_2 and determining the relation factor—Rf. There is a risk of bronchoconstriction when Rf falls below one [21].

Level 3—tissue, organ or organism level: Observations of the symptoms of AIA. Typical procedure for distinguishing AIA from ATA is the measurement of FEV_1 after NSAID ingestion or inhalation and, the determination of a threshold dose [19, 23]. However, all AIA patients do not undergo the bronchoconstrictive reaction after NSAID dosing and thus, the observations of skin reactions, quick- and long-term nasal responses to NSAIDs are necessary.

The authors who measured gene expressions and those who measured Rf all selected patients according to different symptoms of AIA (usually bronchoconstriction and/or nasal polyps). In this sense they combined the criteria of either level 1 or 2 with level 3. None of them have tried to combine all three levels of criteria. The aim of the model is to combine levels 1 and 2 and to show that there exist significant differences between different AIA populations, which could also explain different symptoms of AIA, different threshold doses and different susceptibilities to NSAIDs of level 3. We propose to interlace all three levels of investigations with the mathematical modelling, which will finally provide much better understanding of the causal links between the symptoms of AIA, threshold doses, as well as the differences in susceptibilities of individual AIA patients to bronchoconstriction according to different types and doses of NSAIDs. Our model already explains some of them. Furthermore, the value of the model is in providing more insights into the molecular mechanisms leading to bronchoconstriction as well as in testing the existing simulations and creating the new ones for different personalized treatment strategies.

In conclusion, the main innovative part of this study is the individualization of the treatment strategy for safe managing of two different NSAIDs, aspirin and ibuprofen, in combination with the oral-stable PGE_2 analogue, nocloprost, to AIA patients, which are classified into three different populations $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$ according to available experimental data on PGHS1, PGHS2 and LTC_4S expressions.

Acknowledgments The authors acknowledge financial support from the state budget by the Slovenian Research Agency (Program No. P1-0055).

References

1. Bogatcheva N, Sergeeva M, Dudek S, Verin A (2005) Arachidonic acid cascade in endothelial pathobiology. Microvasc Res 69:107–127
2. Cowburn AS, Sladek K, Soja A, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose J, Austen KF, Holgate ST, Sampson AP (1998) Over-expression of leukotriene C_4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest 101:834–846
3. Dobovišek A, Fajmut A, Brumen M (2011) Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study. J Pharmacokinet Pharmacodyn 38:261–278
4. Gauvreau GM, Watson RM, O'Byrne PM (1999) Protective effects of inhaled PGE_2 on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med 159:31–36
5. Gonchar M, Sergeeva M, Mevkh A, Varfolomeyev S (1999) Kinetics of prostanoid synthesis by macrophages is regulated by arachidonic acid sources. Eur J Biochem 265:779–787
6. Gupta N, Gresser M, Ford-Hutchinson A (1998) Kinetic mechanism of glutathione conjugation to leukotriene A_4 by leukotriene C_4 synthase. Biochim Biophys Acta 1391:157–168
7. Harizi H, Juzan M, Moreau J-F, Gualde N (2003) Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B_4 production from dendritic cells via an IL-10-dependent mechanism. J Immunol 170:139–146
8. Hartert TV, Dworski RT, Mellen BG, Oates JA, Murray JJ, Sheller JR (2000) Prostaglandin E_2 decreases allergen-stimulated release of prostaglandin D_2 in airways of subjects with asthma. Am J Respir Crit Care Med 162:637–640
9. Heinrich R, Schuster S (1996) The regulation of cellular systems. Chapman & Hall, New York, pp 138–153
10. Klipp E, Herwig R, Kowald A, Wierling C, Lehrach H (2005) Systems biology in practice. Concepts, implementation and application. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 174–196
11. Marhl M, Gosak M, Perc M, Roux E (2010) Importance of cell variability for calcium signaling in rat airway myocytes. Biophys Chem 148:42–50
12. Mastalerz L, Nizankowska E, Sladek K, Szczeklik A (1994) Protective effects of prostaglandin E_2 on airway obstruction induced by aspirin in aspirin-intolerant asthmatics. Eur Respir J 7:434S
13. Maxis K, Delalandre A, Martel-Pelletier J, Pelletier JP, Duval N, Lajeunesse D (2006) The shunt from the cyclooxygenase to lipoxygenase pathway in human osteoarthritic subchondral osteoblasts is linked with a variable expression of the 5-lipoxygenase-activating protein. Arthritis Res Ther 8:R181
14. Meade E, Smith W, DeWitt D (1993) Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268:6610–6614
15. Melillo E, Woolley KL, Manning PJ, Watson RM, O'Byrne PM (1994) Effect of inhaled PGE_2 on exercise-induced bronchoconstriction in asthmatic subjects. Am J Respir Crit Care Med 149:1138–1141
16. Picado C, Fernandez-Morata JC, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, Mullol J (1999) Cyclooxygenase-2 mRNA is down expressed in nasal polyps from aspirin-sensitive asthmatics. Am J Respir Crit Care Med 160:291–296
17. Pierzchalska M, Szabo Z, Sanak M, Soja J, Szczeklik A (2003) Deficient prostaglandin E_2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin induced asthma. J Allergy Clin Immunol 111:1041–1048
18. Rosado A, Vives R, González R, Poza P, Rodríguez J (2000) Intolerance to non-steroidal anti inflammatory drugs with respiratory reaction: clinical and diagnostic features. Alergol Inmunol Clin 15:153–159
19. Sánchez-Borges M (2010) NSAID hypersensitivity (Respiratory, cutaneous, and generalized anaphylactic symptoms). Med Clin North Am 94:853–864
20. Schäfer D, Lindenthal U, Wagner M, Bölcskei PL, Baenkler HW (1996) Effect of prostaglandin E_2 on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa. Thorax 51:919–923
21. Schäfer D, Schmid M, Göde UC, Baenkler HW (1999) Dynamics of eicosanoids in peripheral blood cells during provocation in aspirin-intolerant asthmatics. Eur Respir J 13:638–646
22. Sestini P, Armetti L, Gambaro G, Pieroni M, Refini RM, Sala A, Vaghi A, Folco GC, Bianco S, Robuschi M (1996) Inhaled PGE_2 prevents aspirin-induced bronchoconstriction and urinary LTE_4 excretion in aspirin-sensitive asthma. Am J Respir Crit Care Med 153:572–575
23. Setipane RA, Schrank PJ, Simon RA, Mathison DA, Christiansen SC, Stevenson DD (1995) Prevalence of cross-sensitivity with acetaminophen in aspirin-sensitive asthmatic subjects. J Allergy Clin Immunol 96:480–485
24. Setoguchi H, Nishimura J, Hirano K, Takahashi S, Kanaide H (2001) Leukotriene C_4 enhances the contraction of porcine tracheal smooth muscle trough the activation of Y-27632, a rho kinase inhibitor, sensitive pathway. Br J Pharmacol 132:111–118
25. So O-Y, Scarafia LE, Mak AY, Callan OH, Swinney DC (1998) The dynamics of prostaglandin H synthases. Study with prostaglandin H-synthase 2Y355F unmask mechanisms of time-dependent inhibition and allosteric activation. J Biol Chem 273: 5801–5807
26. Stevenson D, Szczeklik A (2006) Clinical and pathologic perspectives on aspirin sensitivity and asthma. J Allergy Clin Immunol 118:773–786
27. Szczeklik A, Stevenson D (1999) Aspirin-induced asthma: advances in pathogenesis and management. J Allergy Clin Immunol 104:5–13
28. Szczeklik A, Nizankowska E, Bochenek G, Nagraba K, Mejza F, Swierczynska M (2001) Safety of specific COX-2 inhibitor in aspirin-induced asthma. Clin Exp Allergy 31:219–225
29. Täuber U, Brudny–Klöppel M, Jakobs U, Madetzki C, Mahler M (1993) Pharmacokinetics of nocloprost in human volunteers and its relation to dose. Eur J Clin Pharmacol 44:497–500
30. Vane J, Botting R (1987) Inflammation and the mechanism of action of anti inflammatory drugs. FASEB J 1:89–96
31. Varghese M, Lockey RF (2008) Aspirin-exacerbated asthma. Allergy Asthma Clin Immunol 4:75–83

Priloga 3

Samostojni prispevek v znanstveni monografiji (v tisku)

Fajmut A, Dobovišek A, Brumen M (2012) Mathematical modeling in aspirin-induced asthma: theory and clinical applications, Nova Publisher, New York

Mathematical modeling in aspirin-induced asthma: theory and clinical applications

Aleš Fajmut[1], Andrej Dobovišek[1], Milan Brumen[1, 2]

[1]University of Maribor, Faculty of Natural Sciences and Mathematics, Faculty of Medicine, Faculty of Health Sciences, Slomškov trg 15, SI-2000, Maribor, Slovenia

[2]Institute Jožef Stefan, Jamova 39, SI-1000, Ljubljana, Slovenia

Abstract

It is generally known that leukotrienes and prostaglandins, the products of arachidonic acid (AA) metabolism, play a central role in the pathophysiology of asthma. Cysteinyl leukotrienes (cysLTs) are potent pro-inflammatory mediators that are produced by inflammatory cells and cause bronchoconstriction. Eicosanoid production is strongly dependent on the expressions of the key enzymes in AA metabolic pathway and it is particularly important in the pathophysiological condition called aspirin-induced asthma, in which asthmatic attacks can be induced by ingestion of non-steroidal anti-inflammatory drugs (NSAIDs). It has been recently shown that there exist a strong relationship between the occurrence of aspirin-induced asthma and the expressions of enzymes prostaglandin H synthases 1 and 2 (PGHS1 and PGHS2) as well as leukotriene C_4 synthase (LTC_4S). Several researchers try to distinguish between aspirin -tolerant and -intolerant asthmatics by comparing the expressions of these enzymes. On the other hand, there is a group of researchers who try to explain the occurrence of aspirin-induced asthma via the measurements of cysLTs and anti-inflammatory prostaglandins (aiPGs) and by comparisons of their relative productions after NSAID ingestion. According to their hypothesis, the possibility of asthmatic attack largely increases when the production of cysLTs is higher than that of aiPGs.

The aim of our study is to link both experimental approaches by mathematical modeling and computer simulations of eicosanoids production in inflammatory cells in the presence or absence of different NSAIDs. Our approach is systems biology approach in which the knowledge of enzyme gene expressions, molecular interactions, pharmacokinetics of drugs

and their action on the molecular level as well as implications on the organ level are integrated. The model enables simulations of different NSAIDs, exogenous PGE_2 analogues, as well as other drugs used in treatment of asthma acting on the molecular level in AA metabolic pathway, such as 5-lipoxygenase (5-LOX) inhibitors (5-LOXIBs) and selective PGHS2 inhibitors (coxib-s). The impacts of different enzyme expressions on the production of cysLTs and aiPGs in healthy humans as well as in aspirin -tolerant and -intolerant asthmatics are studied. The results of our model identify alterations in expressions of enzymes PGHS1 and LTC_4S as the key elements in explaining bronchoconstriction, whereas alterations in expression of the enzyme PGHS2 could be linked to other symptoms of aspirin-induced asthma. The model is further used for identification of susceptible patient populations for different NSAIDs and for identification of threshold doses that induce bronchoconstriction. The model predicts the time between NSAID ingestion and bronchoconstriction as well as the duration of symptoms for different NSAID doses. A computer simulation of a strategy for safe NSAIDs administration to aspirin-intolerant asthmatic patients is also proposed and evaluated. Finally, a link between our two cell-type mathematical models, i.e. of eicosanoids production in inflammatory cells and Ca^{2+}-dependent force development in airway smooth muscle cells, will be shortly presented and the missing parts in the present knowledge between these two signaling pathways will be discussed.

Introduction

Asthma is an inflammatory condition of the airways characterized by airway smooth muscle hyperreactivity and hypersensitivity, excessive secretion of mucus, and multicellular inflammation involving activated mast cells, eosinophils, neutrophils, macrophages, basophils, and lymphocytes [1]. In a subset of asthmatic patients, exposure to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) temporarily accentuate the inflammatory process, leading to asthma exacerbations [1]. This distinct clinical syndrome that affects around 10-20 % of asthmatic patients is usually called aspirin-induced asthma. Many other terms have been used to describe this respiratory disease: aspirin-sensitive asthma, aspirin hypersensitivity, aspirin idiosyncrasy, aspirin intolerance and aspirin-exacerbated respiratory disease (AERD) [2]. All terms refer to the same disease and the same patients who are afflicted with instantaneous inflammation of both - upper and lower - respiratory tracts accompanied by bronchoconstriction, rhino-sinusitis, swollen eyes, urticaria as well as flushing head and neck after ingestion of typical NSAID doses. The symptoms usually occur 30-60 min after NSAID ingestion [2] and can last up to 9 hours [3].

The process of distinguishing between aspirin-intolerant (AIA) and aspirin-tolerant (ATA) asthmatic patients is performed with provocation tests, in which patients are challenged with different doses of NSAIDs by either ingestion or inhalation. In order to follow the level of bronchoconstriction, FEV_1 is measured. Finally, threshold doses, above which patients react with bronchoconstriction, are determined for each particular NSAID [4]. However, all AIA patients do not undergo bronchoconstrictive reaction after NSAID dosing and thus, the observations of skin reactions, quick- and long- term nasal responses to NSAIDs are necessary. When AIA patients undergo challenges with NSAIDs, very substantial increases in urinary LTE_4 levels and decreases in PGE_2 are recorded during bronchospastic reactions [5] or nasal responses [6]. Increases in LTC_4 and histamine levels in both nasal [7] and bronchial [8] fluids after oral aspirin challenges have also been detected in AIA.

Long term follow-up of AIA patients are nasal polyps, which may occur even ten years after first short-term reactions to NSAIDs [9]. In 1922, the first article appeared, describing the association of aspirin sensitivity, asthma, and nasal polyposis. In the late 1960s, this clinical entity was named "aspirin triad" [10]. In the last years, chronic hyperplastic eosinophilic

sinusitis (CHES) became known as the fourth remarkable symptom of aspirin induced asthma [11]. Aspirin-induced asthma is a major medical problem but, despite decades of research, the exact mechanisms that underlie this condition remain unclear. An unsolved question in the research of aspirin-induced asthma still remains, why ingestion of NSAIDs does not induce bronchoconstrictive reactions and other symptoms in all humans, or at least in all asthmatic patients [11].

Pathogenesis of aspirin-induced asthma

The main role in the occurrence of aspirin-induced asthma is attributed to the increase of the production of cysteinyl leukotrienes (cysLTs) - metabolites of arachidonic acid (AA) that are mainly produced in epithelial cells and in leukocytes, involved in inflammation [2, 11]. Among all leukotrienes (LT), LTC_4 is the most potent mediator of asthma and inflammation. It induces bronchoconstriction and inflammatory cell recruitment in asthmatic patients as well as in healthy humans [12]. It is a product of lipoxygenase pathway of AA degradation, in which AA is first transformed into leukotriene A_4 (LTA_4) by the enzyme 5-lipoxygenase (5-LOX) via short-lived intermediate product 5-hydroperoxyeicosatetraenoic acid (5-HETE) (see Figure 1). Then LTA_4 is further transformed into leukotriene B_4 (LTB_4) by the enzyme LTA_4 hydrolase (LTA_4H) or it is conjugated with glutathione (GSH) into LTC_4 by the enzyme leukotriene C_4 synthase (LTC_4S). In the latter enzymatic reaction, LTA_4 is involved as a substrate and LTC_4 as a product, whereby LTA_4 auto-regulates the velocity of its own degradation [13]. Both leukotrienes LTB_4 and LTC_4 are then exported to extracellular space, whereby LTC_4 is first transformed to LTD_4 and later to LTE_4. The latter three represent a group of cysteinyl leukotrienes (cysLT). LTE_4 is usually found and measured in urine [6, 14]. All cysLTs can bind to two types of G-protein coupled transmembrane receptors, $cysLT_1R$ and $cysLT_2R$, of bronchial smooth muscles, and induce contraction. With respect to other inhaled contractile agonists, LTC_4 and LTD_4 are approximately 3,000-times and LTE_4 approximately 30-times more potent than histamine as bronchoconstrictor agonists in in-vivo healthy humans [15]. Maximal bronchoconstrictive response to LTD_4 is even greater than that to methacholine [15].

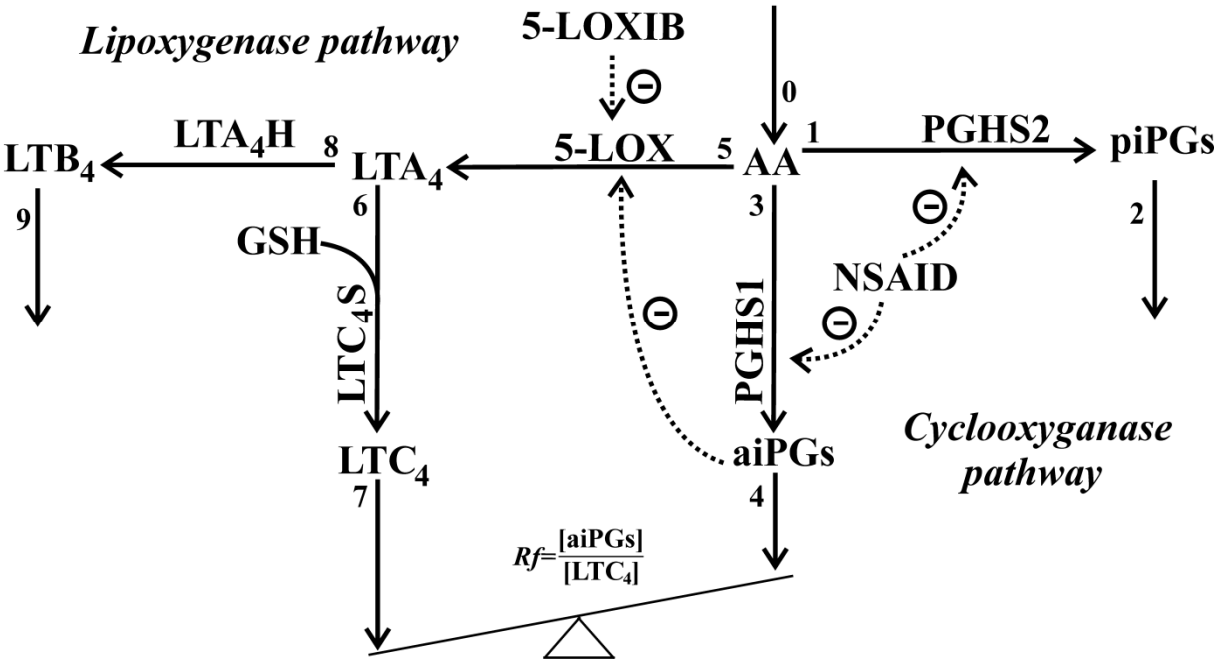


Figure 1 General scheme of eicosanoid production pathways of arachidonic acid (AA) metabolism considered for mathematical modeling. Similar kinetic scheme was considered in our previous publications [16, 17]. Action of NSAID and 5-LOX inhibitors (5-LOXIB) is indicated. The balance between aiPGs and LTC_4 production is schematically presented. Numeration of the fluxes is in accordance with the reaction velocities of the mathematical model.

In the second pathway of AA degradation, cyclooxygenase pathway (see Figure 1), AA is transformed into anti- (aiPGs) and pro-(piPGs) inflammatory prostaglandins by the enzymes prostaglandin H synthase 1 (PGHS1) and prostaglandin H synthase 2 (PGHS2), often referred to as cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) [18]. PGE_2 is the most important representative of aiPGs. In many cells, including endothelial and epithelial cells of the respiratory tract, it has profound regulatory effects: (1) it reduces LT biosynthesis through inhibition of 5-LOX [14, 19, 20]; (2) it prevents discharge of granular mediators from lung mast cells [21]; and (3) it prevents bronchoconstriction [14, 22]. Underproduction of PGE_2 strongly diminishes the inhibitory effects of PGE_2 on 5-LOX, thus, enhances cysLT production, and reduces the inhibitory effect of PGE_2 on mast cells, releasing histamine and tryptase [21, 23, 24]. In this way, decreased levels of PGE_2 might induce bronchoconstriction.

The balance between synthesis of prostaglandins and leukotrienes (see Figure 1) mainly depends on the production of PGE_2 by the inhibition, which PGE_2 exerts on 5-LOX [14, 19,

20]. The target of inhibitory action of PGE_2 is 5-LOX activating protein (FLAP); however, the entire mechanism of this inhibition is not completely revealed [2, 20, 25]. The majority of NSAIDs inhibit both enzymes of cyclooxygenase pathway, PGHS1 and PGHS2 [26, 27], reduce the total prostaglandin production and, thus, also lower the synthesis of PGE_2 [28]. The decrease in PGE_2 synthesis reduces the inhibitory effect on5-LOX, which leads to enhanced synthesis of cysLTs [2, 20, 28]. This seems to be the critical event that tilts AA metabolism in favor of the lipoxygenase pathway and leads to overproduction of LTC_4. However, this mechanism does not explain the different effects of NSAIDs on AIA patients from the ones that are present in ATA patients or healthy humans (HH).

Aspirin, indomethacin and ibuprofen, for example, inhibit both enzymes but are much more potent inhibitors of PGHS1 than PGHS2 [11]. From clinical praxis it is known that these NSAIDs precipitate asthmatic attacks in AIA patients already at low doses [11]. Aspirin (80%) and ibuprofen (41%) were the most commonly reported NSAIDs to elicit prior respiratory reactions [29], and ibuprofen is a drug which was most often confirmed as being the cause of NSAID intolerance [30]. Nimesulide and meloxicam, drugs known to inhibit PGHS2 substantially more than PGHS1 (they are 5-50 -times more selective for PGHS2 than PGHS1), are well tolerated by AIA when given average therapeutic doses, but cause rhinorrhea and mild asthma attacks when ingested in higher doses (e.g. 15 mg of meloxicam) [31, 32]. More recently, several highly selective inhibitors of PGHS2, such as celecoxib, rofecoxib, etoricoxib, parecoxib, lumiracoxib, valedcoxib, were introduced into clinical practice [11]. The idea behind these selective inhibitors is that they inhibit PGHS1 much less than PGHS2 and, in this way, do not reduce inhibitory action of PGE_2 on 5-LOX and, consequently, do not increase LTC_4 levels. As such they should theoretically be safe for AIA patients. In the large majority of studies with these selective drugs, at doses even higher than therapeutic, none of AIA patients reacted to them [33-38]. Although few studies report respiratory reactions to selective PGHS2 inhibitors [39], these drugs still carry the warning label "aspirin triad" as a contraindication and only celecoxib is available in the United States market [2]. Later in this chapter, we will present theoretically predicted limiting doses of celecoxib that represent the limit, above which this drug might potentially induce bronchoconstriction in different AIA patient populations.

A special role for PGE_2 has been attributed to the pathogenesis of AIA [40]. Peripheral blood macrophage cells of some patients with AIA synthesize less PGE_2 at baseline than normal

[41]. This disadvantage of lacking sufficient concentrations of cellular or transcellular PGE_2 leads to overproduction of LTs. There is clear evidence that many, but not all, AIA patients synthesize excessive amounts of cysLTs, before and after exposure to NSAIDs [5, 42]. It is believed that aspirin-induced asthma is largely attributed to LTC_4 overproduction. Limitation of cysLT production is possible by inhibitors of 5-LOX, such as zileuton and ABT-761 [43, 44]. Efficacy of zileuton, most known inhibitor of 5-LOX, has been demonstrated in clinical study [42] and zileuton is in addition to $cysLT_1R$ antagonists ($cysLT_1RA$) (e.g. montelukast, zafirlukast, pranlukast) most frequently used in preventing bronchospastic reactions during oral aspirin challenges [2]. As an example, we will present theoretically predicted strategy for potentially safe administration of ABT-761 and ibuprofen together to different AIA patient populations and the differences that might occur between these populations. Such strategies are common when NSAIDs should be administered to AIA patients because of their other health problems, such as chronic headaches or rheumatic arthritis, for example [45].

In *in-vitro* tests it was evidenced that the effect of exogenous PGE_2 is a reduction of cysLT production in the inflammatory cells from mucosa of human bronchial biopsies [46]. It was also shown that exogenous PGE_2 results in attenuation of the physiologic early and late asthmatic responses. Its main role, either by exogenous administration or by increase in endogenous production, is the protective anti-inflammatory management of allergic asthma in the early phase of asthmatic response [47]. The protective effects of exogenous PGE_2 were studied and confirmed in humans with asthma, induced by NSAIDs [14], allergens [47, 48] or exercise [49]. In all these cases, PGE_2 was administered by inhalation in form of aerosol. Similarly, misoprostol, the oral-stable analogue of PGE_1, also attenuated bronchoconstriction precipitated by aspirin, although its potency was weaker than that of inhaled PGE_2 [36]. The use of nocloprost, an oral-stable form of PGE_2, which is mainly used as a protective prostaglandin in treatment of NSAID-induced gastric ulcers [50], was also proposed for the treatment of AIA [41]. In our recent study [17], we proposed a strategy on the basis of our mathematical model for safe administration of oral stable PGE_2 analogue – nocloprost in combination with either aspirin or ibuprofen to different AIA patient populations. In this chapter, we will summarize our findings and compare this strategy with the one, carried out with ABT-761.

PGE_2 is mainly produced by the enzyme PGHS1 in the cyclooxygenase pathway of AA degradation [2]. Thus, its production rate strongly depends on the enzyme availability.

PGHS1 is a constitutively expressed enzyme that is present in most mammalian cells, including respiratory epithelial cells, as well as inflammatory cells. PGHS2 is expressed mainly in respiratory epithelium and in inflammatory cells and it is an inducible enzyme that is highly upregulated by pro-inflammatory mediators [2]. In bronchi of patients with stable asthma and chronic bronchitis, these two enzymes are not quantitatively up- or down-regulated, but there is evidence that expression of PGHS2 is diminished and its activity is reduced in AIA patients [51, 52]. Reference [51] reports 6-fold lower expression of PGHS2 in nasal polyps and nasal mucosa of AIA patients compared to ATA. Reference [53] reports 3-fold lower expression of PGHS1 in bronchial fibroblasts in AIA patients, compared to those in ATA. Other authors did not observe any significant differences in overall expressions of PGHS1 and PGHS2 in bronchial biopsy specimens, obtained from ATA and AIA patients [54, 55]. Many, but not all, AIA patients demonstrate overexpression of LTC_4S in eosinophils and mast cells in their bronchial biopsy specimens, and in circulating eosinophils [54, 56, 57]. References [11, 54] report 5-fold higher expression of LTC_4S in AIA compared to ATA patients as well as 20-fold higher expression in AIA compared to healthy humans (HH) in bronchial biopsy specimens. These differences between AIA and ATA patients and HH in the expressions of the key enzymes of AA degradation are the basis for the definition of our different model populations. In our recently published work [16], we defined five different model populations. Three of them represent three different aspirin-intolerant asthmatic patient populations ($AIA^{(1)}$, $AIA^{(2)}$, $AIA^{(3)}$), one determines aspirin-tolerant asthmatics (ATA) and another one determines healthy humans (HH).

Common features that are emerging throughout most experimental studies, supposedly grouping AIA patients into subgroups, are that AIA patients produce more LTC_4 and less PGE_2 than ATA and HH. Moreover, they have upregulated expressions of $cysLT_1R$ [58] and LTC_4S [11, 54, 56, 57] and downregulated expressions of E-series prostanoid 2 (EP2) and 3 (EP3) receptors [59], as well as of PGHS1 and PGHS2 [51-53, 55] in their inflammatory cells. However, clear separation between AIA and ATA patient groups has not been accomplished in experimental procedures because of the considerable overlapping between the groups in their inflammatory profiles [5]. To make matters more difficult, different AIA patients present distinct symptoms of AIA and their clinical presentation of the disease can be mild, moderate, or severe [5]. Despite that, a pattern is emerging in which many defects in either overstimulation of inflammation or underproduction of anti-inflammatory mediators as well as overproduction of pro-inflammatory mediators and bronchoconstrictors, especially in

the eicosanoid family, is found in AIA patients [2]. One of such patterns occurred in the study [41], of which the long term goal was to check the possibility of a simple and cheap in-vitro whole blood test for routine diagnosis of aspirin intolerance. Authors of that study observed that selected AIA patients, who had bronchoconstrictive reactions after aspirin inhalation, produced higher amounts of LTC_4 than PGE_2 after NSAID exposure. They introduced the concept of the relation factor (*Rf*), defined as the ratio between PGE_2 and LTC_4 concentrations in peripheral blood cells [41]. *Rf* is for AIA patients with bronchoconstrictive reactions lower than one, whereas for ATA patients as well as healthy humans (HH) it is always more than one [41]. In our model simulations, we use relation factor as a quantitative criterion in predicting bronchoconstriction, limiting drug doses and the strategies for safe drug administration. We defined it as the ratio between the concentrations of aiPGs and LTC_4 [16].

In spite of accumulating and promising experimental and clinical results, as shortly introduced in this chapter above, molecular and cellular mechanism of inducing and developing processes in aspirin induced asthma, pushing the asthmatics to the risk and likely to bronchospastic reactions, are still not completely revealed and understood. It is obvious that experimental and clinical research is running on different molecular, cellular and organ levels as well as on the multiscale level. Our work presented in this chapter is a theoretical contribution to these investigations. A theoretical analysis and predictions attained from mathematical modeling can combine the different levels of investigation and serve for formulation of the framework of experimental observations. In short, the gene and molecular level yields analysis of gene expression for the key enzymes involved in AA metabolism and the search for their genetic polymorphisms as well as the search for overexpression of cysLTR and underexpression of EP2 and EP3 receptors; the cellular-tissue level covers measurements of the inflammatory profiles in terms of eicosanoid production in particular cells and tissues before and after NSAID exposure; and the tissue, organ or organism level is the level of observations of the symptoms of AIA and bronchospastic reactions.

Mathematical model and model populations

The kinetic scheme of AA metabolism and its interaction with NSAID and 5-LOX inhibitor is given in Fig. 1, which was also considered for mathematical modeling in our previous publications [16, 17]. The basic entity of the corresponding mathematical model is a set of six first-order ordinary differential equations, describing the time evolution of system variables,

which are concentrations of metabolites as follows: [AA], [$piPGs$], [$aiPGs$], [LTA_4], [LTB_4] and [LTC_4], i= 1,….,6, respectively :

$$\frac{d[S_i]}{dt} = \sum_{\text{incoming}} v_k - \sum_{\text{outgoing}} v_l \tag{1}$$

The time evolution of a selected system variable [S_i] is taken into account as the difference between the sum of all incoming and the sum of all outgoing metabolic fluxes, both with respect to numeration in the kinetic scheme in Fig. 1. v_k and v_l are also known as reaction velocities.

Reaction velocity v_0 represents the cell influx of AA and its value is taken to be constant in all simulations, v_0=0.7 μMs^{-1}. Reaction velocity v_1 describes the enzyme PGHS2 with the steady-state Michaelis-Menten expression, which takes into account interactions of the enzyme with substrate AA and inhibitor NSAID [27, 60]:

$$v_1 = \frac{v_{\max 1}[AA]}{K_1\left(1+\frac{[NSAID]}{K_{I1}^{(j)}}\right)+[AA]}\ , \tag{2}$$

where $v_{\max 1}$ is the maximal velocity of the reaction, K_1 is the Michaelis-Menten constant and K_{I1} is the equilibrium dissociation constant of inhibitory action of NSAID on PGHS2. Superscript (j) denotes the type of the drug, which is one of NSAIDs (either aspirin, j=ASA, ibuprofen, j=IBU or celecoxib, j=CEL). Reaction velocity v_2, i.e. the piPGs efflux, is described as [61]:

$$v_2 = k_2[piPGs], \tag{3}$$

where k_2 is the reaction rate constant. Reaction velocity v_3 of the enzyme PGHS1 is described as [27, 60]:

$$v_3 = \frac{v_{\max 3}[AA]}{K_3\left(1+\frac{[NSAID]}{K_{I3}^{(j)}}\right)+[AA]}, \tag{4}$$

where $v_{\max 3}$ is the maximal velocity of the reaction and K_3 is the Michaelis-Menten constant. K_{I3} is the equilibrium dissociation constant of inhibitory action of NSAID on PGHS1. Superscript (j) denotes the type of the drug, which is one of NSAIDs (either aspirin, j=ASA, ibuprofen, j=IBU or celecoxib, j=CEL). Reaction velocity v_4 is considered as [61]:

$$v_4 = k_4[aiPGs], \tag{5}$$

where k_4 is the rate constant for aiPGs efflux from the cell. Reaction velocity v_5 describes degradation of AA by the enzyme 5-LOX in terms of Michaelis-Menten kinetics, whereby inhibitory actions of aiPGs and 5-LOX inhibitors (5-LOXIB) on 5-LOX are considered [62]:

$$v_5 = \frac{v_{\max 5}[AA]}{K_5(1+\frac{[aiPGs_{TOT}]}{K_{I5}}+\frac{[5-LOXIB]}{K_{I5}^{(j)}})+[AA]}, \quad (6)$$

where v_{max5} is the maximal velocity of the reaction, K_5 is the Michaelis-Menten constant and K_{I5}, $K_{I5}^{(j)}$ are the equilibrium constants of inhibitory action of total aiPGs (both, native and administered) as well as of other 5-LOX inhibitors, acting in parallel with aiPGs, on 5-LOX, respectively. Superscript (j) denotes the type of 5-LOXIB, which is ABT-761 (j=*ABT*) in one of the simulations. Reaction velocity v_6 describes the mechanism of LTC_4 production, in which LTA_4 and glutathione (GSH) are conjugated with LTC_4S:

$$v_6 = \frac{v_{\max 6}[LTA_4]}{A+B[LTA_4]+C[LTA_4]^2}, \quad (7)$$

A, B and C are the parameters and $v_{\max 6}$ is the maximal reaction velocity. Reaction velocity v_7 describes the efflux of LTC_4 from the cell [63]

$$v_7 = k_7[LTC_4], \quad (8)$$

where $k_7$is the reaction rate constant. Reaction velocity v_8 describes conversion of LTA_4 into LTB_4 by the enzyme LTA_4H as [64]:

$$v_8 = \frac{v_{\max 8}[LTA_4]}{K_8+[LTA_4]}, \quad (9)$$

and reaction velocity v_9 describes efflux of LTB_4 from cells by [65]:

$$v_9 = \frac{v_{\max 9}[LTB_4]}{K_9+[LTB_4]}. \quad (10)$$

In both above equations the meaning of parameters is the same as above.

Time courses of drug concentrations in the plasma are described by the standard pharmacokinetic model that takes into account absorption and elimination phases of the drug:

$$[DRUG] = \frac{D^{(j)}k_a^{(j)}}{(V/F)^{(j)}(k_a^{(j)}-k_e^{(j)})}\left(e^{-k_e^{(j)}t}-e^{-k_a^{(j)}t}\right), \quad (11)$$

where $D^{(j)}$ is the drug dose, $(V/F)^{(j)}$ is the ratio between apparent volume of drug distribution and fraction of the drug absorbed, $k_a^{(j)}$ and $k_e^{(j)}$ are the first order absorption and elimination rate constants, respectively. [*DRUG*] is [*NSAID*] and/or [*5-LOXIB*]. Superscript (j) denotes

the type of the drug, which is one of NSAIDs (either aspirin, $j=ASA$, ibuprofen, $j=IBU$ or celecoxib, $j=CEL$) and/or 5-LOXIBs (ABT-761, $j=ABT$).

Total aiPGs concentration [$aiPGs_{\mathrm{TOT}}$] is given by the equation:

$$[aiPGs_{\mathrm{TOT}}]=[aiPGs]+\frac{D^{(PGE)}k_a^{(PGE)}}{(V/F)^{(PGE)}(k_a^{(PGE)}-k_e^{(PGE)})}(e^{-k_e{}^{(PGE)}t}-e^{-k_a{}^{(PGE)}t}). \quad (12)$$

The first term in the above equation considers aiPGs produced in the cell and the second term describes the level of exogenous PGE_2 analogue, administered orally to the patient, by the absorption and elimination phases. We consider the inhibitory effect of endogenous and exogenous PGE_2 on 5-LOX to be the same since there is strong evidence that PGE_2 exerts the inhibition in an autocrine manner [20, 25, 47].

Relation factor (*Rf*) is according to [41] defined as:

$$Rf=\frac{[aiPGs_{\mathrm{TOT}}]}{[LTC_4]}, \quad (13)$$

and is a measure of critical conditions for the risk of bronchoconstriction in AIA patients. In accordance with the experimental findings [41], bronchoconstriction occurs when $Rf<1$. Descriptions of the model parameters and their values, used in our previous [16, 17] and present model simulations are given in Tables 1 and 2. Model parameters $v_{\max 1}$, $v_{\max 3}$ and $v_{\max 6}$ are defined and described later in this chapter.

Table 1 Kinetic parameters used in model simulations.

Parameter	Value and unit	Description	References
v_0	0.7 μMs^{-1}	AA influx	[16, 17]
K_1	2.5 μM	Michaelis-Menten constant	[66]
$K_{I1}^{(ASA)}$	85 μM	equilibrium dissociation constant of inhibitory action of aspirin on PGHS2	[67]
$K_{I1}^{(IBU)}$	60 μM	equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS2	[67]
$K_{I1}^{(CEL)}$	0.07 μM	equilibrium dissociation constant of inhibitory action of celecoxib on PGHS2	[68]
k_2	0.0028 s^{-1}	rate constant of piPGs efflux	[61]
K_3	3.0 μM	Michaelis-Menten constant	[66]
$K_{I3}^{(ASA)}$	4.0 μM	equilibrium dissociation constant of inhibitory action of aspirin on PGHS1	[67]
$K_{I3}^{(IBU)}$	1.3 μM	equilibrium dissociation constant of inhibitory action of ibuprofen on PGHS1	[67]
$K_{I3}^{(CEL)}$	0.2 μM	equilibrium dissociation constant of inhibitory action of celecoxib on PGHS1	[68]
k_4	0.0028 s^{-1}	rate constant of aiPGs efflux	[61]
v_{max5}	86 μMs^{-1}	maximal velocity of reaction	[69]
K_5	25.4 μM	equilibrium dissociation constant	[62]
K_{I5}	0.03 μM	equilibrium dissociation constant of inhibitory action of aiPGs on 5-LOX	[20]
$K_{I5}^{(ABT)}$	0.018 μM	equilibrium dissociation constant of inhibitory action of ABT-761 on 5-LOX	[70]
A	56 μM	constant	[71]
B	1.4	constant	[71]
C	0.17 μM^{-1}	constant	[71]
k_7	0.0015 s^{-1}	rate constant of LTC_4 efflux	[63]
v_{max8}	3.7 μMs^{-1}	maximal velocity of the reaction	[64]
K_8	27 μM	Michaelis-Menten constant	[64]
v_{max9}	5.74 μMs^{-1}	maximal velocity of the reaction	[65]
K_9	239 μM	half-saturation constant	[65]

Table 2 Values of pharmacokinetic parameters for oral application of aspirin, ibuprofen, PGE_2 analogue – nocloprost and ABT-761. Doses of the drugs ($D^{(j)}$) stand in the text.

Parameter	Value and unit	Description	References
ASPIRIN			
$k_e^{(ASA)}$	$5.5 \cdot 10^{-4}$ s^{-1}	elimination rate constant	[72]
$k_a^{(ASA)}$	$23 \cdot 10^{-4}$ s^{-1}	absorption rate constant	[72]
$V/F^{(ASA)}$	74 L	apparent volume of distribution/fraction of aspirin absorbed	[72]
IBUPROFEN			
$k_e^{(IBU)}$	$1.0 \cdot 10^{-4}$ s^{-1}	elimination rate constant	[73]
$k_a^{(IBU)}$	$2.8 \cdot 10^{-4}$ s^{-1}	absorption rate constant	[73]
$V/F^{(IBU)}$	8.4 L	apparent volume of distribution/fraction of ibuprofen absorbed	[73]
CELECOXIB			
$k_e^{(CEL)}$	$4.4 \cdot 10^{-5}$ s^{-1}	elimination rate constant	[74]
$k_a^{(CEL)}$	$2.0 \cdot 10^{-4}$ s^{-1}	absorption rate constant	[74]
$V/F^{(CEL)}$	242 L	apparent volume of distribution/fraction of celecoxib absorbed	[74]
PGE_2 ANALOGUE - NOCLOPROST			
$k_e^{(PGE)}$	$3.3 \cdot 10^{-4}$ s^{-1}	elimination rate constant	[50]
$k_a^{(PGE)}$	$8.1 \cdot 10^{-4}$ s^{-1}	absorption rate constant	[50]
$V/F^{(PGE)}$	13 L	apparent volume of distribution/fraction of nocloprost absorbed	[50]
ABT-761			
$k_e^{(ABT)}$	$1.4 \cdot 10^{-5}$ s^{-1}	elimination rate constant	[75]
$k_a^{(ABT)}$	$3.2 \cdot 10^{-4}$ s^{-1}	absorption rate constant	[75]
$V/F^{(ABT)}$	60L	apparent volume of distribution/fraction of ABT-761 absorbed	[75]

For theoretical analysis we use three basic populations, healthy humans (HH), aspirin-tolerant (ATA) and aspirin-intolerant (AIA) asthmatic patients, whereby we distinguish and postulate three different AIA model populations: $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$. They are characterized according to experimental findings about the differences in expressions of the key enzymes PGHS2, PGHS1 and LTC_4S [11, 51, 53, 54]. Thus in modeling, the parameters v_{max1}, v_{max3} and v_{max6}, reflecting different expressions of the enzymes PGHS2, PGHS1 and LTC_4S, respectively, are taken as free model parameters and their values were adjusted according to the known experimental data. Hence, AIA model populations differ from each other in the

parameter values v_{max1}, v_{max3} and v_{max6}. Direct proportionalities between the expressions of the enzymes and their maximal activities are considered.

Model population ATA represents the reference model population, which was already determined in our previous study [16]. The reference values of model parameters v_{max1}=0.65 μMs^{-1} and v_{max6}= 0.23 μMs^{-1} were obtained from experimental studies [27, 71].The value v_{max3}=0,096 μMs^{-1} was determined specifically for ATA by obtaining the best agreement between the model [16] and the experiment [41]. Since the parameter value v_{max3} was determined specifically for ATA patients and all other parameters were determined non-specifically (the experimental studies do not report any pathologies), the whole set of parameter values, v_{max1}=0.65 μMs^{-1}, v_{max3}=0.096 μM and v_{max6} =0.23 μMs^{-1}, defines the model population ATA [16].

Different model AIA patient populations are defined with respect to ATA as follows [16]:
i) $AIA^{(1)}$: with 3-fold decreased expression of the enzyme PGHS1: v_{max1}=0.65 μM, v_{max3}=0.032 μM and v_{max6} =0.23 μM. The reduced PGHS1 activity [41] and 3-fold lower expression of PGHS1 [53], both in AIA patients with respect to ATA patients were observed.
ii) $AIA^{(2)}$: with 5-fold increased expression of the enzyme LTC_4S: v_{max1}=0.65 μM, v_{max3}=0.096 μM and v_{max6} =1.15 μM. 5-fold higher expression of LTC_4S in AIA compared to ATA patients was reported [11, 54].
iii) $AIA^{(3)}$: with 6-fold decreased expression of the enzyme PGHS2: v_{max1}=0.11 μM, v_{max3}=0.096 μM and v_{max6} =0.23 μM. Reference [51] reports 6-fold lower expression of PGHS2 in AIA compared to ATA patients.
iv) HH: with 4-fold decreased expression of the enzyme LTC_4S: v_{max1}=0.65 μM, v_{max3}=0.096 μM and v_{max6} =1.15 μM. References [11, 54] report 20-fold lower expression in HH compared to $AIA^{(2)}$.

Software used for all calculations was Berkeley Madonna 8.0.1 (R. Macey and G. Oster, University of California at Berkeley). The system of differential equations, evolved from equation (1) by considering all definitions of the fluxes and plasma drug distributions, was numerically integrated from the initial stationary state, which was determined for each model variable in the absence of NSAID for each particular model population. In simulations, we used numerical integration method with variable integration step.

Results and discussion

Clinical signs such as rhinitis, conjunctivitis and bronchoconstriction in AIA usually occur within 30 to 60 min after ingestion of NSAID irrespective of its type [2]. On the other hand, smooth muscle strips contract approximately 15 min after stimulation with LTC_4 [76]. If we add these 15 min to the value of time, at which relation factor *Rf* drops below one after NSAID dosing (typically 20 – 30 min, depending on the type of NSAID, its dose and AIA model population [16]), we get similar time interval (35 – 45 min) as the one observed in clinical tests (30 – 60 min) [2]. Relation factor (*Rf*) was first introduced by Schäfer et al. in 1999 [41]. The authors showed, by simultaneous measurements of FEV_1 and the concentrations of PGE_2 and LTC_4 in peripheral blood cells of AIA patients, that those AIA patients, for which the value of *Rf* is lower than one, might undergo bronchoconstriction with high probability after aspirin dosing [41]. *Rf* is in our model defined as the ratio between concentrations of aiPGs and LTC_4 and is presented in Figure 2 (dash-dot line) along with the time dependencies of aiPGs and LTC_4 concentrations. Simulation is performed for the model population $AIA^{(1)}$, which represents AIA patient population with 3-fold lower expression of PGHS1, and 200 mg dose of ibuprofen applied at time t=0 h.

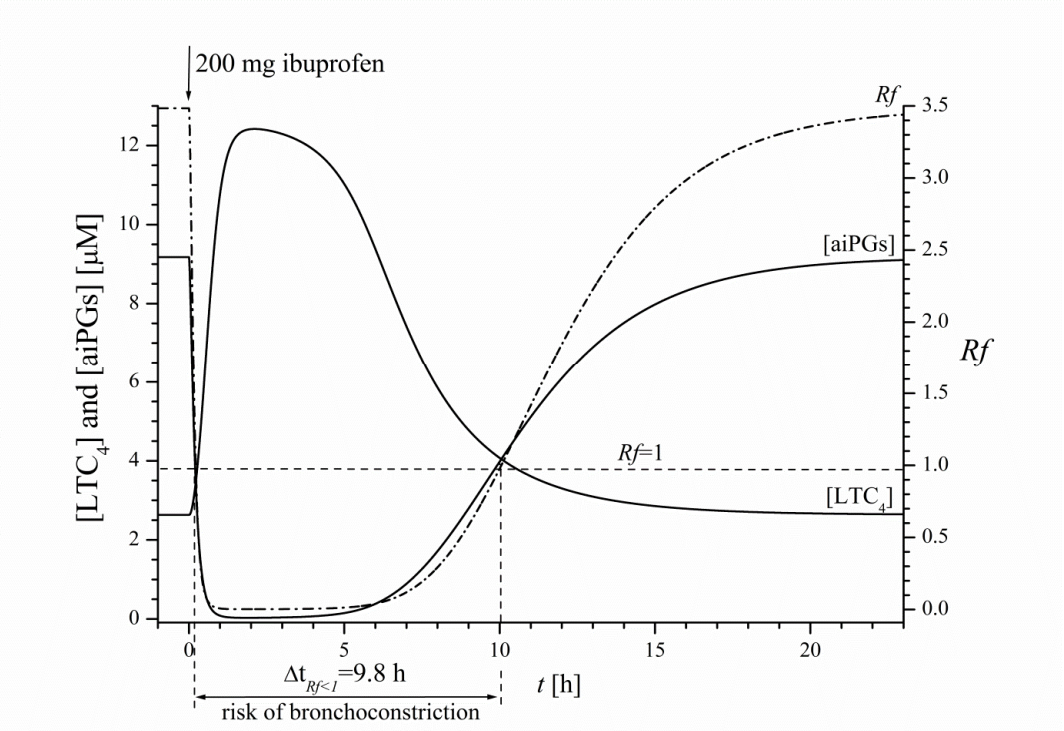


Figure 2 Time course of *[LTC₄]*, *[aiPGs]* and *Rf* after 200 mg ibuprofen dosing.

In Figure 2, the time interval of *Rf*, being less than one, is annotated. Since the value of *Rf* less than one is according to [41] characterized as the state of high risk for bronchoconstriction, the whole time interval is a theoretical estimation for the duration of bronchoconstriction. For the model population AIA$^{(1)}$ and for 200 mg of ibuprofen, the theoretical estimation is 9.8 h. This is in accordance with the value observed in clinical tests (9 h) [3].

Our mathematical model enables the theoretical prediction of NSAID dose for which symptoms of aspirin-induced asthma might appear. We introduced so-called limiting dose, D_{limit}, which could be calculated for any NSAID by knowing its pharmacokinetic parameters and its inhibitory properties on the enzymes PGHS1 and PGHS2. Limiting dose for particular NSAID is determined with the condition $Rf = 1$. D_{limit} is the theoretical estimation for the threshold dose (D_{treshold}) that is determined with provocation tests in clinical praxis. Threshold dose is the dose of NSAID, used in this test, above which significant bronchoconstriction and other symptoms of aspirin-induced asthma occur in AIA patients. Threshold doses are usually determined by measurements of FEV_1 or by observations of different clinical signs of aspirin-induced asthma in AIA patients. By comparing D_{limit} and D_{treshold} we are able to explain which patient population is more susceptible to a particular type of NSAID and to evaluate which of the enzymes PGHS1, PGHS2 or LTC_4S might play the central role in aspirin-induced asthma. In our simulations, three different populations of AIA patients are taken into consideration, which differ in expressions, reflecting the activities, of these enzymes; AIA$^{(1)}$: 3-fold lower PGHS1 activity compared to ATA; AIA$^{(2)}$: 5-fold higher LTC_4S activity compared to ATA; AIA$^{(3)}$: 6-fold lower PGHS2 activity compared to ATA. Table 3 shows theoretically determined limiting doses for aspirin ($D_{\text{limit}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{limit}}^{(\text{IBU})}$) [17] and the intervals or averaged threshold doses from clinical studies [3, 4, 77].

Table 3 Predicted limiting doses for aspirin ($D_{\text{limit}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{limit}}^{(\text{IBU})}$) in AIA model populations and threshold doses for aspirin ($D_{\text{treshold}}^{(\text{ASA})}$) and ibuprofen ($D_{\text{treshold}}^{(\text{IBU})}$) for AIA patients [17].

Dose	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
$D_{\text{limit}}^{(\text{ASA})}$ [mg]	455	1025	5150
$D_{\text{limit}}^{(\text{IBU})}$ [mg]	16	35	175
$D_{\text{treshold}}^{(\text{ASA})}$ [mg]	3 – 600 [4] 325-650 [3]		
$D_{\text{treshold}}^{(\text{IBU})}$ [mg]	≅ 400 [77]		

Model results suggest that different AIA model populations show significantly different susceptibilities to aspirin and ibuprofen. Among all three, $AIA^{(1)}$ and partially $AIA^{(2)}$ could be considered as susceptible to aspirin in terms of bronchoconstriction. According to these results, decreased expression of PGHS1 and increased expression of LTC_4S could be considered as the most important factors responsible for the occurrence of bronchoconstriction in AIA patients after aspirin dosing. On the other hand, all three populations could be considered as susceptible to ibuprofen. This is in accordance with the clinical observations in which ibuprofen was recognized as the most frequent initiator for intolerance to NSAIDs [30]. Very high predicted value of $D_{\text{limit}}^{(\text{ASA})}$ in model population $AIA^{(3)}$ indicates that decreased expression of PGHS2 might not be the reason for bronchoconstrictive reactions after challenges with therapeutic doses of aspirin. However, this does not mean that other symptoms of AIA will not occur in this case. In fact, our results suggest the occurrence of chronic symptoms of AIA for this population, since LTC_4 level in the basal state without NSAIDs in this case is several fold higher compared to those in $AIA^{(1)}$ and $AIA^{(2)}$ [16]. The values $D_{\text{limit}}^{(\text{ASA})}$ are much higher than $D_{\text{limit}}^{(\text{IBU})}$. Ibuprofen is thus recognized as a more potent inhibitor of the enzymes PGHS1 and PGHS2 as aspirin. Predicted values of $D_{\text{limit}}^{(\text{IBU})}$ for all three AIA populations are lower than $D_{\text{treshold}}^{(\text{IBU})}$, which is app. 400 mg [77].

It is widely accepted that PGHS1 is a housekeeping enzyme involved in the production of aiPGs [2]. Inflammatory processes in the organism are mainly mediated by piPGs, which are excessively produced by PGHS2 [2]. Usual types of NSAIDs, such as aspirin or ibuprofen,

inhibit both enzymes PGHS1 and PGHS2; however, they inhibit PGHS1 more than PGHS2. This is visible from the values of inhibitory constants for PGHS1 (K_{I3}) and for PGHS2 (K_{I1}) for each particular NSAID. For both, aspirin and ibuprofen, K_{I3} is lower than K_{I1}. Since the values of K_I-s reflect the concentration of inhibitor that suppresses the enzyme activity by half (IC50), the lower K_I means the stronger inhibition. Inhibitory action of NSAIDs on both enzymes of the cyclooxygenase pathway reduces the total production of prostaglandins. However, since in the case of aspirin and ibuprofen, PGHS1 is more inhibited than PGHS2, the reduction in aiPGs production is more significant than that in piPGs. The most important aiPG is PGE_2, which regulates the production of LTC_4 through the inhibition of the enzyme 5-LOX. This is believed to increase the production of LTC_4, which finally leads to clinical signs of aspirin-induced asthma in AIA patients. In this context, aspirin-induced asthma could be viewed as a side effect of equal inhibitory actions of aspirin and ibuprofen on both enzymes, however, this does not explain *per se*, why side effects are present only in a part of asthmatic patients. Our model results [16, 17] show and many experiments confirm [11, 51-57] that altered expressions of the key enzymes of AA degradation, PGHS1, PGHS2 and LTC_4S, are essential in occurrence of AIA.

There exist also selective NSAIDs, which inhibit enzyme PGHS2 stronger than PGHS1. These are for example celecoxib, valdecoxib and rofecoxib… Celecoxib is most widely used selective inhibitor in clinical studies [2]. In several studies AIA patients were challenged with a typical therapeutic dose of celecoxib (200 mg) [37, 38] or twice as much [34], and none of the patients reacted. However, there exist rare cases in which AIA patients reacted to these drugs [2]. One reference [39] reports about the person, who did not tolerate 15 mg of celecoxib and 10 mg of aspirin. In what follows, we will compare these clinical data with our theoretically predicted limiting doses for celecoxib ($D_{\text{limit}}^{(\text{CEL})}$) for all three AIA patient populations and will also try to explain this rare case with the model.

Table 4 Theoretically predicted limiting doses for celecoxib for three different AIA patient populations and their comparison with the doses used in provocation tests with AIA patients.

Dose	$\text{AIA}^{(1)}$	$\text{AIA}^{(2)}$	$\text{AIA}^{(3)}$
$D_{\text{limit}}^{(\text{CEL})}$ [mg]	115	223	1300
$D_{\text{provocation}}^{(\text{CEL})}$ [mg]	200 mg [37, 38] 400 mg [34]		

Values of $D_{\text{limit}}^{(\text{CEL})}$ presented in Table 4 show that different AIA model populations exhibit different susceptibilities to celecoxib. Population AIA$^{(1)}$ is the most and AIA$^{(3)}$ is the least susceptible to celecoxib. These results are generally in accordance with our previous studies [16, 17], in which we recognized the population AIA$^{(1)}$ as being the most and AIA$^{(3)}$ as being the least susceptible to aspirin and ibuprofen. Since predicted $D_{\text{limit}}^{(\text{CEL})}$ for AIA$^{(3)}$ is higher than doses used in provocation tests [34, 37, 38], population AIA$^{(3)}$ will likely not undergo bronchoconstriction for typical therapeutic doses of celecoxib. For this patient population, bronchoconstriction or symptoms of AIA might occur at higher celecoxib doses than those used in clinical practice. Predicted $D_{\text{limit}}^{(\text{CEL})}$ for population AIA$^{(1)}$ is 115 mg and is lower than the reported values [34, 37, 38] and, $D_{\text{limit}}^{(\text{CEL})}$ for AIA$^{(2)}$, which is 223 mg, falls directly within the reported interval of the doses, used in the provocation tests. The model thus predicts that already typical therapeutic doses of celecoxib might be potentially unsafe for selected AIA populations. It is also interesting how the model can explain a rare case reporting susceptibility of a person to 15 mg dose of celecoxib and 10 mg of aspirin. Our model simulations show that this special case can be explained by approximately 6-fold lower activity of PGHS1 compared to ATA. In comparison to AIA$^{(1)}$, which is defined by 3-fold lower expression of PGHS1 than ATA, this means additional 2-fold lower expression of PGHS1. In this case, model predicts $D_{\text{limit}}^{(\text{CEL})}$=15 mg and $D_{\text{limit}}^{(\text{ASA})} \cong 10$ mg, as reported [39]. We searched also for other possible theoretical solutions. By taking into account the higher activity of LTC_4S and the lower activity of PGHS2 in the model, other theoretical predictions are possible, but very unlikely. For example, $D_{\text{limit}}^{(\text{CEL})}$=15 mg could be achieved with 40-times and $D_{\text{limit}}^{(\text{ASA})}$=10 mg with 120-times higher activity of LTC_4S. Values for PGHS2, needed for achieving these limiting doses, are of several ranges of magnitude lower than those in ATA. However, in avoidance of misinterpretations of the model results, it is also important to note, that not only PGHS1, PGHS2 and LTC_4S activities are those who contribute to high susceptibilities of AIA patients to NSAIDs compared to ATA, but also the increased number of cysLTR [58] or the decreased number of EP receptors [59] might be the reason for that.

Several researchers proposed different strategies that would enable safe administration of NSAIDs to AIA patients. Such strategies are important for AIA patients with frequent headaches and chronic rheumatic diseases [45]. In these cases, continuous therapy with

NSAIDs is required. Since there is strong evidence that increased production of cysLTs plays the most important role in the occurrence of inflammatory reactions in aspirin-induced asthma [2], strategies are mainly focused on the inhibition of the enzyme 5-LOX and the decreases in cysLTs biosynthesis. Typical inhibitors of 5-LOX are native PGE_2, synthetic PGE_2 analogue (nocloprost), synthetic PGI2 analogue (misoprostol) and the drugs such as zileuton and ABT-761. The basic common idea of all the strategies is to decrease the production of cysLTs by inhibiting 5-LOX after NSAID dosing. In this way, bronchoconstriction and other symptoms of aspirin-induced asthma in AIA patients might be avoided. Several clinical studies report that exogenous PGE_2, administered by inhalation in form of aerosol, has protective effects in asthma induced by NSAIDs [14], allergens [47, 48] or exercise [49] in humans. Inhaled PGE_2 prevents aspirin-induced bronchoconstriction and strongly reduces urinary LTE_4 levels in AIA patients [14]. In another study [42], in which AIA patients were treated with 600 mg of zileuton four times daily, it was shown that zileuton inhibits urinary LTE_4 levels, inhibits aspirin-induced bronchoconstriction, diminishes nasal dysfunction and even returns the sense of smell. ABT-761 is another 5-LOX inhibitor that could be used in strategies for safe managing of AIA patients with NSAIDs. This is a novel and potent second-generation 5-LOX inhibitor, which may offer significant improvements in terms of the potency of 5-LOX inhibition and the duration of reduced cysLT biosynthesis as well as in pharmacokinetic properties compared to zileuton [75]. ABT-761 has very slow elimination phase compared to aspirin and ibuprofen, as well as compared to PGE_2 analogue – nocloprost (see Figure 3).

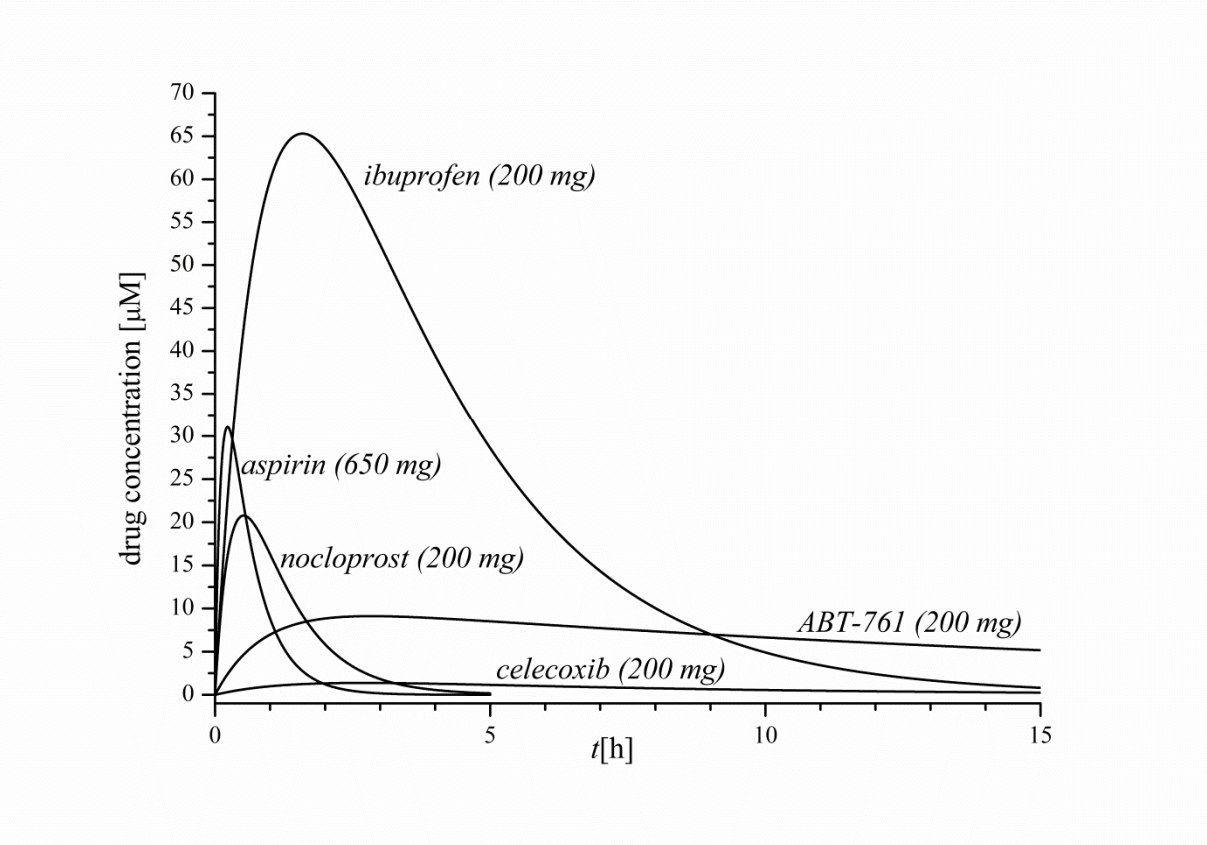


Figure 3 Time courses of plasma drug concentrations for typical doses.

The best strategy for NSAID dosing in combination with ABT-761 is to apply ibuprofen at the time when ABT-761 concentration in blood is maximal; approximately 2.5 hours after ABT-761. This is due to rather slow absorption phase of ABT-761 compared to ibuprofen. In this way, the predicted dose of ABT-761 that should avoid the risk of bronchoconstriction is smaller than in case of taking the drugs at the same time. Predicted doses of ABT-761 in combination to 200 mg in different AIA patient populations are: 510 mg for $AIA^{(1)}$, 700 mg for $AIA^{(2)}$ and 36 mg for $AIA^{(3)}$. The dose of ABT-761 is such, that the relation factor (*Rf*) in our model simulations does not fall below one during the whole period of NSAID action. Although ibuprofen has one of the slowest elimination phases among NSAIDs, there is no need for successive dosing of ABT-761 in combination with ibuprofen.

In our recent study [17], we theoretically analyzed another strategy for safe aspirin and ibuprofen dosing to AIA patients; this time in combination with orally stable PGE_2 analogue – nocloprost. The main goal of this study was to determine the exact protocol of PGE_2 analogue dosing in terms of the doses and the times of dosing after ingestion of either aspirin or ibuprofen. In contrast to ABT-761, PGE2 analogue (nocloprost) has much faster absorption

and elimination phases so that successive doses after aspirin or ibuprofen are required. The protocol was again such that the value of *Rf* did not drop below one during the whole interval of NSAID presence in plasma. Our model results presented in Table 5 show, that the protocol with PGE2 analogue (nocloprost) is dependent on: i) the population of AIA patients, ii) the differences/similarities of pharmacokinetic properties of NSAID and PGE_2 analogue – nocloprost iii) the dose of NSAID.

Table 5 Protocols for PGE_2 analogue – nocloprost dosing in combination with either 1000 mg of aspirin or 200 mg or 400 mg of ibuprofen in all AIA model populations [17].

Model population	$t_1 = 0$ $D_1^{(PGE_2)}$ [mg]	$t_2 = 2h$ $D_2^{(PGE_2)}$ [mg]	$t_3 = 4h$ $D_3^{(PGE_2)}$ [mg]	$t_4 = 6h$ $D_4^{(PGE_2)}$ [mg]	$t_5 = 8h$ $D_5^{(PGE_2)}$ [mg]
1000 mg aspirin					
$AIA^{(1)}$	15	/	/	/	/
200 mg ibuprofen					
$AIA^{(1)}$	130	100	70	70	/
$AIA^{(2)}$	300	210	150	/	/
$AIA^{(3)}$	30	/	/	/	/
400 mg ibuprofen					
$AIA^{(1)}$	160	120	100	70	60
$AIA^{(2)}$	420	300	210	150	/
$AIA^{(3)}$	160	70	/	/	/

The differences in the predicted protocols for aspirin and ibuprofen as well as for different AIA model patient populations suggest that this protocol, if applied in clinical praxis, should certainly be personalized. Partially, this is due to differences in pharmacokinetic properties of aspirin and ibuprofen compared to PGE_2 analogue, and, partially due to differences in susceptibilities between model AIA patient populations. Since absorption and elimination phases of aspirin are similar to those of PGE_2 analogue, the effects of both drugs on AA metabolism appear approximately at the same time. Therefore, already one rather low dose of PGE_2 analogue, applied together with aspirin, is sufficient to prevent bronchoconstriction in all AIA model populations. Since ibuprofen has slower elimination phase compared to PGE_2 analogue, several consecutive doses of PGE_2 analogue are needed to prevent the risk of bronchoconstriction. Also, the doses for ibuprofen are higher, since ibuprofen is stronger inhibitor of both enzymes PGHS1 and PGHS2. Differences also occur between AIA model populations. $AIA^{(2)}$ requires the highest doses and $AIA^{(3)}$ the lowest of PGE_2 analogue – nocloprost.

Conclusion

The mathematical model considers AA degradation via lipoxygenase and cyclooxygenase pathways, resulting in a production of major eicosanoids as well as interaction of cyclooxygenase pathway with NSAID. In this chapter, our previous versions of the model [16, 17] are upgraded with the involvement of 5-LOX inhibitor. The model incorporates all the main players in AA metabolism and relies on theoretical and experimental biochemical, as well as clinical data in terms of model parameters and variables. Differences between AIA and ATA patients and healthy humans (HH) in expressions of the key enzymes of AA degradation, LTC_4S, PGHS1and PGHS2, are the basis for the definition of our model populations and the proposal for different characterization and subgrouping of AIA patients. In principle, our approach is a systems biology approach in which knowledge from enzyme gene expressions, molecular interactions, pharmacokinetics of drugs and their action on the molecular level as well as implications on the organ level are integrated in the mathematical model, simulations and interpretations of the model results. Furthermore, the model enables simulations of different drugs used in clinical praxis: NSAIDs, selective COX inhibitors, exogenous PGE_2 analogues and 5-LOX inhibitors. It helps to understand the molecular mechanisms and causal links between the symptoms of AIA, effects of different drugs, their threshold doses, as well as the differences in susceptibilities of individual AIA patients to bronchoconstriction according to different types and doses of NSAIDs. As seen from the results, presented in this chapter and those published elsewhere [16, 17], our model already explains many of them. Furthermore, the additional value of the model is in testing the existing simulations and creating the new ones for different personalized treatment strategies [16, 17].

In addition to the development of the model to such a level that it would be applicable in clinical praxis for the individualized treatment of AIA patients, the future perspective is also to couple the present model of eicosanoid production with the cell model of bronchial smooth muscle contraction in the view of multiscale modeling [78, 79]. For the purpose of the whole-lung modeling [80], we already developed several whole-cell mathematical models of airway tracheal smooth muscle [81-84], some of them based on the intensive experimental research [81, 83], in which isometric force development in response to cholinergic stimulation is theoretically studied by using computational methods that incorporate biochemical, biophysical and also some anatomical information on cells and tissues. The models introduce

an upgrade to the original 4-state latch bridge model, first published by Hai and Murphy [85]. It is upgraded in terms of more sophisticated description of Ca^{2+}-calmodulin (Ca^{2+}-CaM) dependent activation of myosin light chain kinase (MLCK) [86, 87] and its involvement in myosin light chain (MLC) phosphorylation/dephosphorylation process in concordance with myosin light chain phosphatase (MLCP) [81-84]. Based on the measurements of Ca^{2+}-dynamics, performed by our colleges from the Laboratory for physiology of respiratory cells at the University of Bordeaux 2 in France [81, 83, 88-91], we are developing and constantly upgrading the models of Ca^{2+} handling in airway smooth muscle cells [90, 92-94]. Therefore, the only missing part, required for the complete integration of the two models (the model of eicosanoid production and the model of bronchial smooth muscle Ca^{2+}-contraction coupling), is probably the link between them. A lot is known about cysLTs binding to cysLTRs and their inhibitors as well as about the effects of both on airway smooth muscle contraction. However, very little is known about the signaling pathways and the molecular mechanisms downstream the cysLTRs. It is hypothesized that in porcine tracheal smooth muscle, for example, LTC_4 might enhance the contraction via the activation of the Rho – Rho-kinase pathway by increasing the Ca^{2+}-responsiveness of the contractile apparatus in a MLC-phosphorylation dependent manner [76]. All this is not far from our very recent experimental and theoretical research, in which we measured the developed force and several other variables. Furthermore, we mathematically modeled the signaling pathways in which Rho-kinase was involved as activator/suppressor of MLCP. Our theoretical results, strongly supported by our measurements [81], evaluate the contribution of the Rho-kinase to the early phase of the Ca^{2+}-dependent airway smooth muscle contraction and give good perspectives for coupling the two models in the view of predictions of the level and the duration of bronchoconstriction after drug dosing.

References

1. Babu, K.S. and S.S. Salvi, *Aspirin and asthma.* Chest, 2000. **118**(5): p. 1470-1476.
2. Stevenson, D. and A. Szczeklik, *Clinical and pathologic perspectives on aspirin sensitivity and asthma.* J Allergy Clin Immunol, 2006. **118**(4): p. 773-786.
3. Settipane, R.A., et al., *Prevalence of Cross-Sensitivity with Acetaminophen in Aspirin-Sensitive Asthmatic Subjects.* Journal of Allergy and Clinical Immunology, 1995. **96**(4): p. 480-485.
4. Sanchez-Borges, M., *NSAID hypersensitivity (respiratory, cutaneous, and generalized anaphylactic symptoms).* Med Clin North Am, 2010. **94**(4): p. 853-864.
5. Daffern, P.J., et al., *Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses.* J Allergy Clin Immunol, 1999. **104**(3 Pt 1): p. 559-564.
6. Micheletto, C., et al., *Changes in urinary LTE4 and nasal functions following nasal provocation test with ASA in ASA-tolerant and -intolerant asthmatics.* Respir Med, 2006. **100**(12): p. 2144-2150.
7. Ferreri, N.R., et al., *Release of leukotrienes, prostaglandins, and histamine into nasal secretions of aspirin-sensitive asthmatics during reaction to aspirin.* Am Rev Respir Dis, 1988. **137**(4): p. 847-854.
8. Sladek, K., et al., *Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge.* Am J Respir Crit Care Med, 1994. **149**(4 Pt 1): p. 940-946.
9. Settipane, G., *Asthma, aspirin intolerance and nasal polyps.* N Engl Reg Allergy Proc, 1986. **7**(1): p. 32-37.
10. Samter, M. and R.F. Beers, Jr., *Concerning the nature of intolerance to aspirin.* J Allergy, 1967. **40**(5): p. 281-293.
11. Szczeklik, A. and D. Stevenson, *Aspirin-induced asthma: advances in pathogenesis and management.* J Allergy Clin Immunol, 1999. **104**(1): p. 5-13.
12. Vargaftig, B.B. and M. Singer, *Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13.* Am J Respir Cell Mol Biol, 2003. **28**(4): p. 410-419.
13. Gupta, N., M. Gresser, and A. Ford-Hutchinson, *Kinetic mechanism of glutathione conjugation to leukotriene A4 by leukotriene C4 synthase.* Biochim Biophys Acta, 1998. **1391**(2): p. 157-68.
14. Sestini, P., et al., *Inhaled PGE2 prevents aspirin-induced bronchoconstriction and urinary LTE4 excretion in aspirin-sensitive asthma.* Am J Respir Crit Care Med, 1996. **153**(2): p. 572-575.
15. Drazen, J.M., *Leukotrienes as mediators of airway obstruction.* Am J Respir Crit Care Med, 1998. **158**(5 Pt 3): p. S193-200.
16. Dobovisek, A., A. Fajmut, and M. Brumen, *Role of expression of prostaglandin synthases 1 and 2 and leukotriene C4 synthase in aspirin-intolerant asthma: a theoretical study.* J Pharmacokinet Pharmacodyn, 2011. **38**(2): p. 261-278.
17. Dobovisek, A., A. Fajmut, and M. Brumen, *Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE2 analogue: a theoretical approach.* Submitted to: Medical and Biological Engineering and Computing, 2011.
18. Smith, W.L., E.A. Meade, and D.L. Dwitt, *Pharmacology of prostaglandin endoperoxide synthase isozymes-1 and-2a.* Annals of the New York Academy of Sciences, 1994. **714**(1): p. 136-142.

19. Christman, B.W., et al., *Prostaglandin E2 limits arachidonic acid availability and inhibits leukotriene B4 synthesis in rat alveolar macrophages by a nonphospholipase A2 mechanism.* J Immunol, 1993. **151**(4): p. 2096-2104.
20. Harizi, H., et al., *Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B4 production from dendritic cells via an IL-10-dependent mechanism.* J Immunol, 2003. **170**(1): p. 139-146.
21. Kay, L., W. Yeo, and P. Peachell, *Prostaglandin E2 activates EP2 receptors to inhibit human lung mast cell degranulation.* Br J Pharmacol, 2006. **147**(7): p. 707-713.
22. Hartney, J.M., et al., *Prostaglandin E2 protects lower airways against bronchoconstriction.* Am J Physiol Lung Cell Mol Physiol, 2006. **290**(1): p. L105-113.
23. Nguyen, M., et al., *Receptors and signaling mechanisms required for prostaglandin E2-mediated regulation of mast cell degranulation and IL-6 production.* J Immunol, 2002. **169**(8): p. 4586-4593.
24. Szczeklik, A. and M. Sanak, *The broken balance in aspirin hypersensitivity.* Eur J Pharmacol, 2006. **533**(1-3): p. 145-155.
25. Maxis, K., et al., *The shunt from the cyclooxygenase to lipoxygenase pathway in human osteoarthritic subchondral osteoblasts is linked with a variable expression of the 5-lipoxygenase-activating protein.* Arthritis Res Ther, 2006. **8**(6): p. R181.
26. Meade, E., W. Smith, and D. DeWitt, *Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti- inflammatory drugs.* J. Biol. Chem., 1993. **268**(9): p. 6610-6614.
27. So, O.-Y., et al., *The dynamics of prostaglandin H synthases. Studies with prostaglandin H synthase 2 Y355F unmask mechanisms of time-dependent inhibition and allosteric activation.* J Biol Chem, 1998. **273**(10): p. 5801-5807.
28. Vane, J. and R. Botting, *Inflammation and the mechanism of action of anti-inflammatory drugs.* FASEB J, 1987. **1**(2): p. 89-96.
29. Berges-Gimeno, M.P., R.A. Simon, and D.D. Stevenson, *The natural history and clinical characteristics of aspirin-exacerbated respiratory disease.* Ann Allergy Asthma Immunol, 2002. **89**(5): p. 474-478.
30. Rosado, A., et al., *Intolerance to non-steroidal anti inflammatory drugs with respiratory reaction: clinical and diagnostic features.* Alergol Inmunol Clin, 2000. **15**: p. 153-159.
31. Bianco, S., et al., *Efficacy and tolerability of nimesulide in asthmatic patients intolerant to aspirin.* Drugs, 1993. **46 Suppl 1**: p. 115-120.
32. Kosnik, M., et al., *Relative safety of meloxicam in NSAID-intolerant patients.* Allergy, 1998. **53**(12): p. 1231-1233.
33. Stevenson, D.D. and R.A. Simon, *Lack of cross-reactivity between rofecoxib and aspirin in aspirin-sensitive patients with asthma.* J Allergy Clin Immunol, 2001. **108**(1): p. 47-51.
34. Gyllfors, P., et al., *Biochemical and clinical evidence that aspirin-intolerant asthmatic subjects tolerate the cyclooxygenase 2-selective analgetic drug celecoxib.* J Allergy Clin Immunol, 2003. **111**(5): p. 1116-1121.
35. Martin-Garcia, C., et al., *Safety of a cyclooxygenase-2 inhibitor in patients with aspirin-sensitive asthma.* Chest, 2002. **121**(6): p. 1812-1817.
36. Szczeklik, A., et al., *Safety of a specific COX-2 inhibitor in aspirin-induced asthma.* Clin Exp Allergy, 2001. **31**(2): p. 219-225.
37. Woessner, K.M., R.A. Simon, and D.D. Stevenson, *The safety of celecoxib in patients with aspirin-sensitive asthma.* Arthritis Rheum, 2002. **46**(8): p. 2201-2206.

38. Yoshida, S., et al., *Selective cyclo-oxygenase 2 inhibitor in patients with aspirin-induced asthma.* J Allergy Clin Immunol, 2000. **106**(6): p. 1201-1202.
39. Baldassarre, S., et al., *Asthma attacks induced by low doses of celecoxib, aspirin, and acetaminophen.* J Allergy Clin Immunol, 2006. **117**(1): p. 215-217.
40. Szczeklik, A., *Prostaglandin E2 and aspirin-induced asthma.* Lancet, 1995. **345**(8956): p. 1056.
41. Schafer, D., et al., *Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics.* Eur Respir J, 1999. **13**(3): p. 638-646.
42. Dahlen, B., et al., *Benefits from adding the 5-lipoxygenase inhibitor zileuton to conventional therapy in aspirin-intolerant asthmatics.* Am J Respir Crit Care Med, 1998. **157**(4 Pt 1): p. 1187-1194.
43. Rao, N.L., et al., *Anti-inflammatory activity of a potent, selective leukotriene A4 hydrolase inhibitor in comparison with the 5-lipoxygenase inhibitor zileuton.* J Pharmacol Exp Ther, 2007. **321**(3): p. 1154-1160.
44. Wong, S., et al., *Pharmacokinetics and pharmacodynamics of single and multiple oral doses of a novel 5-lipoxygenase inhibitor (ABT-761) in healthy volunteers.* Clin Pharmacol Ther, 1998. **63**(3): p. 324-31.
45. Knowles, S.R., et al., *Management options for patients with aspirin and nonsteroidal antiinflammatory drug sensitivity.* The Annals of Pharmacotherapy, 2007. **41**(7): p. 1191-1200.
46. Schafer, D., et al., *Effect of prostaglandin E2 on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa.* Thorax, 1996. **51**(9): p. 919-923.
47. Hartert, T.V., et al., *Prostaglandin E(2) decreases allergen-stimulated release of prostaglandin D(2) in airways of subjects with asthma.* Am J Respir Crit Care Med, 2000. **162**(2 Pt 1): p. 637-640.
48. Gauvreau, G.M., R.M. Watson, and P.M. O'Byrne, *Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation.* Am J Respir Crit Care Med, 1999. **159**(1): p. 31-36.
49. Melillo, E., et al., *Effect of inhaled PGE2 on exercise-induced bronchoconstriction in asthmatic subjects.* Am J Respir Crit Care Med, 1994. **149**(5): p. 1138-1141.
50. Tuber, U., et al., *Pharmacokinetics of nocloprost in human volunteers and its relation to dose.* Eur J Clin Pharmacol, 1993. **44**(5): p. 497-500.
51. Picado, C., et al., *Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics.* Am J Respir Crit Care Med, 1999. **160**(1): p. 291-296.
52. Pujols, L., et al., *Dynamics of COX-2 in nasal mucosa and nasal polyps from aspirin-tolerant and aspirin-intolerant patients with asthma.* J Allergy Clin Immunol, 2004. **114**(4): p. 814-819.
53. Pierzchalska, M., et al., *Deficient prostaglandin E2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma.* J Allergy Clin Immunol, 2003. **111**(5): p. 1041-1048.
54. Cowburn, A.S., et al., *Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma.* J Clin Invest, 1998. **101**(4): p. 834-846.
55. Sousa, A., et al., *Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma.* Thorax, 1997. **52**(11): p. 940-945.
56. Sanak, M., et al., *Enhanced expression of the leukotriene C(4) synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma.* Am J Respir Cell Mol Biol, 2000. **23**(3): p. 290-296.

57. Sampson, A., et al., *Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin-intolerant asthmatic patients.* Int Arch Allergy Immunol, 1997. **113**(1-3): p. 355-357.
58. Sousa, A.R., et al., *Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis.* N Engl J Med, 2002. **347**(19): p. 1493-1499.
59. Ying, S., et al., *Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells.* J Allergy Clin Immunol, 2006. **117**(2): p. 312-318.
60. Callan, O.H., O.-Y. So, and D.C. Swinney, *The kinetic factors that determine the affinity and selectivity for slow binding inhibition of human prostaglandin H synthase 1 and 2 by indomethacin and flurbiprofen.* J Biol Chem, 1996. **271**(7): p. 3548-3554.
61. Gonchar, M., et al., *Kinetics of prostanoid synthesis by macrophages is regulated by arachidonic acid sources.* Eur J Biochem, 1999. **265**(2): p. 779-787.
62. Aharony, D. and R. Stein, *Kinetic mechanism of guinea pig neutrophil 5-lipoxygenase.* J Biol Chem, 1986. **261**(25): p. 11512-11519.
63. Owen, W.F., Jr., et al., *Synthesis and release of leukotriene C4 by human eosinophils.* J Immunol, 1987. **138**(2): p. 532-538.
64. Haeggstrom, J., et al., *Guinea-pig liver leukotriene A4 hydrolase. Purification, characterization and structural properties.* Eur J Biochem, 1988. **174**(4): p. 717-724.
65. Lam, B.K., et al., *The mechanism of leukotriene B4 export from human polymorphonuclear leukocytes.* J Biol Chem, 1990. **265**(23): p. 13438-13441.
66. Meade, E.A., W.L. Smith, and D.L. DeWitt, *Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs.* J Biol Chem, 1993. **268**(9): p. 6610-6614.
67. Mitchell, J.A., et al., *Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase.* Proc Natl Acad Sci U S A, 1993. **90**(24): p. 11693-11697.
68. Goltsov, A., et al., *In silico screening of nonsteroidal anti-inflammatory drugs and their combined action on prostaglandin H synthase-1.* Pharmaceuticals, 2010. **3**(7): p. 2059-2081.
69. Noguchi, M., et al., *Interfacial kinetic reaction of human 5-lipoxygenase.* Eur J Biochem, 1994. **222**(2): p. 285-292.
70. Bell, R.L., et al., *ABT-761 attenuates bronchoconstriction and pulmonary inflammation in rodents.* J Pharmacol Exp Ther, 1997. **280**(3): p. 1366-1373.
71. Gupta, N., M.J. Gresser, and A.W. Ford-Hutchinson, *Kinetic mechanism of glutathione conjugation to leukotriene A4 by leukotriene C4 synthase.* Biochim Biophys Acta, 1998. **1391**(2): p. 157-168.
72. Rowland, M., et al., *Absorption kinetics of aspirin in man following oral administration of an aqueous solution.* J Pharm Sci, 1972. **61**(3): p. 379-385.
73. Klueglich, M., et al., *Ibuprofen extrudate, a novel, rapidly dissolving ibuprofen formulation: relative bioavailability compared to ibuprofen lysinate and regular ibuprofen, and food effect on all formulations.* J Clin Pharmacol, 2005. **45**(9): p. 1055-1061.
74. Paulson, S.K., et al., *Pharmacokinetics of celecoxib after oral administration in dogs and humans: effect of food and site of absorption.* J Pharmacol Exp Ther, 2001. **297**(2): p. 638-645.
75. Wong, S.L., et al., *Dose-proportional pharmacokinetics of a new 5-lipoxygenase inhibitor, ABT-761, in healthy volunteers.* Biopharm Drug Dispos, 1998. **19**(3): p. 159-162.

76. Setoguchi, H., et al., *Leukotriene C(4) enhances the contraction of porcine tracheal smooth muscle through the activation of Y-27632, a rho kinase inhibitor, sensitive pathway.* Br J Pharmacol, 2001. **132**(1): p. 11111-8.
77. Varghese, M. and R.F. Lockey, *Aspirin-exacerbated asthma.* Allergy Asthma Clin Immunol, 2008. **4**(2): p. 75-83.
78. Hunter, P., P. Robbins, and D. Noble, *The IUPS human Physiome Project.* Pflugers Arch, 2002. **445**(1): p. 1-9.
79. Hunter, P.J. and T.K. Borg, *Integration from proteins to organs: the Physiome Project.* Nat Rev Mol Cell Biol, 2003. **4**(3): p. 237-243.
80. Tawhai, M.H., E.A. Hoffman, and C.L. Lin, *The lung physiome: merging imaging-based measures with predictive computational models.* Wiley Interdiscip Rev Syst Biol Med, 2009. **1**(1): p. 61-72.
81. Mbikou, P., et al., *Contribution of Rho kinase to the early phase of the calcium-contraction coupling in airway smooth muscle.* Exp Physiol, 2011. **96**(2): p. 240-258.
82. Fajmut, A. and M. Brumen, *MLC-kinase/phosphatase control of Ca2+ signal transduction in airway smooth muscles.* J Theor Biol, 2008. **252**(3): p. 474-481.
83. Mbikou, P., et al., *Theoretical and experimental investigation of calcium-contraction coupling in airway smooth muscle.* Cell Biochem Biophys, 2006. **46**(3): p. 233-252.
84. Fajmut, A., A. Dobovisek, and M. Brumen, *Mathematical modeling of the relation between myosin phosphorylation and stress development in smooth muscles.* J Chem Inf Model, 2005. **45**(6): p. 1610-1615.
85. Hai, C.M. and R.A. Murphy, *Cross-bridge phosphorylation and regulation of latch state in smooth muscle.* Am J Physiol, 1988. **254**(1 Pt 1): p. C99-106.
86. Fajmut, A., M. Jagodic, and M. Brumen, *Mathematical modeling of the myosin light chain kinase activation.* J Chem Inf Model, 2005. **45**(6): p. 1605-9.
87. Fajmut, A., M. Brumen, and S. Schuster, *Theoretical model of the interactions between Ca2+, calmodulin and myosin light chain kinase.* FEBS Lett, 2005. **579**(20): p. 4361-4366.
88. Belouchi, N.E., et al., *Effect of chronic hypoxia on calcium signalling in airway smooth muscle cells.* Eur Respir J, 1999. **14**(1): p. 74-79.
89. Belouchi, N.E., et al., *Interaction of extracellular albumin and intravenous anaesthetics, etomidate and propofol, on calcium signalling in rat airway smooth muscle cells.* Fundam Clin Pharmacol, 2000. **14**(4): p. 395-400.
90. Marhl, M., et al., *Importance of cell variability for calcium signaling in rat airway myocytes.* Biophys Chem, 2010. **148**(1-3): p. 42-50.
91. Roux, E., et al., *Calcium signaling in airway smooth muscle cells is altered by in vitro exposure to the aldehyde acrolein.* Am J Respir Cell Mol Biol, 1998. **19**(3): p. 437-444.
92. Brumen, M., et al., *Mathematical modelling of Ca2+ oscillations in airway smooth muscle cells.* Journal of Biological Physics, 2005. **31**(3): p. 515-524.
93. Roux, E. and M. Marhl, *Role of sarcoplasmic reticulum and mitochondria in Ca2+ removal in airway myocytes.* Biophys J, 2004. **86**(4): p. 2583-2595.
94. Haberichter, T., et al., *The influence of different InsP(3) receptor isoforms on Ca(2+) signaling in tracheal smooth muscle cells.* Bioelectrochemistry, 2002. **57**(2): p. 129-138.

DELOVNI ŽIVLJENJEPIS DOKTORSKEGA KANDIDATA

Datum in kraj rojstva

7. 3. 1980, Ptuj

Izobraževanje

1987−1995 Osnovna šola Olge Meglič Ptuj

1995−1999 Srednja poklicna in tehniška strojna šola Ptuj

1999−2003 Univerza v Mariboru, Pedagoška fakulteta
Smer študija: enopredmetni pedagoški študij fizike
Pridobljen naziv: profesor fizike

2004−2007 Univerza v Mariboru, Fakulteta za naravoslovje in matematiko
Smer študija: podiplomski magistrski študijski program fizika, področje izobraževanja
Pridobljen naziv: magister znanosti iz področja fizike, področje izobraževanja

Zaposlitev

2004 Univerza v Mariboru,
Fakulteta za naravoslovje in matematiko in Medicinska fakulteta
Naziv: asistent za fiziko (3. izvolitev)

Strokovna izpopolnjevanja v tujini

2006 Udeležba na 9. mednarodni biofizikalni šoli v Rovinju na Hrvaškem.
2009−2010 Trije enotedenski delovni obiski Oddelka za fiziko Fakultete za naravoslovje na Univerzi v Splitu v okviru raziskovalnega bilateralnega projekta.

Priznanja in nagrade

2005 Perlachova nagrada za diplomsko delo

Raziskovalno delo

Znanstvena področja: Teoretična biofizika
Znanstveno-raziskovalne metode: Matematično modeliranje

Področja dela:

- kalcijeva signalizacija v procesu krčenja gladkih mišičnih celic dihalnih poti,
- optimizacija encimskih reakcij,
- aspirinska intoleranca pri astmatičnih bolnikih.

OSEBNA COBISS BIBLIOGRAFIJA

Izvirni znanstveni članki

BRUMEN, Milan, FAJMUT, Aleš, DOBOVIŠEK, Andrej, ROUX, Etienne. Mathematical modelling of Ca^{2+} oscillations in airway smooth muscle cells. Journal of biological physics, 2005, 31, str. 515−524.

FAJMUT, Aleš, DOBOVIŠEK, Andrej, BRUMEN, Milan. Mathematical modeling of the relation between myosin phosphorylation and stress development in smooth muscles. J. chem. inf. mod., 2005, 45, str. 1610−1615.

ŽUPANOVIĆ, Paško, KUIĆ, Domagoj, JURETIĆ, Davor, DOBOVIŠEK, Andrej. On the problem of formulating principles in nonequilibrium thermodynamics. Entropy (Basel, Online), 2010, 4, str. 926−931.

DOBOVIŠEK, Andrej, ŽUPANOVIĆ, Paško, BRUMEN, Milan, BONAČIĆ LOŠIĆ, Željana, KUIĆ, Domagoj, JURETIĆ, Davor. Enzyme kinetics and the maximum entropy production principle. Biophysical chemistry, 2011, 154, str. 49−55.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Role of expression of prostaglandin synthases 1 and 2 and leukotriene C_4 synthase in aspirin-intolerant asthma: a theoretical study. Journal of pharmacokinetics and pharmacodynamics, 2011, 38, str. 261−278.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Strategy for NSAID administration to aspirin-intolerant asthmatics in combination with PGE [sub] 2 analogue: a theoretical approach. Med. biol. eng. comput. 2012, 50, str. 33−42.

Strokovni članki

DOBOVIŠEK, Andrej, VAUPOTIČ, Nataša. Merjenje moči mišic nog. *Proteus*, feb. 2012, letn. 74, št. 6, str. 255−259.

Objavljeni povzetki znanstvenih prispevkov na konferenci

BRUMEN, Milan, FAJMUT, Aleš, DOBOVIŠEK, Andrej, ROUX, Etienne. The Mathematical Modelling of [Ca^{2+}] Oscillations in Airway Smooth Muscle Cells. V: The 5th International Conference on Biological Physics, August 23−August 27, 2004, [Chalmers Conference Center], Göteborg, Sweden. ICBP 2004 : abstracts. [Göteborg: University: Chalmers, 2004], str. 23.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Theoretical Study of Force Development in Smooth Muscle Cells. V: 15th IUPAB & 5th EBSA International Biophysics Congress, August 27th−September 1st 2005, Montpellier, France. Abstracts, (European biophysics journal, Vol. 34, no. 6, August 2005). [Heidelberg]: Springer: EBSA, 2005, str. 686.

DOBOVIŠEK, Andrej. Theoretical Study of Force Development in Smooth Muscle Cells. V: Book of abstracts. Będlewo: Banach Center of the Mathematical Institute of the Polish Academy of Sciences, 2005, str. 3.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Mathematical Modelling of Relation between Myosin Phosphorylation and Force Development in Smooth Muscles. V: ABRAMOVIĆ, Zrinka (ur.), DOGŠA, Iztok (ur.). Regionalno srečanje biofizikov 2005, Zreče, Slovenija, 16.−20. marec 2005. str. 52.

DOBOVIŠEK, Andrej, JAGODIČ, Marko, FAJMUT, Aleš, BRUMEN, Milan. Modeliranje sklopitve stimulacije in skrčitve gladkih mišic: ROBNIK, Marko (ur.), KOROŠAK, Dean (ur.). 4. simpozij fizikov Univerze v Mariboru, Hotel Piramida, Maribor, 15. in 16. december 2005. Zbornik povzetkov. Maribor: CAMTP, 2005.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Mathematical Modelling of the Myosin and Actin Filament regulated Smooth Muscle Contraction. V: Ninth International Summer School on Biophysics, September 16−18, 2006, Rovinj, Croatia. Supramolecular structure and function : book of abstracts. Zagreb: Ruđer Bošković Institute, 2006, str. 108.

DOBOVIŠEK, Andrej, FAJMUT, Aleš, BRUMEN, Milan. Matematično modeliranje krčitve gladkih mišic. V: TKALEC, Uroš (ur.), ŠKARABOT, Miha (ur.), MUŠEVIČ, Igor (ur.). 5. konferenca fizikov v osnovnih raziskavah, Gozd Martuljek, 10. november 2006. Zbornik povzetkov, (Publikacija DMFA, št. 1638). Ljubljana: DMFA, založništvo, 2006.

DOBOVIŠEK, Andrej, ONIŠAK, Boris, FAJMUT, Aleš, POTOČNIK, Uroš, BRUMEN, Milan. Mathematical Model of Aspirin Intolerance in Asthma. V: ZIMÁNYI, László (ur.), KÓTA, Zoltán (ur.), SZALONTAI, Balázs (ur.). RBC 2007, Regional Biophysical Conference, 21−25 August 2007, Balatonfüred, Hungary. Book of abstracts. [S. l.]: Hungarian Biophysical Society, 2007, str. 132.

DOBOVIŠEK, Andrej, ONIŠAK, Boris, FAJMUT, Aleš, POTOČNIK, Uroš, BRUMEN, Milan. Mathematical model of aspirin intolerance in asthma. V: POKLAR ULRIH, Nataša (ur.), ABRAM, Veronika (ur.), CIGIĆ, Blaž (ur.). 7. srečanje Slovenskega biokemijskega društva z mednarodno udeležbo, 26.−29. september 2007. Zbornik povzetkov. Ljubljana: Slovensko biokemijsko društvo, str. 147.

DOBOVIŠEK, Andrej, BRUMEN, Milan, ŽUPANOVIĆ, Paško, JURETIĆ, Davor. Maximum entropy production principle (MEPP) in generalized Michaelis-Menten kinetics. V:7th EBSA European Biophysics Congress, July 11th−15th 2009, Genoa, Italy. Abstracts, European biophysics journal, 38, suppl. 1, Jul. 2009. [Heidelberg]: Springer, 2009, str. S121.

DOBOVIŠEK, Andrej, KUIĆ, Domagoj, BONAČIĆ LOŠIĆ, Željana, BRUMEN, Milan, ŽUPANOVIĆ, Paško, JURETIĆ, Davor. Entropy production in enzymatic reactions. V:Regional biophysics conference 2009 : February 10−14, 2009, Linz, Austria: programme and abstract book. [S.l.: s.n.], 2009, str. 142.

UNIVERZA V MARIBORU

Fakulteta za naravoslovje in matematiko

IZJAVA DOKTORSKEGA KANDIDATA

Podpisani **Andrej Dobovišek**, vpisna številka **N0002510**

izjavljam,

da je doktorska disertacija z naslovom **MATEMATIČNO MODELIRANJE VPLIVA NESTEROIDNIH ANTIREVMATIKOV NA ASPIRINSKO INTOLERANCO ASTME**

- rezultat lastnega raziskovalnega dela,
- da predložena disertacija v celoti ali v delih ni bila predložena za pridobitev kakršnekoli izobrazbe po študijskem programu druge fakultete ali univerze,
- da so rezultati korektno navedeni,
- da nisem kršil avtorskih pravic in intelektualne lastnine drugih.

Podpis doktorskega kandidata