UNIVERZA V MARIBORU
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Tadej Emeršič

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Modelna napoved forsiranega izdihanega volumna v prvi sekundi (FEV1) pred in po zaužitju nesteroidnih antirevmatikov

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IZJAVA

Podpisani Tadej Emeršič, rojen 27. 1. 1990, študent Fakultete za naravoslovje in matematiko Univerze v Mariboru, študijskega programa Fizika, izjavljam, da je magistrsko delo z naslovom **Modelna napoved forsiranega izdihanega volumna v prvi sekundi (FEV1) pred in po zaužitju nesteroidnih antirevmatikov** pri mentorju doc. dr. Alešu Fajmutu avtorsko delo. V magistrskem delu so uporabljeni viri in literatura korektno navedeni; teksti in druge oblike zapisov niso uporabljeni brez navedbe avtorjev.

Maribor, avgust 2015 Podpis:_____

Emeršič T.: Modelna napoved forsiranega izdihanega volumna v prvi sekundi (FEV1) pred in po zaužitju nesteroidnih antirevmatikov

Magistrsko delo, Univerza v Mariboru, Fakulteta za naravoslovje in matematiko, Oddelek za fiziko, 2015.

IZVLEČEK:

Skupina zdravil nesteroidni antirevmatiki (NSAR) lahko po zaužitju pri posameznih osebah povzroči značilne klinične znake, poznane kot aspirinska intoleranca. Astmatika, ki je preobčutljiv na NSAR in ima dva značilna klinična znaka – bronhokonstrikcijo in nosne polipe, imenujemo aspirinsko intolerantni astmatik. Za pojav aspirinske intolerance je ključna razgradnja arahidonske kisline (AA) v belih krvnih celicah. V splošnem ta poteka po ciklooksigenazni in lipoksigenazni poti. Po prvi poti se tvorijo prostanoidi, po drugi pa cisteinil levkotrieni. NSAR učinkujejo na ciklooksigenazno pot tako, da inhibirajo encime in posledično zmanjšujejo produkcijo prostanoidov, kar naj bi vodilo do povišanja produkcije cisteinil levkotrienov. Znižana produkcija prostanoidov in zvišana produkcija cisteinil levkotrienov sta tipični značilnosti aspirinske intolerance na metabolomski ravni. Raziskave, ki ločujejo aspirinsko intolerantne astmatike od aspirinsko tolerantnih astmatikov in neastmatikov, potekajo na različnih nivojih človeškega organizma. Na proteomskem nivoju potekajo raziskave v smeri študija razlik v ekspresiji posameznih ključnih encimov v metabolni mreži AA, na metabolomskem nivoju pa potekajo raziskave v smeri meritev in analize razmerij med posameznimi eikozanoidi. Raziskave na nivoju tkiv in organov so usmerjene v določanje povečane bronhialne reaktivnosti in preobčutljivosti pacientov na vrste in doze NSAR. To se izvaja s t. i. provokacijskimi testi, pri čemer se s spirometrijo meri forsirani izdihani volumen zraka v prvi sekundi (FEV1).

V magistrski nalogi smo s fizikalno-matematičnim modelom povezali proteomski in metabolomski nivo na ravni celice ter napovedali dogajanje na ravni tkiva in organa virtualnega pacienta. Na celičnem nivoju smo z modelom napovedali koncentracije eikozanoidov za različna stanja ekspresij ključnih encimov v procesu metabolizma AA, ki bi naj karakterizirale različne skupine in podskupine pacientov – neastmatike ter astmatike tolerantne in intolerantne na aspirin. Na podlagi teh rezultatov smo najprej na celičnem nivoju napovedali razvito silo v gladkih mišičnih celicah dihalnih poti, nato pa še polmer tipične dihalne poti in končno FEV1. Vse izračune smo izvedli v bazalnem

stanju (tj. brez zaužitega zdravila) in po simulaciji oralnega zaužitja različnih vrst NSAR z različnimi dozami. Z modelom smo določili mejne doze simuliranih NSAR (indometacina, ibuprofena, naproksena in celecoxiba) in paracetamola ter ovrednotili njihovo potentnost v smislu povzročitve bronhokonstrikcije in tveganja astmatičnega napada pri različnih modelnih populacijah aspirinsko intolerantnih astmatikov. Vse modelne simulacije smo izvedli v dveh modelnih stanjih. Prvo simulira stanje neinflamacije, drugo pa stanje inflamacije. Na tak način smo z modelom napovedali in simulirali, kako bi se individualni pacient ali skupina pacientov z razpoznavnim vzorcem v produkciji eikozanoidov in z značilnimi razlikami v ekspresiji posameznih ključnih encimov v metabolni mreži AA lahko odzivali na NSAR v primerih nezdravljene in kontrolirane astme, npr. s kortikosteroidi.

Ključne besede: fizikalni in matematični model, farmakokinetični model, encimska kinetika, inhibicija encimov, metabolne mreže, eikozanoidi, nesteroidni antirevmatiki, gladka mišična celica dihalnih poti, bronhokonstrikcija, astma, aspirinska intoleranca, sistemska farmakologija

Emeršič T.: Model prediction of forced expiratory volume in first second (FEV1) before and after ingestion of non-steroidal anti-inflammatory drugs

Master thesis, University of Maribor, Faculty of Natural Sciences and Mathematics, Department of Physics, 2015.

ABSTRACT:

In some individuals ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) induces typical clinical signs known as aspirin intolerance. Asthmatics with two typical symptoms – bronchoconstriction and nasal polyps, and with hypersensitivity to NSAIDs are identified as aspirin intolerant asthmatics. Degradation of arachidonic acid (AA) in white blood cells is essential for the occurrence of aspirin intolerance. Metabolism of AA is generally divided into the cyclooxygenase and the lipoxygenase pathway revealing prostanoids and leukotrienes as metabolites, respectively. NSAIDs have inhibitory impact on the enzymes of the cyclooxygenase pathway resulting in the reduction of the prostanoids production. As a consequence, this might result in higher cysteinyl leukotrienes production. Low prostanoid levels and high cisteinil leukotrienes levels are typical characteristics of aspirin intolerance at the metabolomic level. The occurrence of aspirin intolerance is studied on different levels of human organism. On the proteomic level, study is focused on detecting the differences in the expression of important enzymes in the AA metabolic pathway, and, on the metabolomic level, the ratios between particular eicosanoids are measured and analysed. Research on the tissue and organ level is oriented in determining the patients' bronchial hyperreactivity and hypersensitivity towards the type and the dose of different NSAIDs. This is carried out with provocation tests, whereby forced expiratory volume in first second (FEV1) is measured by spirometry technique.

With our mathematical model, presented in this master's thesis, we coupled proteomic and metabolomic properties at the cellular level, and gave predictions at the tissue and organ level of a virtual patient. On the cellular level we predicted absolute concentrations of eicosanoids for different expressions of the key enzymes in the metabolism of AA, which characterize different groups and subgroups of patients – non-asthmatics as well as asthmatics tolerant and intolerant to aspirin. Based on these results, we first predicted stress developed in the airway smooth muscle cells, then the diameter of a typical airway, and, finally, FEV1. All calculations were carried out in the basal state (i.e. without drug)

and after the simulated oral dosing of different NSAIDs with different doses. We determined and evaluated the limiting doses of each of the studied NSAIDs (indomethacin, ibuprofen, naproxen and celecoxib) and paracetamol in terms of the potency for the occurrence of bronchoconstriction and the risk for asthmatic attack in cases of different model populations of aspirin intolerant asthmatics. All model simulations were carried out in two different model states; the first one, simulating the state of no-inflammation, and the second one, simulating the state of inflammation. In this way we predicted and simulated, how an individual patient or a patient group with a distinguishing pattern in eicosanoid production as well as with characteristic differences in the expression of the key enzymes within the AA metabolic pathway would respond to NSAIDs in cases of untreated and treated asthma, e.g. with corticosteroids.

Key words: physical and mathematical model, pharmacokinetic model, enzyme kinetics, enzyme inhibition, metabolic pathway, eicosanoids, NSAID, airway smooth muscle cell, bronchoconstriction, asthma, aspirin intolerance, systems pharmacology

POGOSTO UPORABLJENE OKRAJŠAVE

NSAR	nesteroidni antirevmatik
AA	arahidonska kislina
COX-1	encim ciklooksigenaza 1
COX-2	encim ciklooksigenaza 2
PGES	encim sintaza prostaglandina E
5-LOX	encim 5-lipoksigenaza
LTC_4S	encima sintaza levkotriena C_4
cisLT	cisteinil levkotrieni
PGE_2	prostaglandin E_2
PGD_2	prostaglandin D_2
LTC_4	levkotrien C_4
NA	modelna populacija neastmatikov
ATA	modelna populacija aspirinsko-tolerantnih astmatikov
$AIA^{(1)}$	modelna populacija aspirinsko-intolerantnih astmatikov z znižano ekspresijo encima PGES
$AIA^{(2)}$	modelna populacija aspirinsko-intolerantnih astmatikov z zvišano ekspresijo encima LTC_4S
$AIA^{(3)}$	modelna populacija aspirinsko-intolerantnih astmatikov z znižano ekspresijo encima PGES in zvišano ekspresijo encima LTC_4S
NI	stanje neinflamacije
I	stanje inflamacije
FEV1	forsirani ekspiratorni volumen zraka, izdihan v prvi sekundi

KAZALO

1 UVOD

Aspirin in druge nesteroidne antirevmatike (NSAR) uporabljamo kot zdravila za lajšanje vnetja in bolečin. Ta skupina zdravil lahko po zaužitju pri posameznih osebah povzroči klinične znake, kot so zoženje bronhijev (bronhokonstrikcija), vnetje sluznice zgornjih in spodnjih dihal, urtikarijo, angioedem, nosne polipe, rino-sinusitis, konjunktivitis idr. Skupek teh kliničnih znakov imenujemo preobčutljivost na NSAR (angl. NSAID-triggered hypersensitivity) oziroma aspirinska intoleranca [1, 2]. Klinični znaki se lahko pojavijo že prej kot v eni uri po zaužitju zdravila [3], v primeru bronhokonstrikcije pa lahko ta traja tudi do 9 ur in več [4]. Aspirinsko intoleranco je leta 1922 prvi opisal Widal [5], pozneje, leta 1960, pa sta jo z natančnejšim kliničnim opisom opredelila Samter in Beers [6]. Znano je, da aspirinska intoleranca prizadene od 0,6 do 2,5 % celotne populacije, od tega največ žensk in odraslih astmatičnih bolnikov [1, 2, 7, 8]. Astmatika, ki je preobčutljiv na NSAR in ima dva značilna simptoma – bronhokonstrikcijo in nosne polipe, imenujemo aspirinsko intolerantni astmatik [9].

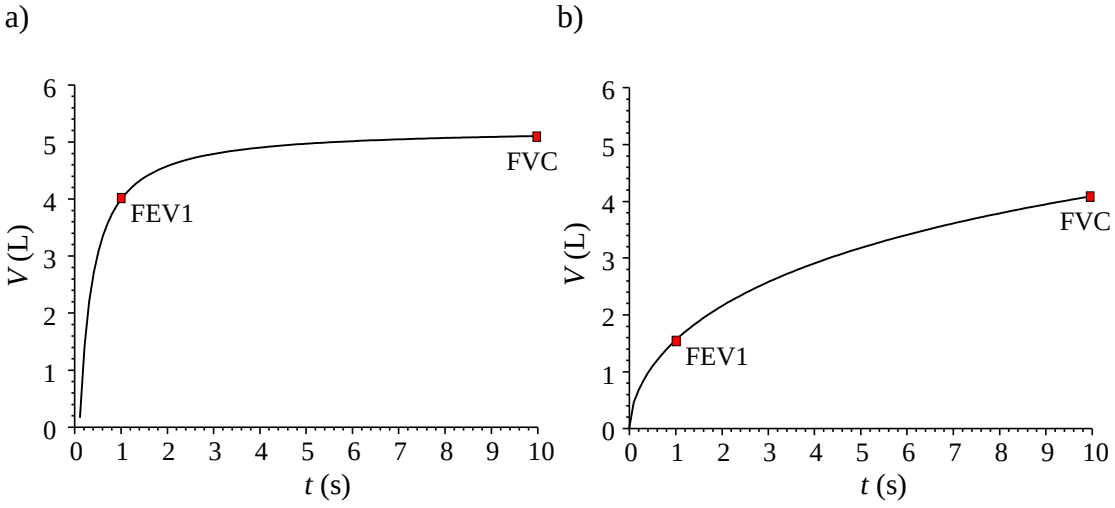
Leta 1971 je J. R. Vane odkril, da je za pojav aspirinske intolerance ključnega pomena inhibicija encimov v procesu razgradnje arahidonske kisline (AA) [10]. To sta encima ciklooksigenaza 1 in ciklooksigenaza 2 (COX-1 in COX-2), imenovana tudi prostaglandin H sintaza 1 in prostaglandin H sintaza 2 (PGHS1 in PGHS2); njun najbolj poznan inhibitor pa je aspirin. Razgradnja AA v celicah poteka po dveh različnih poteh: ciklooksigenazni in lipoksigenazni. Inhibitorni učinek aspirina se kaže v zmanjšani produkciji prostanoidov, ki nastajajo po ciklooksigenazni poti. Pozneje so Szczeklik in sod. potrdili, da tudi ostali NSAR povzročajo enake klinične znake kot aspirin [1]. Odkritja so vodila do spoznanja, da so za pojav aspirinske intolerance poleg prostanoidov pomembni tudi cisteinil levkotrieni (cisLT). To so metaboliti AA, ki nastajajo po lipoksigenazni poti. Izkazalo se je, da inhibicija encimov COX-1 in COX-2 posredno vpliva tudi na njihovo produkcijo [1, 11].

Raziskave aspirinske intolerance, ki ločujejo aspirinsko intolerantne astmatike od aspirinsko tolerantnih astmatikov in neastmatikov, potekajo na različnih nivojih. Na proteomskem nivoju eksperimentalne študije poročajo predvsem o spremenjeni ekspresiji encima sintaze levkotriena C_4 (LTC_4S) [12, 13]. Le-ta je ključen za produkcijo LTC_4. Za

neastmatike je značilna 20-krat nižja ekspresija LTC_4S kot za intolerantne astmatike in 5-krat nižja kot za tolerantne astmatike [12, 13]. Avtorji so v študijah [14, 15] ugotovili razlike tudi v ekspresiji encimov COX-1 in COX-2, vendar rezultati niso enoznačni.

Na metabolomskem nivoju meritve, opravljene na celični ravni, kažejo, da so za razločitev na aspirin tolerantnih in intolerantnih astmatikov ključna razmerja med koncentracijo PGE_2 ([PGE_2]) in cisLT ([*cisLT*]) [11] ter med [PGE_2] in koncentracijo prostaglandina D_2 ([PGD_2]) [14]. Prvo razmerje so analizirali predvsem Schäfer in sod. [11], drugega pa Pierzchalska in sod. [14]. Prvi so meritve opravljali na krvni plazmi različnih astmatikov. Opazili so, da je razmerje pri aspirinsko intolerantnih astmatikih vedno manjše od 1 pred in po doziranju zdravila in se lahko od zdravih osebkov razlikuje tudi za faktor 1000. Avtorji teoretičnih modelov [16-20] so razmerje med [PGE_2] in [*cisLT*] uporabili kot kvantitativni kriterij, na osnovi katerega so poskušali razločevati aspirinsko intolerantne astmatike od tolerantnih in od neastmatikov, le-ti pa se v modelnih simulacijah razlikujejo v ekspresijah posameznih ključnih encimov v procesu razgradnje AA. Z modelom so napovedali tudi mejne doze zdravil, pri katerih bi lahko nastopilo tveganje bronhokonstrikcije [16-20]. Manjša vrednost tega razmerja je predstavljala večje tveganje, saj je povišana produkcija cisLT in zmanjšana produkcija PGE_2 tipična značilnost preobčutljivosti na NSAR [3, 11]. Pierzchalska in sod. [14] so z eksperimentom, izvedenem *in vitro* na izoliranih fibroblastih, odvzetih iz dihalnih poti različnih tipov astmatikov in neastmatikov, analizirali razmerje med [PGE_2] in [PGD_2]. Ugotovili so, da je pri aspirinsko intolerantnih astmatikih to razmerje nižje kot pri aspirinsko tolerantnih astmatikih in neastmatikih. Še večje razlike med temi skupinami pa so se izkazale po tem, ko so v eksperimentu simulirali stanje vnetja z dodatkom mešanice citokinov (t. i. citomiks), ki so jo sestavljali interlevkin 1β (IL-1β), tumorski faktor nekroze (TNF) in lipopolisaharid iz bakterije (LPS) *Pseudomonas aeruginosa*. Produkcija PGE_2 se je pri aspirinsko tolerantnih astmatikih in neastmatikih povišala bistveno bolj kot pri aspirinsko intolerantnih astmatikih, medtem ko se je produkcija PGD_2 pri vseh skupinah približno enako povišala. Razmerje [PGE_2]/[PGD_2] se je po dodatku citomiksa zvišalo pri neastmatikih in aspirinsko tolerantnih astmatikih bolj kot pri aspirinsko intolerantnih astmatikih. Meritve koncentracij metabolitov AA so bile opravljene tudi na drugih vzorcih − kondenzatih izdihanega zraka [21, 22], vendar rezultati niso pokazali značilnih razlik med različnimi astmatičnimi populacijami.

Raziskave na nivoju tkiv in organov potekajo s provokacijskimi testi [2]. Po mestu vnosa zdravila v telo ločimo oralne, bronhialne, nosne in intravenozne provokacijske teste. Njihova izvedba je različna, razlikujejo se v odmerkih zdravila, časovnih intervalih med posameznimi odmerki in kriterijih za pozitivnost testa. Pri tem se bolnikom v telo postopoma vnaša manjša količina NSAR in se opazuje, pri katerih dozah (t. i. mejne doze) se pojavijo značilni simptomi, ali pa se s spirometrijo meri pljučna funkcija bolnika [23]. Spirometrija je zelo pogosta preiskava za merjenje kapacitete in funkcije pljuč. Uporabna je v diagnostiki astme, kronične obstruktivne pljučne bolezni, pljučnega raka ipd. Meritve se opravljajo s spirometrom, napravo, ki meri volumske pretoke in volumne vdihanega in izdihanega zraka. Tipična mera za določitev stanja obstrukcije je maksimalni volumen zraka, ki ga oseba kar se da hitro izdiha v prvi sekundi po maksimalnem vdihu (FEV1) (t. i. forsirani ekspiratorni volumen zraka, izdihan v prvi sekundi). Podatek o FEV1 se lahko izraža v absolutni vrednosti, ali pa kot relativna vrednost glede na forsirano vitalno kapaciteto pljuč (FVC), ki se določi kot volumen izdihanega zraka po maksimalnem možnem vdihu. Za učinkovito meritev FVC izdih traja najmanj 6 sekund [24]. V povprečju lahko zdrava oseba v prvi sekundi izdihne med 70–80 % FVC. V primeru obstrukcije, za katero je značilno blago zoženje dihalnih poti, se ta vrednost zmanjša pod 60 %. Pri osebah z močno bronhokonstrikcijo, ki je lahko posledica astmatičnega napada, pa je vrednost FEV1 manjša od 50 % FVC in lahko v izjemnih primerih pade tudi pod 30 % [24]. Primer časovnega poteka volumna izdihanega zraka pri forsiranem izdihu zdrave osebe in osebe z obstrukcijo prikazuje slika 1.



Slika 1. Primerjava časovnih odvisnosti volumnov izdihanega zraka ter vrednosti FEV1 in FVC za a) zdrave osebe in b) osebe z obstruktivno boleznijo pljuč. Povzeto po [25].

Do sedaj smo lahko s fizikalno-matematičnim modelom [16-20] ob prisotnosti NSAR napovedali stanje, za katerega smo pričakovali oziroma predvidevali zgolj to, da je verjetnost za pojav bronhokonstrikcije povečana. Mera za to je bilo razmerje med [PGE_2] in [*cisLT*] [11, 16-20]. S predlaganim modelom v tej študiji pa želimo preveriti, ali je razmerje [PGE_2]/[*cisLT*] res dobra mera za napoved bronhokonstrikcije, ali pa so za napoved bolj ključne absolutne koncentracije posameznih eikozanoidov. Naša hipoteza je, da se lahko v dihalnih poteh pri enaki vrednosti razmerja med bronhodilatorjem (PGE_2) in bronhokonstriktorji (cisLT) razvije različna sila. Na podlagi 20-odstotnega padca FEV1 od normalne vrednosti bomo določili skladnost z modelom napovedanih in izmerjenih mejnih doz za različne NSAR za različne tipe modelnih skupin astmatičnih bolnikov, saj se na ta način v medicini ugotavlja senzitivnost na NSAR. Pri modelni skupini aspirinsko intolerantnih astmatikov bi moral model potrditi padec FEV1 za 20 % že pri veliko nižji dozi NSAR kot pri na aspirin tolerantni modelni skupini astmatičnih pacientov. Pri modelni skupini neastmatikov do padca FEV1 za 20 % ali več sploh ne bi smelo priti. Pričakujemo tudi, da bo model napovedal padec FEV1 pod 40 % FVC samo pri modelni skupini aspirinsko intolerantnih astmatikov. Dozo zdravila, kjer pride do takšnega zmanjšanja FEV1, imenujemo mejna doza. Nad to mejno dozo bi pri določenem tipu astmatika tvegali močno bronhokonstrikcijo in astmatični napad. Simulacija različnih NSAR bi morala pokazati, da enaka doza zdravila, ki je močnejši inhibitor ciklooksigenaz, povzroči večji padec FEV1, kot tista zdravila, ki so šibkejši inhibitorji. V naši študiji kot najšibkejši inhibitor simuliramo zdravilo paracetamol, znan tudi pod imenom acetaminofen. Kljub temu, da to zdravilo ne deluje protivnetno in ne spada med NSAR, velja za šibkega inhibitorja encimov COX-1 in COX-2 [26].

V nadaljevanju bomo v drugem poglavju najprej opisali produkcijo prostanoidov in levkotrienov. Zatem v tretjem poglavju sledi podroben opis fizikalno-matematičnega modela, s katerim povežemo proteomski in metabolomski nivo na raven celice in napovemo dogajanje na ravni tkiva in organa. V poglavju *Rezultati in diskusija* z modelom napovemo koncentracije eikozanoidov v mišičnem intersticiju dihalnih poti za različna stanja ekspresij ključnih encimov v procesu metabolizma AA, ki bi naj okarakterizirala različne skupine astmatičnih bolnikov. Na podlagi teh koncentracij napovemo razvito silo na nivoju gladke mišičnine dihalnih poti, njen tipičen polmer in vrednost FEV1. Nato z modelom napovemo dozo različnih zdravil, pri kateri vrednost

FEV1 pade pod 40 % FVC. Prav tako določimo dozo zdravila, pri kateri se FEV1 pri določenem tipu modelnega astmatika zniža za 20 % od njegove začetne vrednosti, izmerjene brez zaužitega zdravila.

2 PRODUKCIJA EIKOZANOIDOV

Eikozanoidi so metaboliti arahidonske kisline (AA), ki je polinenasičena ω-6 maščobna kislina z dvajsetimi C-atomi. Ta s pomočjo encima fosfolipaza A_2 (PLA_2) nastaja pretežno iz fosfolipidov, ki so sestavni del celične membrane. Razgradnja AA v celici poteka po dveh različnih poteh, ciklooksigenazni in lipoksigenazni poti. Njeni metaboliti so prostanoidi, levkotrieni, hidroksi(peroksi)eikozatetraenojske (H(P)ETE) kisline, lipoksini (Lx), eoxini (Ex) in trioksilini (TrX). Veliko število njenih metabolitov ima pomembne biološke učinke v telesu. Pomembno vlogo imajo pri bolečinah, vročini ter kontrakciji gladkih mišic in vnetnih obolenjih, kot sta astma in artritis. V različnih celicah so ključni encimi v procesu nastajanja različnih eikozanoidov različno izraženi. Biosintezo posameznih eikozanoidov lahko spodbujajo specifični citokini, rastni faktorji in številni drugi dražljaji. Eikozanoidi po biosintezi celico zapustijo, biološko aktivne oblike pa se vežejo na specifične receptorje tarčnih celic. Praviloma gre za receptorje, ki so sklopljeni z G-proteini (GPCR). Njihovo delovanje je lahko parakrino ali avtokrino, vsem pa je skupno, da je njihova biološka učinkovitost časovno omejena, saj se v zunajceličnem prostoru še naprej pretvarjajo v metabolite, ki pa niso več biološko aktivni. Tipični učinek vezave eikozanoidov na tarčne celice je sprožitev znotrajceličnih signalizacijskih poti, ki lahko vodijo do spremembe koncentracije znotrajceličnega kalcija (Ca^{2+}), ali pa sprožijo tvorbo signalne molekule znotrajceličnega cikličnega adenozin monofosfata (cAMP) [27].

2.1 PROSTANOIDI

Prostanoidi se sintetizirajo po ciklooksigenazni poti razgradnje AA v večini celic našega telesa. Med prostanoide prištevamo prostaglandine PGE_2, PGD_2 in $PGF_{2\alpha}$, prostaciklin I_2 (PGI_2) in tromboksan (TX) A_2 (TXA_2) [28, 29], kot prikazuje slika 2. Po biosintezi v celici s pomočjo transporterja prostaglandinov (PGT) preidejo v zunajcelično okolje, kjer se vežejo na specifične receptorje tarčnih celic [30]. Njihova funkcija v človeškem telesu je različna. Tvorijo se po potrebi in se ne shranjujejo. So avtokrini in parakrini lipidni

mediatorji in navadno učinkujejo v neposredni bližini svojega nastanka [31]. Prostanoidi nastajajo v celici iz skupnega substrata prostaglandina H_2 (PGH_2). Pretvorbo AA v PGH_2 v dveh korakih katalizirata izoencima COX-1 in COX-2, ki imata dve katalitični funkciji – ciklooksigenazno in peroksidazno. Oba encima najprej s ciklooksigenazno aktivnostjo pretvorita AA v prostaglandin G_2 (PGG_2), nato pa s peroksidazno aktivnostjo PGG_2 v PGH_2 [29]. Encim COX-1 je v večini celic stalno prisoten, COX-2 pa inducirajo različni vnetni mediatorji in citokini [31, 32]. Posledično se v vnetnem stanju oziroma stanju inflamacije zaradi povišane aktivnosti COX-2 poviša produkcija prostanoidov [33]. Aktivnost encimov COX-1 in COX-2 pa se zmanjša z učinkovanjem NSAR (slika 2), kar vodi do zmanjšane produkcije prostanoidov [1, 10].

PGE_2 se katalizira iz PGH_2 s pomočjo encima PGES (slika 2). Ta encim je izražen v epitelnih in endotelnih celicah, gladkih mišičnih celicah dihalnih poti, makrofagih, fibroblastih, levkocitih in drugih krvnih celicah [34]. Njegova vloga v pljučih je zelo pomembna. Nastaja predvsem v področju spodnjih dihalnih poti, kjer je njegova vloga proti vnetna in bronhodilatorna tako pri zdravih osebah [35] kot pri astmatikih in bolnikih s kroničnim bronhitisom [36]. PGE_2 inhibira sproščanje ostalih eikozanoidov iz mastocitov, monocitov, nevtrofilcev in eozinofilcev [37]. Veže se lahko na 4 različne izoforme receptorjev tipa EP (EP1, EP2, EP3 in EP4) in jih aktivira [38]. Vezava na receptorja EP2 in EP4, ki sproži znotrajcelično signalizacijo, posredovano preko cAMP, preprečuje bronhokonstrikcijo tako, da relaksira gladke mišice dihalnih poti in inhibira sproščanje ostalih mediatorjev kontrakcije iz mastocitov in drugih vnetnih celic [38, 39]. Aktivacija receptorjev EP1 in EP3 ima kontraktilni učinek [38], saj poviša koncentracijo znotrajceličnega Ca^{2+} [40]. V spodnjih dihalnih poteh so najpogosteje izraženi receptorji tipa EP2, medtem ko so receptorji tipa EP1 pogosteje izraženi v gladkih mišičnih celicah žil [41]. V pljučih tako PGE_2 pripisujemo predvsem bronhodilatorni učinek.

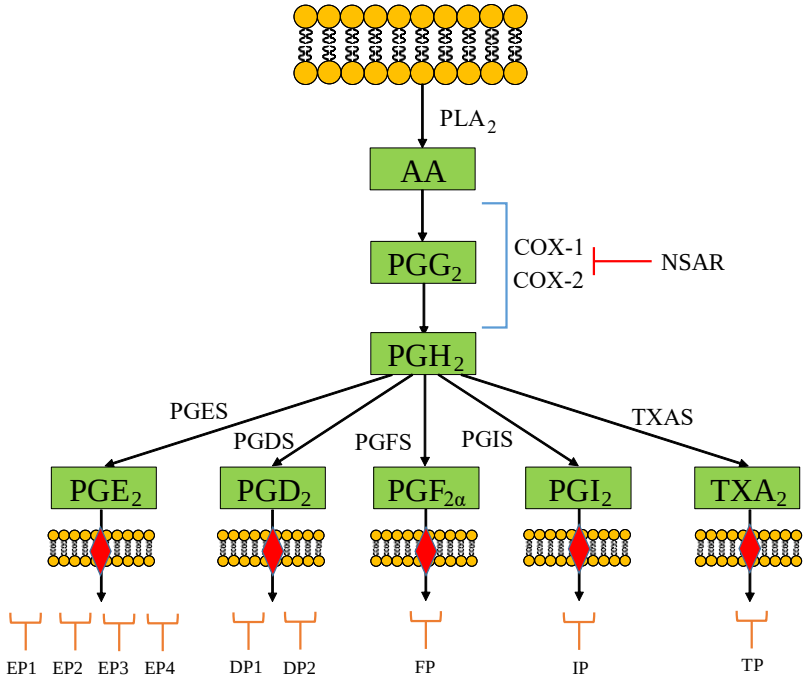
Pretvorbo PGH_2 v PGD_2 katalizira encim sintaza prostaglandina D (PGDS) (slika 2), ki je izražen predvsem v mastocitih in limfocitih Th2 [42, 43]. PGD_2 sodeluje pri različnih fizioloških procesih, kot so vazodilacija, bronhokonstrikcija [44], regulacija bolečine [45], spanje [46], in boleznih, ki jih spremljajo vnetja, kot sta astma [47] in ateroskleroza [48]. PGD_2 se veže na dva receptorja tipa DP (DP1 in DP2) in ju aktivira. Aktivacija receptorja DP1 vodi do povišanja cAMP [49], medtem ko aktivacija DP2 vodi ali do

znižanja cAMP in/ali do povišanja koncentracije znotrajceličnega Ca^{2+} [50]. V *in vitro* poskusih, izvedenih na izoliranih gladkih mišičnih celicah dihalnih poti, so pokazali, da se lahko PGD_2 veže tudi na receptorje za tromboksan (TP) in sproži kontrakcijo [51]. V spodnjih dihalnih poteh mu pripisujemo predvsem bronhokonstrikcijsko vlogo.

$PGF_{2\alpha}$ se iz PGH_2 sintetizira s pomočjo encima sintaze prostaglandina F (PGFS) (slika 2). Ta je izražen v različnih celicah v pljučih in v limfocitih [52]. $PGF_{2\alpha}$ ob vezavi na receptor tipa FP na gladkih mišičnih celicah dihalnih poti poviša znotrajcelično koncentracijo Ca^{2+} [33], zato mu pripisujemo bronhokonstrikcijsko vlogo [53]. Sinteza PGFS se še posebej poveča ob porodu in menstrualnem ciklu žensk [54]. Prav tako kot PGD_2 se tudi $PGF_{2\alpha}$ v gladkih mišicah dihalnih poti veže na receptorje za tromboksan (TP) in učinkuje bronhokonstrikcijsko, kar so demonstrirali v *in vitro* poskusih [51].

Prostaciklin PGI_2 se sintetizira iz PGH_2 s pomočjo encima sintaze prostaglandina I (PGIS), kot prikazuje slika 2. Ta encim je močno izražen v celicah srca, pljuč, raznih gladkih mišic, ledvic in prostate [55]. Vezava PGI_2 na receptor tipa IP poviša raven cAMP [56], kar ima v pljučih bronhodilatorni učinek. Eksperimenti *in vitro* kažejo, da je PGI_2 manj učinkovit bronhodilator kot PGE_2 [57]. PGI_2 med drugim tudi preprečuje nastajanje krvnih strdkov in je antagonist tromboksana (TX) [58].

Tromboksan TXA_2 je glavni metabolit AA v trombocitih. Pretvorbo PGH_2 v TXA_2 katalizira encim sintaza tromboksana A (TXAS) (slika 2), ki je membranski protein endoplazmatskega retikuluma. Njegova ekspresija je še posebej izrazita v raznih celicah pljuč, jeter, ledvic in krvnih celicah [59]. TXA_2 nastaja predvsem v trombocitih, monocitih, makrofagih, nevtrofilcih in pljučnem parenhimu [60]. TXA_2 z vezavo na receptor TP povzroči povišanje koncentracije znotrajceličnega Ca^{2+} in aktivacijo PKC, kar vodi do skrčitve gladkih mišic [59]. Ker je TXA_2 zelo kratkoživ metabolit, se v celicah pogosto detektira in analizira njegov metabolit TXB_2. Ta iz TXA_2 nastane z neencimsko pretvorbo [61].



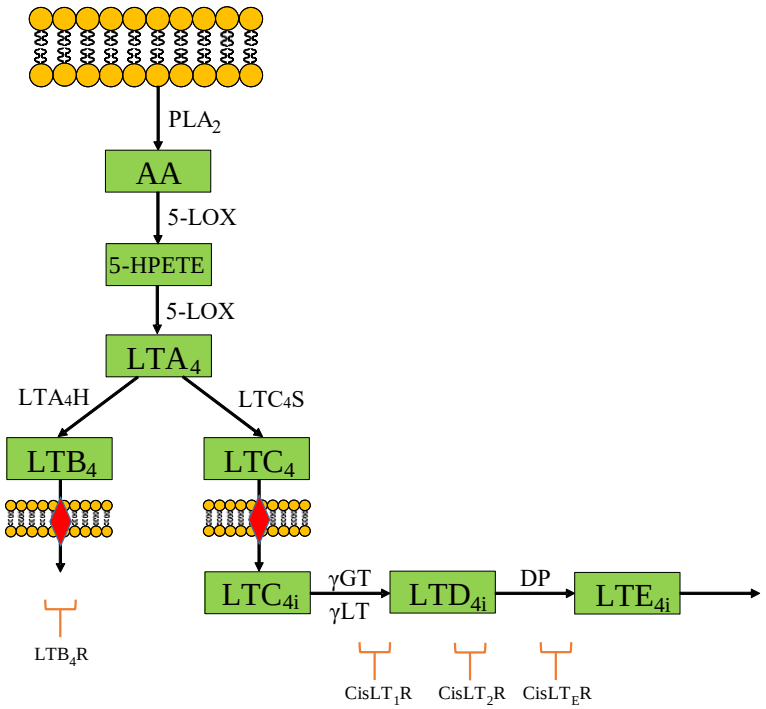
Slika 2. Shematski prikaz nastanka metabolitov AA po ciklooksigenazni poti (prostanoidov) iz skupnega prekurzorja PGH_2 s pomočjo specifičnih encimov. Prostanoidi iz celice prehajajo preko transporterjev (PGT) (rdeči rombi na sliki) in se na tarčnih celicah vežejo na zanje specifične receptorje. NSAR znižujejo aktivnost encimov COX-1 in COX-2 tako, da se nanju reverzibilno ali ireverzibilno vežejo in jih inhibirajo. Za ostale simbole in opise glej tekst.

2.2 LEVKOTRIENI

Po lipoksigenazni poti se AA razgrajuje do levkotrienov in različnih vrst hidro(peroksi)eikozatetranoičnih kislin (H(P)ETE). Levkotrieni nastajajo tako, da encim 5-lipoksigenaza (5-LOX) najprej pretvori AA do 5-hidroperoksieikozatetraenoične kisline (5-HPETE), enak encim pa katalizira še naslednji korak do nastanka levkotriena A_4 (LTA_4). Ta se s pomočjo encima LTA_4 hidrolaza (LTA_4H) naprej pretvori v levkotrien B_4 (LTB_4) ali pa s pomočjo encima LTC_4S ob prisotnosti glutationa (GSH) v LTC_4 [62]. Tako LTB_4 kot LTC_4 z aktivnim transportom preideta v zunajcelično okolje [63]. LTC_4 se izven celice s pomočjo encimov γ-glutamil transpeptidaza (γGT) ali γ-glutamil levkotrinaza (γLT) pretvori v levkotrien D_4 (LTD_4), ta pa se s pomočjo encima dipeptidaza (DP) naprej pretvori v levkotrien E_4 (LTE_4), kot je prikazano na sliki 3. LTE_4

je stabilen metabolit, ki se izloča z urinom. LTC_4, LTD_4 in LTE_4 zaradi podobne strukture in podobnega biološkega učinka imenujemo cisteinil levkotrieni (cisLT) [27]. So mediatorji vnetja in bronhokonstriktorji ter imajo pomembno vlogo v patologiji astme [64]. Tvorijo se predvsem v celicah, ki so udeležene v procesih vnetja. V največji meri jih proizvajajo eozinofilci, bazofilci in mastociti [65].

Po izstopu iz celice LTC_4 in njegov metabolit LTD_4 učinkujeta na dva tipa receptorjev za cisteinil levkotriene ($CisLT_1R$ in $CisLT_2R$), ki se nahajajo v celičnih membranah tarčnih celic. Pri tem ima LTD_4 10-krat večjo afiniteto do $CisLT_1R$ kot LTC_4, njuna afiniteta do $CisLT_2R$ pa je enaka [66, 67]. Študije *in vitro* kažejo, da sta približno 1000-krat močnejša bronhokonstriktorja kot histamin [68]. Bronhokonstrikcijo povzroča tudi metabolit LTE_4, ki učinkuje predvsem na receptor $CisLT_E$ [69]. LTB_4 se po izstopu iz celice veže na receptor LTB_4R, vendar nima učinka na gladke mišične celice in ne povzroča bronhokonstrikcije [37]. Aktivacija receptorjev $CisLT_1R$ in $CisLT_2R$ naj bi povzročila povišanje koncentracije znotrajceličnega Ca^{2+} ali znižanje ravni cAMP, vendar natančna signalna pot učinkovanja vezave cisLT na ta receptorja še ni popolnoma raziskana [27].



Slika 3. Shematski prikaz lipoksigenazne poti razgradnje AA, po kateri nastajata dva tipa levkotrienov – cisteinil levkotrien (LTC_4) in levkotrien B_4 (LTB_4) s pomočjo specifičnih encimov. Oba tipa levkotrienov iz celice prehajata preko aktivnega transporta. LTC_4 se v

zunajceličnem okolju s pomočjo encimov pretvarja v metabolit LTD_4, ta pa naprej v LTE_4, ki ju uvrščamo med cisLT. Vsi se na tarčnih celicah vežejo na zanje specifične receptorje. Na sliki so cisLT v intersticiju označeni z indeksom i. Za dodatne opise glej tekst.

Razgradnja AA po lipoksigenazni poti, opisana in prikazana na sliki 3, je lahko še kompleksnejša. Obstajajo še druge izoforme lipoksigenaz (LOX), kot sta 12-LOX in 15-LOX, s pomočjo katerih AA metabolira do vmesnih produktov 12-HPETE in 15-HPETE. Ti produkti metabolirajo do končnih metabolitov lipoksinov (LX), eoksinov (EX) in številnih drugih hidroksieikozatetranoičnih kislin (HETE). Njihovega podrobnega mehanizma ne podajamo, saj ti nimajo tako natančno definiranih in poznanih bioloških aktivnosti kot levkotrieni, ki nastajajo po 5-lipoksigenazni poti [70].

3 FIZIKALNO-MATEMATIČNI MODEL ZA NAPOVED FEV1 PO ORALNEM DOZIRANJU NSAR V RAZLIČNIH MODELNIH POPULACIJAH

V tem poglavju opišemo matematični model za napoved FEV1. Osnovo tega modela predstavlja model, ki smo ga avtorji Aleš Fajmut, Tadej Emeršič, Andrej Dobovišek, Nataša Antić, Dirk Schäfer in Milan Brumen objavili v članku [20] z naslovom »*Dynamic model of eicosanoid production with special reference to non-steroidal anti-inflammatory drug-triggered hypersensitivity*« v reviji IET Systems Biology. Kopija članka se v celoti nahaja v prilogi 2, na katero se v tem delu občasno sklicujemo. Osnovo tega kompleksnejšega modela [20] je predstavljal poenostavljeni model, ki so ga prvič predstavili Dobovišek v doktorski disertaciji [16] ter Dobovišek in sod. v z doktorsko disertacijo povezanih izvirnih znanstvenih člankih [17-19].

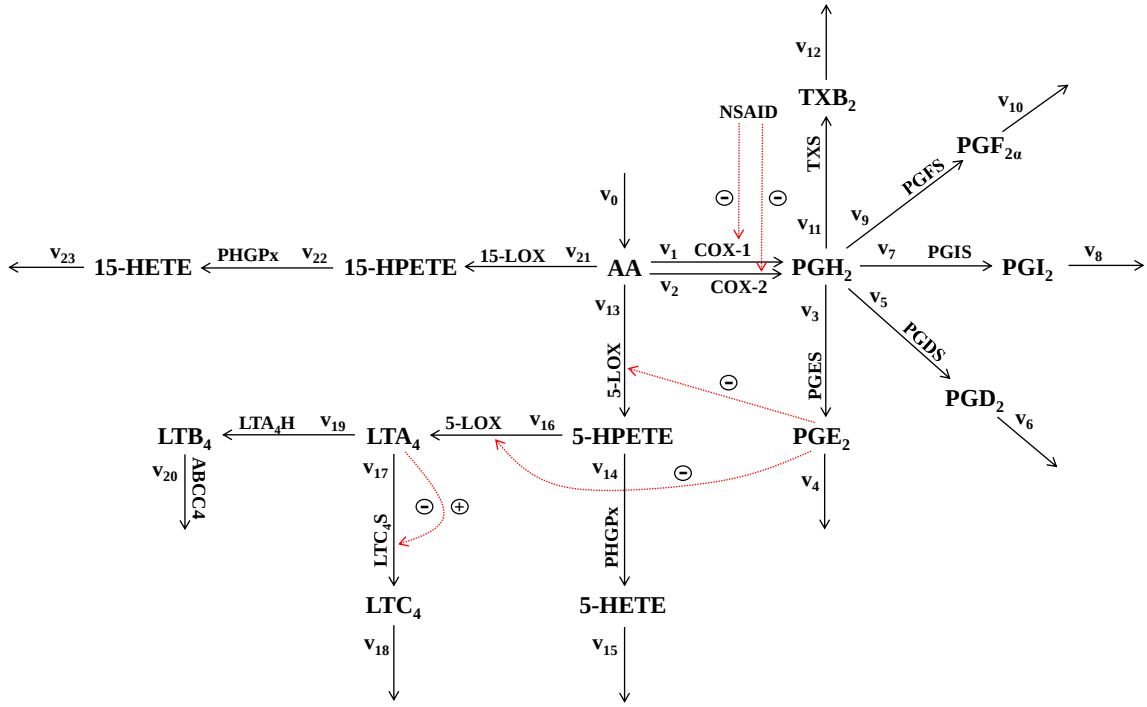
Dinamični fizikalno-matematični model, ki je predstavljen v tem članku, je namreč v tem delu še nadgrajen glede na predhodni kompleksnejši model [20] tako, da je s celostnim modelom moč napovedati stopnjo bronhokonstrikcije na podlagi FEV1 za različne modelne populacije. Le-te smo že predhodno definirali na podlagi razlik v ekspresijah različnih ključnih encimov v procesu metabolizma AA [20]. Prav tako smo definirali dve stanji modela – to sta stanji inflamacije in neinflamacije [20], ki sta osnovani na podlagi

številnih meritev in eksperimentalnih študij na proteomski in metabolomski ravni. Tukaj predstavljena nadgradnja modela omogoča, poleg napovedi absolutnih koncentracij vseh eikozanoidov in njihovih razmerij na ravni celice, še napoved njihovih absolutnih koncentracij v intersticiju dihalnih poti. Le-te so vhodni podatek za izračun sile kot posledice učinkovanja eikozanoidov na tarčne celice, tj. gladke mišične celice dihalnih poti, kjer učinkujejo tako, da povzročajo kontrakcijo ali relaksacijo teh celic. Sila je v modelu izračunana kot relativna vrednost glede na holinergično stimulacijo s supramaksimalno koncentracijo agonista. Končno na podlagi razvite sile v gladkih mišičnih celicah dihalnih poti z modelom napovemo še FEV1 in ga simuliramo za različne doze različnih vrst NSAR. S tem lahko simuliramo razmere, kakršne v realnosti na pacientih izvajajo pri provokacijskih testih, in z modelom napovemo jakost odziva hipotetičnega pacienta za posamezno dozo določenega NSAR. S tem model omogoča globlji kvantitativni vpogled v biološko učinkovitost zdravil in fiziološki odziv neastmatika ter na aspirin tolerantnega in intolerantnega astmatika na ta zdravila.

3.1 PODMODEL ZA NAPOVED PRODUKCIJE EIKOZANOIDOV NA RAVNI CELICE

Produkcijo eikozanoidov v celici opišemo s fizikalno-matematičnim modelom [20] (priloga 2), ki obravnava kompleksno razgradnjo AA po ciklooksigenazni in lipoksigenazni poti v levkotriene (LTA_4, LTB_4, LTC_4), hidro(peroksi)eikozatetraenoične kisline (5-H(P)ETE, 15-H(P)ETE), prostanoide (PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2, TXB_2) ter njihov transport v zunajcelično okolje, kot je opisano v poglavju 2. V ciklooksigenazni poti razgradnje AA upoštevamo, da le-ta s pomočjo encimov COX-1 in COX-2 metabolira v PGH_2, ta pa naprej z ustreznimi encimi v različne prostanoide. Pri tem upoštevamo inhibicijo COX-1 in COX-2 z NSAR, katerega koncentracijo v krvni plazmi simuliramo s farmakokinetičnim modelom. Po lipoksigenazni poti se AA ob prisotnosti encimov 5- ali 15-LOX pretvori v metabolit 5- ali 15- HPETE, ta dva pa naprej v 5- ali 15-HETE. 5-HPETE se lahko tudi s pomočjo 5-LOX pretvori v LTA_4, ta pa naprej ali v LTB_4 ali v LTC_4. V modelu upoštevamo, da se vsi končni nastali eikozanoidi v celici izločijo v zunajcelično okolje. Vpliv ciklooksigenazne poti na lipoksigenazno pot upoštevamo z inhibitornim učinkom metabolita PGE_2 na encim 5-LOX [11, 16-19]. Po zaužitju in učinkovanju NSAR se zmanjša aktivnost COX-1 in COX-2. Posledično se zmanjša produkcija vseh prostanoidov, med drugim tudi PGE_2. Tako je zaradi manjše

produkcije PGE_2 njegov inhibitorni učinek na 5-LOX manjši. To vodi do povišane produkcije levkotrienov, kar je tipična značilnost aspirinske intolerance [11]. Kinetična shema fizikalno-matematičnega modela za napoved produkcije eikozanoidov na ravni celice je prikazana na sliki 4.



Slika 4. Kinetična shema fizikalno-matematičnega modela za napoved produkcije eikozanoidov na ravni celice. Črne polne puščice z ustreznimi encimi predstavljajo smer tokov, rdeče črtkane puščice pa inhibicijski ali aktivacijski učinek metabolitov na encime. Za podrobnejši opis glej tekst.

Obravnavani fizikalno-matematični model (slika 4) sestoji iz 14 navadnih diferencialnih enačb prvega reda, ki opisujejo časovno spreminjanje vseh spremenljivk v sistemu. To so koncentracija AA ([*AA*]) in koncentracije vseh njenih metabolitov: ([PGH_2], [PGE_2], [PGD_2], [PGI_2], [$PGF_{2\alpha}$], [TXB_2], [*5-HPETE*], [*5-HETE*], [LTA_4], [LTC_4], [LTB_4], [*15-HPETE*] in [*15-HETE*]). Časovni odvod izbrane spremenljivke v sistemu (M) je enak razliki med vsoto vseh pritekajočih tokov ($\sum v_{\text{in}}$) in vsoto vseh odtekajočih tokov ($\sum v_{\text{out}}$):

$$\frac{\mathrm{d}[M]}{\mathrm{d}t} = \sum v_{\text{in}} - \sum v_{\text{out}} . \qquad (1)$$

Tokove i = 3, 5, 7, 9, 11, 14, 19, 20, 21 in 22, označene s polnimi črnimi puščicami na sliki 4, opišemo z Michaelis-Mentenino encimsko kinetiko:

$$v_i = \frac{v_{\max i}[S]}{K_i + [S]}. \qquad (2)$$

V enačbi (2) je $v_{\max i}$ maksimalna hitrost rekcije, K_i Michaelis-Mentenina konstanta in S ustrezen substrat posamezne reakcije. Encimske reakcije, v katerih se na encim reverzibilno veže inhibitor, opišemo s kinetiko reverzibilne kompetitivne inhibicije encima:

$$v_i = \frac{v_{\max i}[S]}{K_i\alpha + [S]}, \text{ kjer je } \alpha = \left(1 + \frac{[I]}{K_{Ii}}\right). \qquad (3)$$

Uporabimo jo za tokove i = 1, 2, 13, 16; $v_{\max i}$ je maksimalna hitrost rekcije, K_i je Michaelis-Mentenina konstanta, S ustrezen substrat posamezne reakcije, I inhibitor in K_{Ii} ravnotežna disociacijska konstanta vezave inhibitorja na encim. Z enačbo (3) opišemo inhibitorni učinek NSAR na encima COX-1 in COX-2 ter posredni inhibitorni učinek PGE_2 na encim 5-LOX. Časovni potek koncentracije NSAR v krvni plazmi po oralnem doziranju ([*NSAR*]) opisuje enačba (6). Aktivnost encima LTC_4S opišemo s kinetiko avtoinhibicije encima s substratom; pri tem pa ima hitrost encimske reakcije tipično obliko zvonaste krivulje v odvisnosti od količine substrata, ki je v tem primeru LTA_4:

$$v_i = \frac{v_{\max i}[LTA_4]}{A + B[LTA_4] + C[LTA_4]^2} \quad . \qquad (4)$$

To velja za i = 17, kjer so A, B in C ustrezni parametri in $v_{\max i}$ maksimalna hitrost rekcije. Tokove večine metabolitov (razen LTC_4 in LTB_4) iz celice opišemo z linearno odvisnostjo:

$$v_i = k_i[M]. \qquad (5)$$

Za i = 4, 6, 8, 10, 12, 15, 18, 23, kjer je k_i hitrostna konstanta prehajanja metabolitov preko celične membrane, [M] pa ustrezni posamezni končni metabolit AA.

Časovni potek koncentracije aktivne zdravilne učinkovine (krajše zdravila) v krvni plazmi [*NSAR*] pri oralnem zaužitju NSAR opišemo s standardnim dvoshrambnim farmakokinetičnim modelom:

$$[NSAR] = \frac{Dk_a}{MV\left(k_a - k_e\right)}\left(e^{-k_e t} - e^{-k_a t}\right). \qquad (6)$$

Model upošteva, da zdravilo z zaužitjem preide v prebavni trakt, iz katerega se absorbira v krvno plazmo, od koder se nato eliminira. V enačbi (6) je D doza oziroma masa zaužitega zdravila, M molekulska masa zdravila, V navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila, k_a hitrost absorpcije zdravila iz prebavnega trakta v kri in k_e hitrost eliminacije zdravila iz krvi.

3.1.1 Modelne populacije v stanju inflamacije in v stanju neinflamacije

Za simulacijo učinkovanja zdravil definiramo različne modelne populacije na podlagi poznanih razlik v ekspresijah posameznih ključnih encimov v procesu metabolizma AA. Prvi so na tak način modelne populacije definirali Dobovišek v doktorski disertaciji [16] ter Dobovišek in sod. v z doktorsko disertacijo povezanih člankih [17-19]. Njihove teoretične analize z modelom so pokazale, da imajo pri pojavu aspirinske intolerance najpomembnejšo vlogo razlike v ekspresijah encima LTC_4S, ki so bile tudi eksperimentalno potrjene [12]. V aktualni model [20] smo v definicijo modelnih populacij vključili še razlike v ekspresiji encima PGES, ki sicer niso bile direktno eksperimentalno potrjene, so pa na te razlike kazali eksperimentalni rezultati produkcije PGE_2 pri na aspirin tolerantnih in intolerantnih astmatikih [14]. V našem modelu tako definiramo modelno kontrolno skupino neastmatikov (NA) in 4 različne modelne astmatične populacije, pri čemer ena karakterizira aspirinsko tolerantne astmatike (ATA), ostale tri pa aspirinsko intolerantne astmatike ($AIA^{(1)}$, $AIA^{(2)}$, $AIA^{(3)}$). Modelne populacije astmatikov se med seboj razlikujejo po ekspresiji encimov PGES in/ali LTC_4S, kar bo predstavljeno v nadaljevanju. Naloga modela je, da karakterizira, katera izmed omenjenih razlik na proteomskem nivoju rezultira obnašanje na metabolomskem nivoju, ki je značilno za aspirinsko intoleranco. Po vrhu vsega simuliramo vsako posamezno modelno populacijo v dveh različnih stanjih – v stanju inflamacije (I) in v stanju neinflamacije (NI). Na ta način simuliramo 10 različnih modelnih stanj. Natančen opis

definicije modelnih populacij in modelnih stanj inflamacije in neinflamacije je podan v prilogi 3. Tukaj zelo na kratko povzamemo postopek. V procesu izgradnje modela najprej definiramo set parametrov za opis referenčnega stanja modela. To je simulacija neastmatika (NA) v stanju neinflamacije (NI), krajše NA-NI. To referenčno stanje modela je osnova za definiranje vseh ostalih modelnih populacij in za definicijo stanja inflamacije. Pri definiciji stanja inflamacije za referenčno stanje neastmatika smo se opirali na številne eksperimentalne podatke: izmerjene poraste koncentracij metabolitov PGE_2 in PGD_2 ter izmerjene spremembe razmerja $[PGE_2]/[PGD_2]$ v *in vitro* eksperimentu, v katerem so stanje inflamacije simulirali s citomiksom [14], izmerjene poraste ekspresij posameznih encimov v stanjih inflamacije v študijah, izvedenih na izoliranih celicah ali tkivih ter v *in vivo* živalskih modelih [14, 71-81] ter nenazadnje tudi na senzitivnostno analizo našega modela, predstavljene v prilogi 3. Podrobnejši opis definicije stanja inflamacije je podan v prilogi (Appendix-u) naše študije [20] (glej prilogo 3).

Na podlagi eksperimentalnih ugotovitev [12] v modelu aspirinsko tolerantne astmatike (ATA) razlikujemo od zdrave populacije (NA) po 4-krat višji ekspresiji encima LTC_4S, populacijo aspirinsko intolerantnih astmatikov 2 in 3 ($AIA^{(2)}$ in $AIA^{(3)}$) pa od aspirinsko tolerantnih razlikujemo po 5-krat višji ekspresiji LTC_4S. Modelni aspirinsko intolerantni astmatiki 1 ($AIA^{(1)}$) in aspirinsko tolerantni astmatiki se med seboj ne razlikujejo po ekspresiji LTC_4S, temveč po PGES. Po eksperimentalnih meritvah Pierzchalske in sod. [14] v modelu predpostavimo nižjo ekspresijo PGES pri aspirinsko intolerantnih astmatikih 1 in 3 ($AIA^{(1)}$ in $AIA^{(3)}$), pri na aspirin intolerantnih astmatikih 2 ($AIA^{(2)}$) pa v stanju neinflamacije predpostavimo enako ekspresijo, kot pri tolerantnih astmatikih in neastmatikih. Razlike med stanjema inflamacije in neinflamacije pa so še v vrednosti ekspresij encimov: PLA_2, COX-2, 5-LOX, 15-LOX in PGES, ki so vse večje v stanju inflamacije. Za podrobnejše vrednosti razlik v ekspresijah encimov glej tabele 1, 2 in 3 v prilogi 2. Pri tem v modelu upoštevamo, da je ekspresija encima sorazmerna njegovi totalni koncentraciji v celici, le-ta pa maksimalni aktivnosti encima (v_{max}). Tako smo vse ekspresije encimov izrazili z v_{max}. Celotni set modelnih parametrov in njihove vrednosti so za referenčno stanje neastmatikov v neinflamaciji podane v tabeli 2 v prilogi 3.

3.2 PODMODEL ZA NAPOVED ABSOLUTNIH KONCENTRACIJ EIKOZANOIDOV V PLJUČNEM INTERSTICIJU

Zgoraj na kratko predstavljeni fizikalno-matematični model za izračun produkcije eikozanoidov na ravni ene vnetne celice [20] tukaj nadgradimo tako, da model omogoča izračun absolutnih koncentracij v intersticiju spodnjih dihalnih poti za zgoraj definirane modelne populacije NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$. Ta podmodel predstavlja enega izmed izvirnih delov tega magistrskega dela.

V nadgradnji upoštevamo, da so vnetne celice, ki v največji meri prispevajo k produkciji eikozanoidov, eozinofilci, ki so infiltrirani v stene dihalnih poti [82]. Po zgledu Demina in sod. [83] število vnetnih celic izrazimo z volumnom vseh aktivnih eozinofilcev (V_a) v dihalnih poteh, ki so sposobni producirati eikozanoide:

$$V_a = C_a N_A V_{avg} V_i \,. \tag{7}$$

Pri tem je C_a koncentracija vseh aktivnih eozinofilcev, N_A Avogadrovo število, V_{avg} povprečni volumen eozinofilca in V_i volumen mišičnega intersticija pljuč [83]. Vrednosti omenjenih parametrov so podane v tabeli 1 v prilogi 1. Ker je v stanju inflamacije število aktivnih infiltriranih eozinofilcev v dihalne poti večje kot v stanju neinflamacije [84], je V_a novi parameter, po katerem se stanji med seboj razlikujeta. Na podlagi ocene iz meritev [84] je v modelnem stanju inflamacije parameter V_a 2-krat večji kot v neinflamaciji. Ta parameter (V_a) upoštevamo pri opisu prehoda eikozanoidov iz celice v mišični intersticij (slika 5), kot je prikazano v enačbah (8), (11) in (12).

V modelu upoštevamo, da se LTC_4 po prehodu preko celične membrane v intersticij s pomočjo encimov pretvori najprej v LTD_4, ta pa na enak način še naprej v LTE_4 (glej sliko 3, kjer vse cisLT v intersticiju označujemo z indeksom i). LTE_4 se nato eliminira iz intersticija, medtem ko eliminacije LTC_4 in LTD_4 iz intersticija posebej ne upoštevamo. Po zgledu Demina in sod. [83] pretvorbe cisLT iz ene v drugo obliko v intersticiju opišemo z linearnimi nastavki. Časovno spreminjanje koncentracij cisLT v intersticiju ($[LTC_{4i}]$, $[LTD_{4i}]$ in $[LTE_{4i}]$) tako opišemo z naslednjimi tremi diferencialnimi enačbami:

$$\frac{\mathrm{d}[LTC_{4\mathrm{i}}]}{\mathrm{d}t} = \frac{V_{\mathrm{a}}\ k_{\mathrm{LTC\_exp}}\ [LTC_4] - V_{\mathrm{d\_LTC}}\ k_{\mathrm{LTC}}\ [LTC_{4\mathrm{i}}]}{V_{\mathrm{d\_LTC}}}, \tag{8}$$

$$\frac{\mathrm{d}[LTD_{4\mathrm{i}}]}{\mathrm{d}t} = \frac{V_{\mathrm{d\_LTC}}\ k_{\mathrm{LTC}}\ [LTC_{4\mathrm{i}}] - V_{\mathrm{d\_LTD}}\ k_{\mathrm{LTD}}\ [LTD_{4\mathrm{i}}]}{V_{\mathrm{d\_LTD}}}, \tag{9}$$

$$\frac{\mathrm{d}[LTE_{4\mathrm{i}}]}{\mathrm{d}t} = \frac{V_{\mathrm{d\_LTD}}\ k_{\mathrm{LTD}}\ [LTD_{4\mathrm{i}}] - V_{\mathrm{d\_LTE}}\ k_{\mathrm{LTE\_elim}}\ [LTE_{4\mathrm{i}}]}{V_{\mathrm{d\_LTE}}}, \tag{10}$$

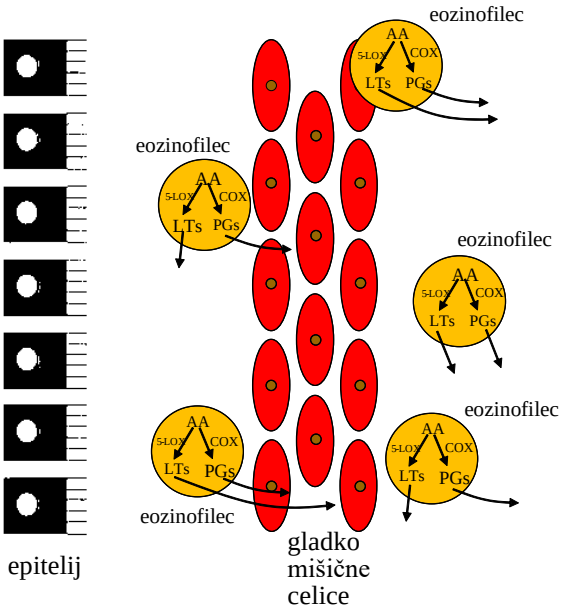
kjer je $k_{\mathrm{LTC\_exp}}$ hitrostna konstanta pretoka LTC_4 iz celice v intersticij [16-20], k_{LTC} hitrostna konstanta pretvorbe $LTC_{4\mathrm{i}}$ v $LTD_{4\mathrm{i}}$, k_{LTD} hitrostna konstanta pretvorbe $LTD_{4\mathrm{i}}$ v $LTE_{4\mathrm{i}}$ in $k_{\mathrm{LTE\_elim}}$ hitrostna konstanta eliminacije $LTE_{4\mathrm{i}}$ iz intersticija [83, 85]. V enačbah (8)–(10) upoštevamo tudi navidezne volumne porazdelitve posameznih metabolitov v dihalnih poteh ($V_{\mathrm{d\_LTC}}$, $V_{\mathrm{d\_LTD}}$, $V_{\mathrm{d\_LTE}}$). Vrednosti parametrov, ki nastopajo v enačbah (8)–(10) in jih povzamemo po Deminu in sod. [83], so podane v tabeli 1 v prilogi 1.

Za PGE_2 in PGD_2 upoštevamo, da se po prehodu iz celic v intersticij iz njega eliminirata, kar lahko pomeni direktno izločanje, ali pa prehod iz biološko aktivnih v neaktivne oblike, poznane pod imenoma M-PGE_2 in M-PGD_2. Spreminjanje koncentracij PGE_2 in PGD_2 v intersticiju ([$PGE_{2\mathrm{i}}$] in [$PGD_{2\mathrm{i}}$]) v diferencialnih enačbah prav tako opišemo z linearnimi nastavki po zgledu enačb (8)–(10):

$$\frac{\mathrm{d}[PGE_{2\mathrm{i}}]}{\mathrm{d}t} = \frac{V_{\mathrm{a}}\ \ k_{\mathrm{PGE\_exp}}\ [PGE_2] - V_{\mathrm{d\_PGE}}\ k_{\mathrm{PGE\_elim}}\ [PGE_{2\mathrm{i}}]}{V_{\mathrm{d\_PGE}}}, \tag{11}$$

$$\frac{\mathrm{d}[PGD_{2\mathrm{i}}]}{\mathrm{d}t} = \frac{V_{\mathrm{a}}\ \ k_{\mathrm{PGD\_exp}}\ [PGD_2] - V_{\mathrm{d\_PGD}}\ k_{\mathrm{PGD\_elim}}\ [PGD_{2\mathrm{i}}]}{V_{\mathrm{d\_PGD}}}, \tag{12}$$

kjer sta $V_{\mathrm{d\_PGE}}$ in $V_{\mathrm{d\_PGD}}$ navidezna volumna porazdelitve $PGE_{2\mathrm{i}}$ in $PGD_{2\mathrm{i}}$ v intersticiju, $k_{\mathrm{PGE\_exp}}$ in $k_{\mathrm{PGD\_exp}}$ hitrostni konstanti pretoka PGE_2 in PGD_2 iz celice v intersticij [16-20], $k_{\mathrm{PGE\_elim}}$ in $k_{\mathrm{PGD\_elim}}$ pa sta hitrostni konstantni eliminacije $PGE_{2\mathrm{i}}$ in $PGD_{2\mathrm{i}}$ iz intersticija. Vrednosti parametrov, ki nastopajo v enačbi (11) in (12), so podane v tabeli 1 v prilogi 1.



Slika 5. Shematski prikaz prereza stene dihalne poti in prehoda eikozanoidov iz eozinofilcev, infiltriranih v mišični intersticij dihalnih poti, ki je medcelična v stenah dihalnih poti.

3.3 PODMODEL ZA NAPOVED RAZVOJA SILE V GLADKIH MIŠICAH DIHALNIH POTI

Tudi ta del magistrskega dela predstavlja dodatno nadgradnjo našega objavljenega modela [20] in je izvirni prispevek.

Kontrakcijo gladkih mišic dihalnih poti lahko sprožijo različni hormoni, zdravila, živčni prenašalci in eikozanoidi. Slednji bi naj igrali pomembno vlogo predvsem pri astmi in vnetnih stanjih, saj je njihova koncentracija v mišičnem intersticiju ob bronhih in bronhiolih takrat povišana. Razvoj sile v gladki mišični celici je prvenstveno odvisen od porasta znotrajcelične koncentracije Ca^{2+}. Čeprav obstajajo zelo dobri modeli [86-89] za opis razvoja sile za primer holinergične stimulacije, kjer vezava acetilholina (Ach) na receptorje sproži produkcijo sekundarnega prenašalca inozitol tri-fosfata (IP_3), ki je odgovoren za izpust Ca^{2+} iz sarkoplazemskega retikuluma v gladki mišični celici in posledičnega dviga njegove koncentracije v citoplazmi, pa je za razvoj sile v primeru stimulacije celice z eikozanoidi ta model nemogoče uporabiti. Znotrajcelične signalne poti po vezavi teh agonistov namreč niso popolnoma poznane, ali pa so zelo kompleksne

in vključujejo več izoform receptorjev, več sekundarnih prenašalcev in regulacijo množice različnih encimov v celici. Znano je sicer, da vezava nekaterih eikozanoidov na receptorje gladkih mišičnih celic lahko poviša koncentracijo znotrajceličnega Ca^{2+}, ali pa vpliva na produkcijo sekundarnega prenašalca cAMP znotraj celice (za pregled glej 2. poglavje). V našem modelu zato izberemo drugačen pristop. Zaobidemo sekundarne prenašalce in Ca^{2+} ter izdelamo matematični izraz za odvisnost sile od koncentracij posameznih eikozanoidov, ki imajo dokazan učinek bronhokonstrikcije ali bronhodilacije, in katerih koncentracije so v našem modelu dobro določene na podlagi meritev. Ta izraz temelji izključno na podlagi izmerjenih odvisnosti sile od posameznih koncentracij agonistov, izvedenih v *in vitro* študijah [90-92]. Kot ključne bronhokonstriktorje upoštevamo LTC_4, LTD_4, LTE_4 in PGD_2, kot ključnega bronhodilatorja pa PGE_2. Ostalih agonistov, kot sta npr. histamin in acetilholin, v modelu ne upoštevamo, saj želimo z modelnimi simulacijami napovedati zgolj učinek na bronhokonstrikcijo po zaužitju NSAR, ne pa npr. odziva na alergične reakcije ipd.

Iz objavljenih *in vitro* oziroma *ex vivo* meritev, izvedenih na spiralnih trakcih traheje [90] in pljučne paranhime morskih prašičkov [92] ter mišje traheje [91], smo s pomočjo orodja Digitize v programu OriginLab pridobili matematične odvisnosti sile, ki se najbolje prilegajo meritvam v odvisnosti od absolutnih koncentracij posameznih bronhokonstriktorjev ($[LTC_{4i}]$, $[LTD_{4i}]$, $[LTE_{4i}]$, $[PGD_{2i}]$) in bronhodilatorja ($[PGE_{2i}]$). Na ta način pridobljene odvisnosti so prikazane na sliki 6a.

Izmerjenim vrednostim sile v odvisnosti od koncentracij bronhokonstriktorjev smo prilagodili Hillovo funkcijo oblike:

$$F = \frac{F_{\max}\left[M\right]^n}{K^n + \left[M\right]^n}, \tag{13}$$

izmerjenim vrednostim sile v odvisnosti od koncentracije bronhodilatorja pa funkcijo oblike:

$$F = F_{\text{start}} + \frac{\left(F_{\text{end}} - F_{\text{start}}\right)\left[M\right]^n}{K^n + \left[M\right]^n}, \tag{14}$$

kjer je F_{max} maksimalna razvita sila, ki se teoretično razvije pri supramaksimalni koncentraciji posameznega eikozanoida (M), K je njegova koncentracija pri do polovice razviti sili v primerjavi z maksimalno (t. i. konstanta polovične zasičenosti), n je Hillov koeficient ter F_{start} in F_{end} sta velikosti sile pri začetni (majhni) in končni (veliki) koncentraciji posameznega eikozanoida. Izmerjene sile so v študijah [90-92] prikazane v procentih glede na maksimalno možno razvito silo v gladki mišičnini dihalnih poti, ki jo povzroči mešanica acetilholina, histamina in kalijevega klorida. Vrednosti parametrov, ki nastopajo v enačbah (13) in (14) za različne eikozanoide, so podane v tabeli 2 v prilogi 1.

S slike 6a je razvidno, da ima izmed vseh upoštevanih bronhokonstriktorjev LTC_{4i} najvišji kontraktilni učinek, in da je polovična vrednost sile dosežena pri nižjih koncentracijah, kot pri ostalih agonistih. Izmed vseh ima PGD_{2i} najmanjši kontraktilni učinek, pa tudi polovična vrednost sile je dosežena pri višjih koncentracijah kot pri ostalih. Maksimalne sile, ki se razvijejo pod vplivom PGD_{2i}, LTD_{4i} in LTE_{4i}, so približno enake. Edini bronhodilator, ki ga v modelu upoštevamo, je PGE_{2i}. Njegov učinek je tak, da povzroči znižanje že predhodno razvite sile. Z drugimi besedami bi lahko rekli, da sistem desenzitizira, saj je za dosego enake razvite sile ob njegovi prisotnosti potrebna višja koncentracija kontraktilnega agonista. Odvisnost sile od koncentracije PGE_{2i} prikazuje padajoča krivulja v diagramu na sliki 6a. Omenjena odvisnost je bila izmerjena pri stalni supramaksimalni stimulaciji [91].

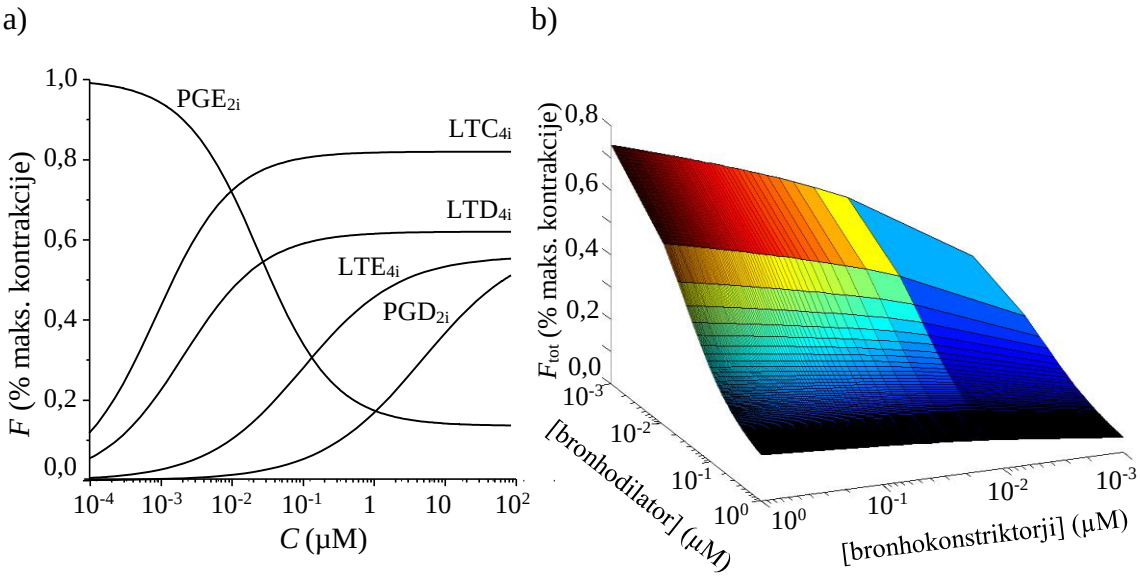
Za izračun skupne sile, ki se razvije kot posledica hkratnega delovanja različnih eikozanoidov na gladke mišične celice dihalnih poti, definiramo en izraz. V njem nastopajo absolutne koncentracije vseh zgoraj naštetih eikozanoidov v intersticiju, ki jih izračunamo z zgornjih dveh podpoglavjih predstavljenima podmodeloma. Najprej definiramo izraz za relativno silo, ki se razvije zgolj pod vplivom kontraktilnih agonistov tako, da posamezne prispevke sil kontraktilnih eikozanoidov (izračunanih po enačbi (13)) med seboj seštejemo, vsoto pa normiramo z vsoto maksimalnih možnih posameznih razvitih sil:

$$f_{\text{contr\_agonist}} = \frac{F_{\text{LTC}_{4i}} + F_{\text{LTD}_{4i}} + F_{\text{LTE}_{4i}} + F_{\text{PGD}_{2i}}}{F_{\text{max\_LTC}_{4i}} + F_{\text{max\_LTD}_{4i}} + F_{\text{max\_LTE}_{4i}} + F_{\text{max\_PGD}_{2i}}}. \qquad (15)$$

Celotno silo (F_{tot}), ki vključuje še relaksacijski prispevek, nato izračunamo tako, da relativno silo, razvito pod vplivom kontraktilnih agonistov ($f_{contr\_agonist}$), uporabimo kot normalizacijski faktor relaksacijskega prispevka, ki je podan z enačbo (14). Tako dobimo:

$$F_{tot} = f_{contr\_agonist} F_{PGE_{2i}} . \qquad (16)$$

Omeniti velja, da je tudi ta sila izražena kot relativna sila glede na maksimalno možno razvito silo, ki je dosežena s stimulacijo s supramaksimalnimi koncentracijami agonistov acetilholina, histamina in kalijevega klorida. Odvisnost celotne sile kot posledice delovanja kontraktilnih in relaksacijskih eikozanoidov (F_{tot}) v odvisnosti od njihovih absolutnih koncentracij v intersticiju dihalnih poti prikazuje slika 6b.



Slika 6. a) Odvisnost razvite sile (F), ki jo povzroči posamezni eikozanoid v dihalnih poteh, od absolutne koncentracije (C) posameznega eikozanoida. Krivulje predstavljajo matematični odvisnosti (13) za bronhokonstriktorje in (14) za bronhodilator. Le-te so bile pridobljene z digitalizacijo izmerjenih podatkov iz referenc [90-92] in z najbolj optimalnim prilagajanjem teh dveh matematičnih izrazov izmerjenim vrednostim. b) Odvisnost celotne sile (F_{tot}) v dihalnih poteh od absolutnih koncentracij bronhokonstriktorjev (cisLT in PGD_{2i}) in bronhodilatorja (PGE_{2i}). 2D ploskev predstavlja matematično odvisnost (16). Na ordinatni osi prikazana sila v obeh primerih predstavlja delež maksimalne možne razvite sile, ki je dosežena s supramaksimalno stimulacijo z acetilholinom, histaminom in kalijevim kloridom.

3.4 ELASTOMEHANIČNI MODEL BRONHIJEV IN PODMODEL ZA IZRAČUN FEV1

Za namen izračuna spremembe FEV1 kot posledice učinkovanja različnih NSAR pri različnih modelnih populacijah izdelamo na podlagi elastičnih, mehanskih in geometrijskih lastnosti spodnjih dihalnih poti fizikalni model in ga sklopimo s podmodelom za napoved sile v dihalnih poteh. Shema modela, ki ga delno povzamemo po Deminu in sod. [83], je prikazan na sliki 7c in d. Ta obravnava obroč gladke mišičnine z vezivnim tkivom, pripetim na notranjo stran hrustanca, ki predstavlja trdno steno bronhija. Model temelji na dveh predpostavkah. Prva predpostavka je, da se volumen obročka gladke mišičnine med krčenjem ne spreminja. Druga predpostavka pa je, da sila, ki se razvije v gladkih mišičnih celicah pod vplivom agonistov, zmanjša polmer obročka iz gladke mišičnine, premer hrustanca pa ostane nespremenjen. Ta predpostavka temelji na eksperimentalnih dognanjih [93], prikazanih na sliki 7a in b, ki pričajo o tem, da se ob kontrakciji gladke mišičnine in zmanjšanju notranjega polmera dihalnih poti (r_{in} na sliki 7c in d) zmanjša tudi zunanji polmer obročka gladke mišičnine (r_{out} na sliki 7c in d) na račun raztega vezivnega tkiva. Le-to ima tudi svoje elastične lastnosti, zato ga lahko modeliramo z elastičnimi vzmetmi, kot prikazuje slika 7d.

Razvita sila v gladki mišičnini opravi delo (A), ki se porabi za razteg vezivnega tkiva. Tako lahko zapišemo, da je delo enako spremembi prožnostne energije vezivnega tkiva (ΔW_{pr}) pred in po kontrakciji:

$$\Delta W_{pr} = A. \tag{17}$$

Ob upoštevanju Hookovega zakona za elastične lastnosti vezivnega tkiva lahko enačbo (17) preoblikujemo v:

$$\frac{1}{2}k\left(R - r_{out}\right)^2 - \frac{1}{2}k\left(R - r_{out} - \Delta r\right)^2 = F\Delta l\,, \tag{18}$$

kjer je k konstanta vzmeti, R polmer hrustančastega obročka, Δr sprememba polmera zunanjega roba obročka gladke mišičnine pri kontrakciji ter Δl sprememba obsega zunanjega roba obročka gladke mišičnine pri kontrakciji (glej sliko 7c in d). Δl je podan z izrazom:

$$\Delta l = 2\pi\Delta r\,. \tag{19}$$

Iz enačb (18) in (19) ob predpostavki, da je Δr majhen, kar nam omogoči zanemariti kvadratni člen Δr^2, dobimo odvisnost zunanjega polmera obročka gladke mišičnine (r_{out}) od razvite sile (F_{tot}):

$$r_{\text{out}} = R - \frac{2\pi F_{\text{tot}}}{k}\,. \tag{20}$$

Na podlagi predpostavke, da se volumen obročka gladke mišičnine ob krčenju in relaksaciji ne spreminja, lahko izrazimo še zvezo med polmerom njenega zunanjega roba (r_{out}) in polmerom njenega notranjega roba (r_{in}). Volumen obročka gladke mišičnine pri popolnoma relaksiranem stanju (V_{GM1}) je enak volumnu gladke mišičnine v stanju kontrakcije (V_{GM2}):

$$V_{\text{GM 1}} = 2\pi^2\left(\frac{R - R_{\text{in}}}{2}\right)R\,, \tag{21}$$

$$V_{\text{GM 2}} = 2\pi^2\left(\frac{r_{\text{out}} - r_{\text{in}}}{2}\right)^2 r_{\text{out}}\,. \tag{22}$$

V enačbi (21) je R_{in} polmer notranjega roba gladke mišičnine pri popolnoma relaksiranem stanju. Z enačenjem enačb (21) in (22) dobimo odvisnost r_{in} od r_{out} in s tem posredno tudi odvisnost notranjega polmera dihalne poti (r_{in}) od sile (F_{tot}):

$$r_{\text{in}} = r_{\text{out}} - \left(R - R_{\text{in}}\right)\sqrt{\frac{R}{r_{\text{out}}}}\,. \tag{23}$$

Pri izračunu FEV1 upoštevamo, da je ta premo sorazmeren prečnemu preseku bronhija, kar pomeni, da je premo sorazmeren kvadratu notranjega polmera obročka gladke mišičnine:

$$FEV1 = \alpha\left(r_{\text{in}}\right)^2, \tag{24}$$

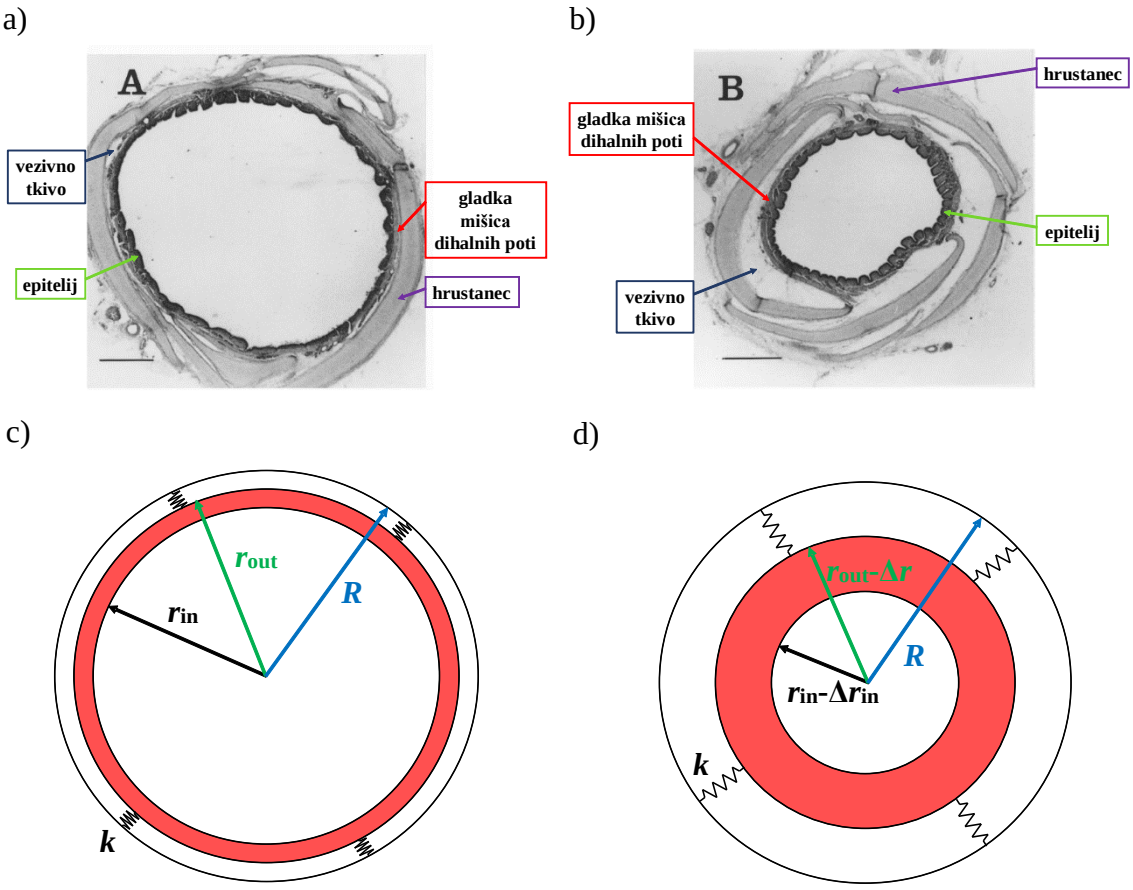
kjer je α premo sorazmernostni faktor. Ta faktor izrazimo iz izraza za maksimalno vrednost FEV1. Le-ta je dosežena, ko so mišice dihalnih poti popolnoma relaksirane in je $r_{in}= R_{in}$:

$$FEV1_{MAX} = \alpha \left(R_{in} \right)^2 . \tag{25}$$

Z združitvijo enačb (24) in (25) dobimo:

$$FEV1 = FEV1_{MAX} \left(\frac{r_{in}}{R_{in}} \right)^2 . \tag{26}$$

Vsi modelni parametri podmodela za napoved FEV1 so podani v tabeli 3 v prilogi 1.

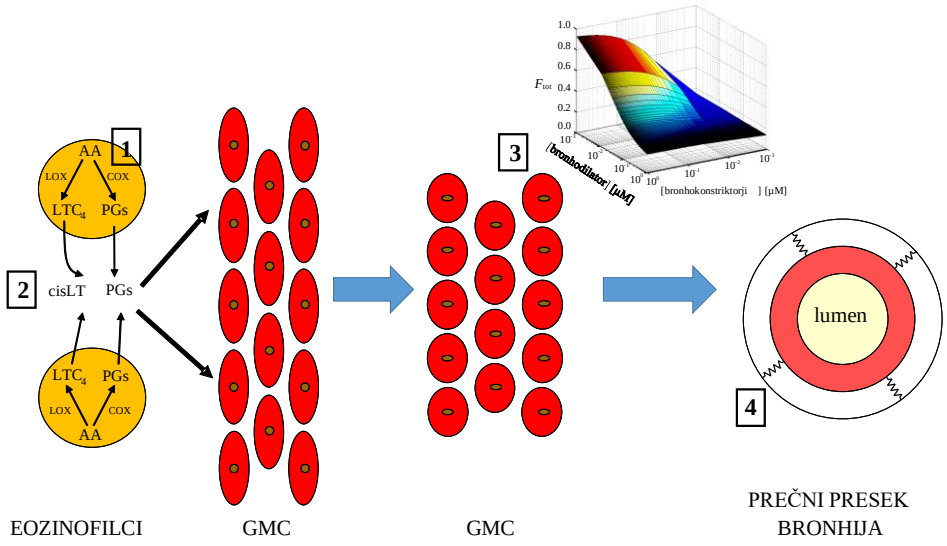


Slika 7. Prikaz prečnega preseka bronhija in njegove zgradbe pred (a, c) in po kontrakciji (b, d). Sliki a in b sta izvzeti in prirejeni iz eksperimentalne študije [93], sliki c in d pa prikazujeta elastomehanični model bronhija, delno povzetega in prirejenega po Deminu in sod. [83]. Oznake na sliki so opisane v tekstu.

3.5 POVZETEK SKLOPITVE PODMODELOV

V tem poglavju na kratko povzamemo model za izračun FEV1 kot celoto. Njegova postopna izgradnja je shematsko prikazana na sliki 8, podrobneje pa opisana v poglavjih 3.1, 3.2, 3.3 in 3.4.

Osnovo celotnega modela predstavlja model za napoved eikozanoidov na ravni celice [20] (oznaka 1 na sliki 8). Z njim napovemo absolutne koncentracije eikozanoidov in njihova razmerja v eni vnetni celici. Z upoštevanjem števila vseh aktivnih eozinofilcev, infiltriranih v dihalnih poteh, v katerih se producirajo eikozanoidi in izločajo v zunajcelično okolje, z modelom napovemo absolutne koncentracije eikozanoidov in njihova razmerja v mišičnem intersticiju pljuč (oznaka 2 na sliki 8). Eikozanoidi se po prehodu iz eozinofilcev lahko vežejo na specifične receptorje na gladkih mišičnih celicah (kratica GMC na sliki 8) in v njih povzročijo razvoj sile. Z modelom iz absolutnih koncentracij bronhokonstriktorjev (cisLT in PGD_2) ter bronhodilatorja (PGE_2) v mišičnem intersticiju izračunamo razvito silo v gladkih mišičnih celicah (oznaka 3 na sliki 8). Razvito silo nato povežemo še s premerom bronhijev (oznaka 4 na sliki 8), letega pa s forsiranim ekspiratornim volumnom zraka, izdihanem v prvi sekundi (FEV1).



Slika 8. Shematski prikaz postopne izgradnje celotnega modela za napoved FEV1. Podrobnosti so napisane v tekstu.

V nalogi prikažemo modelne rezultate različnih modelnih populacij. Pri tem v skladu z definicijo populacij spreminjamo ekspresije ključnih encimov in simuliramo različne doze različnih NSAR. S tem se spreminjajo absolutne koncentracije eikozanoidov v intersticiju, kar se odraža na razviti sili v dihalnih poteh, premeru bronhija in posledično na FEV1.

3.6 KALIBRACIJA MODELA

V celostnem fizikalno-matematičnem modelu nastopajo posamezni parametri, katerih vrednosti eksperimentalno niso znane, zato jih določimo s pomočjo kalibracije sistema. Natančnejša določitev vrednosti neznanih parametrov podmodela za produkcijo eikozanoidov v eni vnetni celici je na kratko nakazana v poglavju 3.1 in natančneje opisana v naši študiji [20] (priloga 2) in v njeni prilogi (priloga 3). Z nadgradnjo modela do izračuna absolutnih koncentracij eikozanoidov v mišičnem intersticiju pa k_{PGE_elim} in k_{PGD_elim} postaneta nova neznana parametra. Zato ju določimo s fizikalno-matematičnim modelom s pomočjo referenčne modelne populacije neastmatika v stanju neinflamacije (NA-NI) ob odsotnosti NSAR tako, da sta razmerji $[PGE_{2i}]/[cisLT_i]$ in $[PGE_{2i}]/[PGD_{2i}]$ enaki tistim v celici. Tako dobljeni vrednosti parametrov nato uporabimo za izračune pri ostalih modelnih populacijah v obeh stanjih, stanju inflamacije in neinflamacije Vrednosti parametrov so podane v tabeli 1 v prilogi 1.

Z nadgradnjo modela do izračuna FEV1 postane vrednost konstante elastičnosti vezivnega tkiva (k) nov neznani parameter. Le-tega prav tako določimo s kalibracijo modela tako, da FEV1 pri modelni populaciji aspirinsko tolerantnih astmatikov v stanju neinflamacije (ATA-NI) pri supramaksimalni dozi enega izmed najmočnejših inhibitorjev COX (v našem primeru indometacina) ne pade pod 80 % bazalne vrednosti FEV1 (tj. brez zdravila). S tem v modelu zagotovimo, da je modelna populacija aspirinsko tolerantnih astmatikov (ATA) dejansko tolerantna na NSAR v smislu bronhokonstrikcije. Padec vrednosti za 20 % izberemo na podlagi standardov, ki se uporabljajo pri metaholinskih testih, kjer padec FEV1 pod 80 % bazalne vrednosti že pomeni hipersenzitivnost na metaholin. Vrednost konstante k je podana v tabeli 3 v prilogi 1.

4 REZULTATI IN DISKUSIJA

Z obstoječimi fizikalno-matematičnimi modeli [16-20] je bilo moč napovedati zgolj razmerje med [PGE_2] in [LTC_4] [20] oziroma razmerje med antiinflamatornimi prostaglandini ([*aiPG*]) in [LTC_4] [16-19] v celici. V eksperimentih je bilo izmerjeno razmerje med koncentracijo PGE_{2i} in celokupno koncentracijo cisteinil levkotrienov $cisLT_i$ ([PGE_{2i}]/[cisLT_i]) v supernatantu krvne plazme [11]. To razmerje je veljalo kot kriterij za razločevanje na aspirin tolerantnih od intolerantnih astmatikov [11]. Okvirna vrednost [PGE_{2i}]/[cisLT_i], ki bi naj razločevala obe skupini astmatičnih bolnikov, je bila 1 [11]. Tipične izmerjene vrednosti v bazalnem stanju brez dodatka NSAR so bile pri na aspirin intolerantnih astmatikih (AIA) še za pribl. faktor 2 do 6 manjše od 1 in so se gibale med vrednostima 0,16 in 0,63 (slika 9). Vrednosti razmerja pri na aspirin tolerantnih astmatikih (ATA) so se gibala med 10 in 20, kar je veliko več od 1. Tako je vrednost 1 postala okvirna mera za oceno obstoja intolerance na aspirin, hkrati pa tudi mera, ki je napovedovala tveganje za bronhokonstrikcijo. V primeru, da je razmerje [PGE_{2i}]/[$cisLT_i$] veliko manjše kot 1, se tveganje za bronhokonstrikcijo poveča in obratno. Z nadaljnjim študijem se je izkazalo, da je razmerje med [PGE_{2i}] in [$cisLT_i$] lahko zgolj okvirna mera za napoved bronhokonstrikcije, saj stopnja bronhokonstrikcije ni nujno odvisna zgolj od razmerja med koncentracijami PGE_{2i}, ki je bronhodilator, in $cisLT_i$, ki so bronhokonstriktorji, saj učinki enih in drugih niso linearni. To pomeni, da lahko enako razmerje [PGE_{2i}]/[$cisLT_i$] povzroči drugačno silo v gladkih mišicah dihalnih poti in s tem sproži različno stopnjo bronhokonstrikcije. Izkaže se, da so za napoved sile in posledične zožitve dihalnih poti pomembne predvsem absolutne koncentracije teh metabolitov. Z natančnim izračunom absolutnih koncentracij eikozanoidov v intersticiju je tako omogočena bolj realistična modelna napoved vrednosti FEV1, s čimer je moč razlikovati med učinki zdravil v smislu bronhokonstrikcije na različne modelne hipotetične modelne populacije astmatikov in neastmatikov. Še več, modelne simulacije lahko pokažejo tudi, kako stanje inflamacije v posamezni modelni populaciji vpliva na bronhokonstrikcijo po zaužitju NSAR. Na podlagi rezultatov modela [20], ki je vključeval zgolj analizo razmerja [PGE_2]/[LTC_4], tega ni bilo mogoče zelo jasno in enolično razbrati.

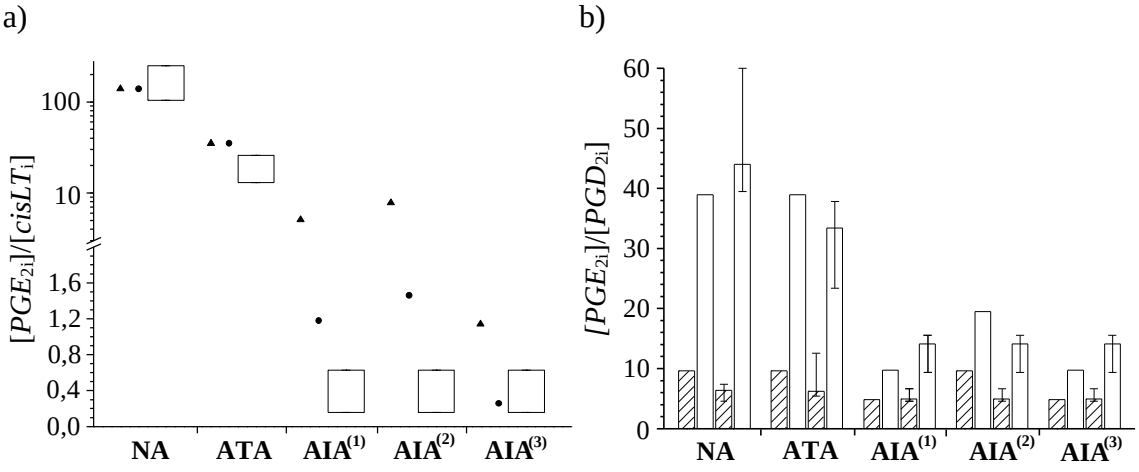
Kot rezultat v tej magistrski nalogi v nadaljevanju najprej prikažemo in diskutiramo o modelnih izračunih razmerij eikozanoidov, njihovi absolutni koncentraciji, razviti

relativni sili v dihalnih poteh, FEV1 in o polmerih dihalnih poti različnih modelnih populacij v stanju inflamacije in stanju neinflamacije v bazalnem stanju, tj. ob odsotnosti NSAR. Za tem predstavimo in diskutiramo o rezultatih, pridobljenih z upoštevanjem oralnega doziranja različnih doz različnih vrst NSAR. Z modelom določimo doze posameznega NSAR, pri katerih vrednost FEV1 pade pod 40 % FVC. Pri tako nizkih vrednostih FEV1 že lahko govorimo o zelo močni bronhokonstrikciji, ki je značilna tudi za astmatični napad. V modelu predpostavimo, da je doza, ki povzroči tak padec v FEV1, mejna doza zdravila, nad katero bi pri določenem tipu na aspirin intolerantnega astmatika tvegali zelo močno bronhokonstrikcijo in astmatični napad. Prav tako določimo dozo zdravila, pri katerem se FEV1 pri določeni modelni skupini zniža za 20 % od njegove začetne vrednosti brez zdravila. Tako se v medicini s provokacijskimi testi ugotavlja senzitivnost astmatikov na NSAR. Enak kriterij, tj. padec FEV1 pod 80 % začetne vrednosti, uporabljajo tudi za ugotavljanje hiperodzivnosti dihalnih poti na metaholin.

4.1 REZULTATI MODELA V BAZALNEM STANJU, TJ. OB ODSOTNOSTI NSAR

V tem podpoglavju primerjamo modelne rezultate razmerij med absolutnimi koncentracijami eikozanoidov v mišičnem intersticiju z izmerjenimi vrednostmi v krvni plazmi [11] in supernatantu, pridobljenem iz celične kulture fibroblastov iz dihalnih poti [14]. Sliki 9a in 9b prikazujeta primerjavo z modelom napovedanih razmerij $[PGE_{2i}]/[cisLT_i]$ in $[PGE_{2i}]/[PGD_{2i}]$ v intersticiju gladke mišičnine. Prvo razmerje je bilo izmerjeno v krvni plazmi [11], drugo pa v supernatantu fibroblastov [14]. Njuni vrednosti smo študirali in analizirali že v naši nedavno objavljeni publikaciji [20] (priloga 2), le da smo tam izmerjene vrednosti primerjali z modelnimi napovedmi na ravni ene same celice. V prilogi 2 smo referenčno modelno populacijo NA definirali tako, da se razmerje $[PGE_{2i}]/[cisLT_i]$ med stanjema neinflamacije in inflamacije ni razlikovalo. Kasnejša analiza ostalih modelnih populacij je pokazala, da se tudi pri ATA razmerje $[PGE_{2i}]/[cisLT_i]$ pri prehodu iz stanja neinflamacije v stanje inflamacije ohranja. Pri modelnih populacijah AIA ($AIA^{(1)}$, $AIA^{(2)}$, $AIA^{(3)}$) pa se pojavijo razlike, saj je omenjeno razmerje v stanju neinflamacije večje kot v stanju inflamacije. Samo za modelno populacijo $AIA^{(3)}$, za katero je značilna znižana ekspresija PGES in povišana ekspresija LTC_4S, je razmerje v stanju inflamacije manjše od 1 in hkrati znotraj izmerjenega intervala med 0,16 in 0,63 [11]. Modelni rezultati na sliki 9b pokažejo, da je razmerje

[PGE_{2i}]/[PGD_{2i}] pri vseh modelnih populacijah v stanju inflamacije večje kot v stanju neinflamacije. Tudi to razmerje je pri vseh modelnih populacijah AIA nekoliko manjše kot pri ATA in NA. Iz primerjave rezultatov med modelnimi populacijami opazimo še, da imata NA in ATA enako vrednost tega razmerja v stanju inflamacije in nižjo vrednost razmerja v stanju neinflamacije. Podobno velja za modelni populaciji AIA$^{(1)}$ in AIA$^{(3)}$. Z obeh slik je razvidno, da se modelne napovedi dokaj dobro ujemajo z izmerjenimi [11, 14].



Slika 9. Primerjava z modelom napovedanih vrednosti razmerij za različne modelne populacije v stanjih inflamacije (I) in neinflamacije (NI) z izmerjenimi. a) [PGE_{2i}]/[$cisLT_i$], kjer polni trikotniki predstavljajo modelne napovedi za stanje NI, polni krogi pa za stanje I. Spodnja in zgornja stranica pravokotnikov predstavljata mejne izmerjene vrednosti Schäferja in sod. [11], kjer stanja NI in I niso razločevali. b) [PGE_{2i}]/[PGD_{2i}], kjer črtkani stolpci predstavljajo vrednosti v stanju NI, beli stolpci pa v stanju I. Stolpci brez oznak napak so napovedi našega modela, stolpci, označeni z napako, pa so izmerjene vrednosti Pierzchalske in sod. [14], kjer je stanje inflamacije simulirano z dodatkom citomiksa v celično kulturo.

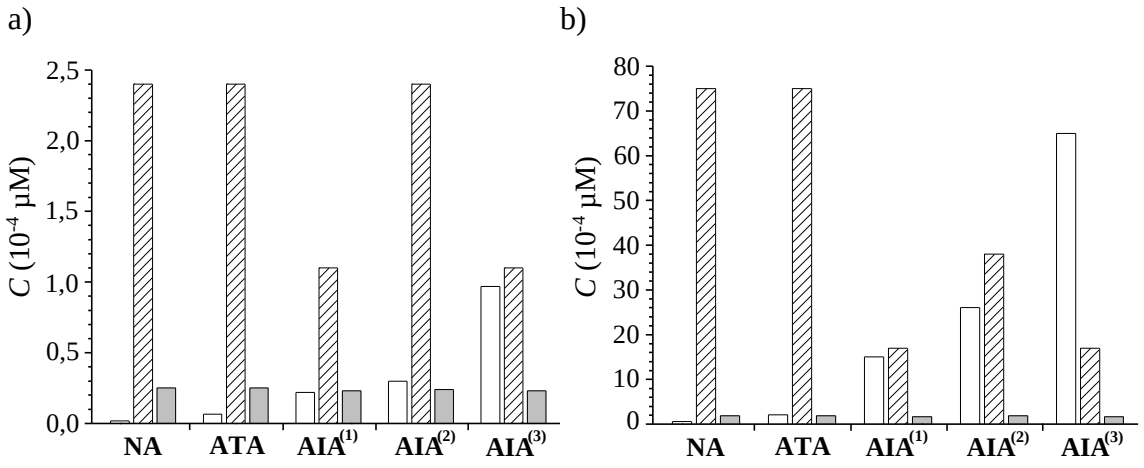
Tako očitne razlike v razmerjih med posameznimi eikozanoidi v različnih modelnih populacijah – bodisi v stanju neinflamacije bodisi v stanju inflamacije – so posledice velikih razlik v absolutnih koncentracijah posameznih eikozanoidov. To je eksplicitno razvidno tudi iz meritev Pierzchalske in sod. [14], kjer pokažejo, da je sprememba razmerja [PGE_{2i}]/[PGD_{2i}] iz stanja neinflamacije v inflamacijo pretežno posledica spremembe v [PGE_{2i}]. Vrednosti [PGD_{2i}] se pri tem pri vseh populacijah dvignejo za

približno enak in nizek odstotek v primerjavi s porastom [PGE_{2i}] [14]. Od tod tudi ideja, da se naše modelne populacije med seboj razlikujejo v ekspresiji encima PGES, saj je le na ta način možno razložiti razlike v razmerju [PGE_{2i}]/[PGD_{2i}]. Na sliki 10 so prikazane modelne napovedi absolutnih koncentracij PGE_{2i}, PGD_{2i} in $cisLT_i$ v pljučnem intersticiju. V slednjem so skupaj seštete vrednosti absolutnih koncentracij LTC_{4i}, LTD_{4i} in LTE_{4i}, saj je na tak način bilo definirano razmerje [PGE_{2i}]/[$cisLT_i$] v referenci [11]. Primerjava rezultatov na sliki 10 a) in b) razkrije, da se pri ATA in NA, kljub ohranjenemu razmerju [PGE_{2i}]/[$cisLT_i$] pri prehodu iz stanja neinflamacije v inflamacijo absolutne koncentracije v teh dveh stanjih močno razlikujejo. V stanju inflamacije so v vseh modelnih stanjih koncentracije PGE_{2i} in $cisLT_i$ za enega ali celo za več redov velikosti višje. Ker je stopnja odziva v obliki kontrakcije ali relaksacije gladkih mišičnih celic odvisna od absolutnih koncentracij agonistov in se celice različno odzivajo na različne agoniste, je samo iz razmerja [PGE_{2i}]/[$cisLT_i$], ki predstavlja razmerje med ključnim bronhodilatorjem (PGE_2) in ključnimi bronhokonstriktorji (cisLT), nemogoče napovedati stopnjo bronhokonstrikcije. Naš tukaj predstavljeni fizikalno-matematični model zato upošteva učinek absolutne koncentracije vsakega posameznega eikozanoida na kontrakcijo/relaksacijo gladkih mišičnih celic dihalnih poti in posledičnega FEV1.

Modelni rezultati na sliki 10 pokažejo, da se vrednosti [PGD_{2i}] med različnimi modelnimi populacijami tako v neinflamaciji kot v inflamaciji bistveno ne razlikujejo, kar je v skladu z meritvami [14]. V neinflamaciji se modelne populacije NA, ATA in $AIA^{(2)}$ med seboj ne razlikujejo po vrednosti [PGE_{2i}]; enaki, a nižji vrednosti [PGE_{2i}] pa imata $AIA^{(3)}$ in $AIA^{(1)}$. V neinflamaciji je vrednost [$cisLT_i$] najvišja pri $AIA^{(3)}$, saj je ta modelna populacija definirana z najvišjo ekspresijo encima LTC_4S. Kljub temu, da je tudi populacija $AIA^{(2)}$ definirana z enako ekspresijo LTC_4S kot $AIA^{(3)}$, pa je vrednost [$cisLT_i$] pri $AIA^{(1)}$ in $AIA^{(2)}$ enaka in nekoliko nižja kot pri $AIA^{(3)}$, vendar višja kot pri ATA in NA.V stanju inflamacije je vrednost [PGE_{2i}] enaka pri NA in ATA, pri $AIA^{(2)}$ pa je za faktor dva manjša kot pri slednjih. To je posledica dejstva, da smo v definiciji modelnih stanj predpostavili, da inflamacija v modelni populaciji $AIA^{(2)}$ poviša ekspresijo PGES manj kot v modelnih populacijah NA in ATA. Enako, a dosti nižjo vrednost [PGE_{2i}] v inflamaciji imata tudi $AIA^{(1)}$ in $AIA^{(3)}$. Pri teh dveh modelnih populacijah je iz stanja neinflamacije v inflamacijo sprememba ekspresije PGES enaka. Velikost modelno napovedanega porasta [PGE_{2i}] iz stanja neinflamacije v inflamacijo se pri NA in ATA

ujema z eksperimentalnimi meritvami Pierzchalske in sod. [14], pri AIA pa je rezultat modelne napovedi približno 2-krat večji od izmerjene [14]. Tudi v stanju inflamacije je vrednost [$cisLT_i$] najvišja pri AIA$^{(3)}$. Vrednost [$cisLT_i$], ki je bila pri AIA$^{(1)}$ in AIA$^{(2)}$ v neinflamaciji enaka, je v inflamaciji večja pri AIA$^{(2)}$. To je posledica dejstva, da je populacija AIA$^{(2)}$ definirana s povišano ekspresijo LTC_4S v primerjavi z AIA$^{(1)}$. Z modelom napovedane vrednosti [$cisLT_i$] za modelne populacije AIA v stanju inflamacije se do reda velikosti natančno ujemajo z vrednostmi podanimi v študiji [83], kar ponovno kaže na ustreznost modela v smislu napovedi absolutnih koncentracij eikozanoidov.

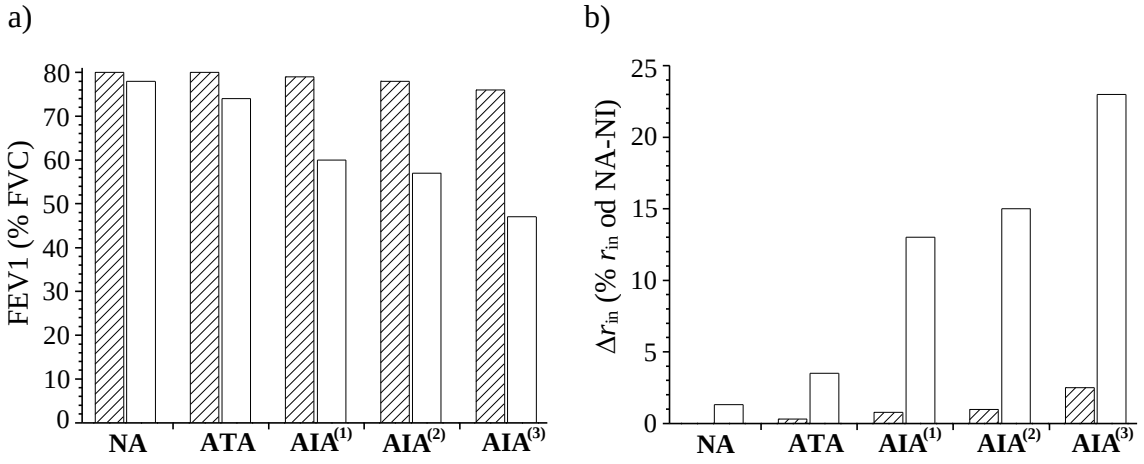
Splošne značilnosti naših hipotetičnih modelnih populacij AIA na ravni produkcije PGE_{2i} in $cisLT_i$ so torej naslednje: AIA$^{(1)}$ izkazuje v neinflamaciji in inflamaciji nizko raven [PGE_{2i}] in [$cisLT_i$], AIA$^{(2)}$ izkazuje v neinflamaciji in v inflamaciji visoko raven [PGE_{2i}] ter povišano raven [$cisLT_i$] v inflamaciji, AIA$^{(3)}$ izkazuje v neinflamaciji in v inflamaciji nizko raven [PGE_{2i}] in v vseh primerih najvišjo raven [$cisLT_i$].



Slika 10. Z modelom napovedane absolutne koncentracije $cisLT_i$ (prazni stolpci), PGE_{2i} (črtkani stolpci) in PGD_{2i} (sivi stolpci) v mišičnem intersticiju pljuč za različne modelne populacije v stanjih a) neinflamacije in b) inflamacije.

Kako se te različne značilnosti na metabolomskem nivoju odražajo na ravni bronhokonstrikcije, preverimo z izračunom FEV1 za vsako posamezno modelno populacijo v odsotnosti zdravila in v primeru oralnega doziranja NSAR. Simulacije izvedemo v obeh modelnih stanjih – neinflamaciji in inflamaciji. Rezultati modela na sliki 11a, ki prikazujejo FEV1 v bazalnem stanju, tj. ob odsotnosti zdravila, pokažejo, da

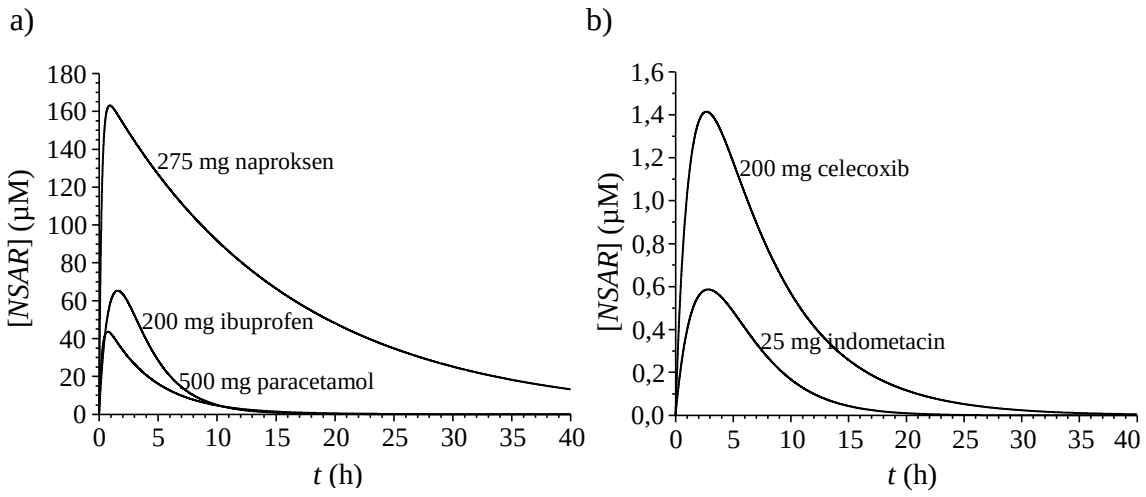
je pri vseh modelnih populacijah v stanju inflamacije FEV1 nekoliko nižji kot v stanju neinflamacije. Najnižja vrednost je pri AIA$^{(3)}$ v inflamaciji in znaša 47 % FVC. To bi lahko okarakterizirali kot nekakšno stanje nezdravljene astme. Po drugi strani so v stanju neinflamacije vrednosti FEV1 vseh modelnih populacij približno enake in znašajo normalnih 80 % FVC. Nižja vrednost FEV1 je posledica večje zožitve dihalnih poti, kar je razvidno iz modelnih napovedi na sliki 11b. Ta prikazuje relativno zmanjšanje polmera tipične dihalne poti, izraženo v %, pri posamezni modelni populaciji glede na referenčno stanje, tj. neastmatik v stanju neinflamacije (NA-NI). V modelnih stanjih NA in ATA je v inflamaciji polmer tipičnih dihalnih poti manjši za nekaj %, medtem ko so zmanjšanja pri vseh modelnih populacijah AIA večja in znašajo med 15 in 25 %. Iz te primerjave je razvidno, da inflamacija zelo pomembno vpliva na zoženje dihalnih poti pri modelnih populacijah aspirinsko intolerantnih astmatikov (AIA), medtem ko pri modelnih populacijah neastmatikov (NA) in na aspirin tolerantnih astmatikov (ATA) nima bistvenega vpliva. Zanimiva je tudi primerjava, da je sprememba pri ATA v stanju inflamacije približno enaka spremembi pri AIA$^{(3)}$ v neinflamaciji, prav tako je sprememba pri NA v stanju inflamacije približno enaka spremembi pri AIA$^{(1)}$ in AIA$^{(2)}$ v neinflamaciji.



Slika 11. Modelna napoved a) FEV1 in b) relativne spremembe (izražene v %) polmera dihalnih poti (Δr_{in}) modelnih populacij v stanju inflamacije (prazni stolpci) in stanju neinflamacije (črtkani stolpci) glede na neastmatika v neinflamaciji (NA-NI). Za vse modelne populacije predpostavimo enak izhodiščni FVC, kot ga ima NA-NI.

4.2 FARMAKOKINETIKA NSAR

V modelu z enačbo (6) simuliramo doziranje različnih NSAR: indometacina, ibuprofena, naproksena in celecoxiba ter paracetamola. Na sliki 12 so prikazani časovni poteki koncentracij posameznih zdravilnih učinkovin v krvni plazmi po oralnem zaužitju tipičnih doz. Te časovne odvisnosti so vhodni podatki za modelni izračun koncentracij eikozanoidov. Oblike krivulj odražajo farmakokinetične lastnosti posameznih zdravil. Razvidno je, da se izmed vseh učinkovin naproksen in paracetamol najhitreje absorbirata v kri, saj je vrh njune koncentracije v krvni plazmi dosežen najhitreje. Pri ibuprofenu, indometacinu in celecoxibu je vrh dosežen nekoliko kasneje. V primerjavi z drugimi NSAR se naproksen nekoliko počasneje eliminira iz krvi in se hkrati v največjem deležu absorbira v kri, saj je pri dozi zdravila 275 mg koncentracija v krvni plazmi najvišja. V najmanjši meri se v kri absorbira celecoxib, ki doseže pri dozi 200 mg maksimalno koncentracijo, ki je 100-krat manjša od tiste za naproksen pri podobni dozi (275 mg). Opomniti pa velja, da sama koncentracija zdravilne učinkovine še ne korelira direktno z jakostjo inhibicije, saj imajo različna zdravila različne afinitete za vezavo na encima COX-1 in COX-2, s čimer je dosežena njuna inhibicija. Vrednosti kinetičnih parametrov za simulacijo časovnih potekov koncentracij posameznih NSAR in paracetamola v krvni plazmi po enačbi (6), so podane v tabeli 4 v prilogi 1.

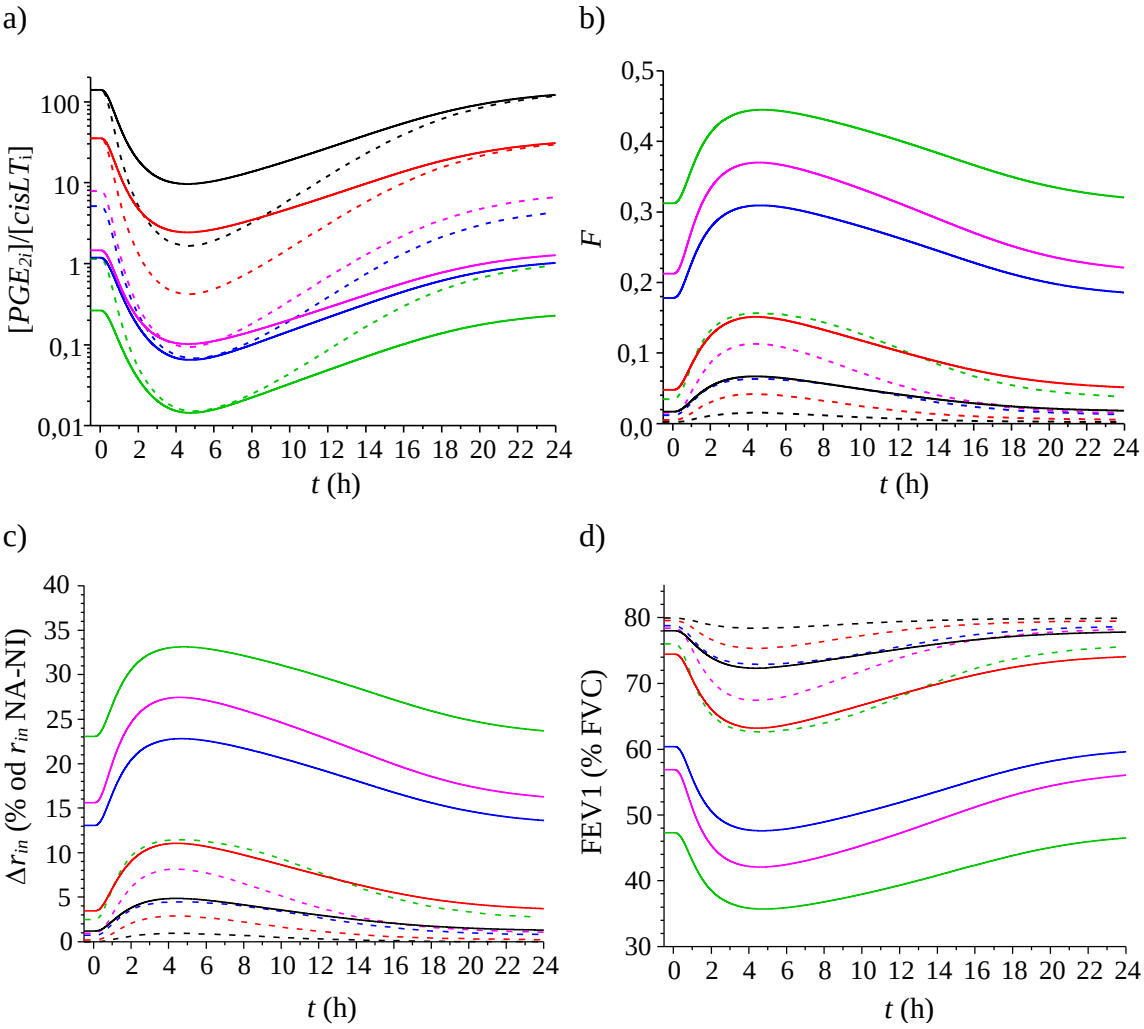


Slika 12. Časovni potek koncentracij a) naproksena, ibuprofena, paracetamola (acetaminofena) in b) celecoxiba ter indometacina v krvni plazmi ([*NSAR*]) po oralnem zaužitju tipičnih doz. Časovna odvisnost [*NSAR*] odraža farmakokinetične lastnosti posamezne zdravilne učinkovine.

4.3 MODELNA SIMULACIJA DOZIRANJA INDOMETACINA IN PARACETAMOLA

Za primerjavo z modelom najprej napovemo časovno spreminjanje razmerja [PGE_{2i}]/[$cisLT_i$] in relativne sile v dihalnih poteh različnih modelnih populacij po oralnem doziranju 25 mg indometacina in 500 mg paracetamola. Prvi predstavlja enega močnejših, drugi pa enega šibkejših inhibitorjev COX. Slika 13 prikazuje časovne poteke obravnavanih spremenljivk po oralnem doziranju 25 mg indometacina za vse modelne populacije v obeh stanjih – neinflmaciji in inflamaciji. Pri tem opazimo, da je padec razmerja [PGE_{2i}]/[$cisLT_i$] po doziranju zdravila pri NA in ATA večji v stanju neinflamacije kot inflamacije. Ob upoštevanju, da bi bilo razmerje [PGE_{2i}]/[$cisLT_i$] kvantitativni kriterij, na osnovi katerega bi lahko napovedovali tveganje bronhokonstrikcije, sklepamo, da bi bili modelni populaciji NA in ATA v stanju neinflamacije bolj izpostavljeni tveganju bronhokonstrikcije kot v stanju inflamacije. Vrednost [PGE_{2i}]/[$cisLT_i$] pa se po doziranju NSAR pri modelnih populacijah $AIA^{(1)}$, $AIA^{(2)}$ in $AIA^{(3)}$ med stanjem inflamacije in neinflamacije bistveno ne razlikuje. Na podlagi zgornje predpostavke bi sklepali, da stanje inflamacije ne pomeni večjega tveganja bronhokonstrikcije pri modelnih AIA. To pa je v nasprotju s pričakovanji, saj je hipoteza, da se v stanju inflamacije senzitivnost na zaužiti NSAR poveča pri vseh modelnih populacijah, še posebej pa pri modelnih populacijah AIA. Modelne dokaze, da ta hipoteza drži, predstavljajo rezultati na slikah 13b do 13d. Rezultati na sliki 13b, ki prikazujejo časovni potek relativne sile po zaužitju 25 mg indometacina, pokažejo, da je pri vseh populacijah v stanju inflamacije razvita sila v dihalnih poteh večja kot v stanju neinflamacije. Med drugim je tudi razvidno, da so relativni porasti iz bazalnega stanja (brez zdravila) do maksimuma (z zdravilom) v stanju inflamacije večji. To ima za posledico večjo zožitev dihalnih poti (slika 13c), kar posledično pomeni večji padec FEV1 (slika 13d) po zaužitju NSAR. S slike 13b je razvidno, da se po zaužitju zdravila največja sila razvije pri modelni populaciji $AIA^{(3)}$ v stanju inflamacije, najmanjša pa pri NA v stanju neinflamacije. S slike 13c je razvidno, da se prav tako pri $AIA^{(3)}$ zaradi največje razvite sile notranji polmer dihalne poti najbolj zmanjša. Najmanjši je 4–5 ur po zaužitju 25 mg indometacina in znaša približno 34 % notranjega polmera gladke mišičnine neastmatika v bazalnem stanju. Posledično je vrednost FEV1 za populacijo $AIA^{(3)}$ v stanju inflamacije v tem časovnem obdobju po zaužitju zdravila najnižja in znaša približno 36 % FVC (slika 13d). To pomeni zelo visoko stopnjo bronhokonstrikcije, ki je

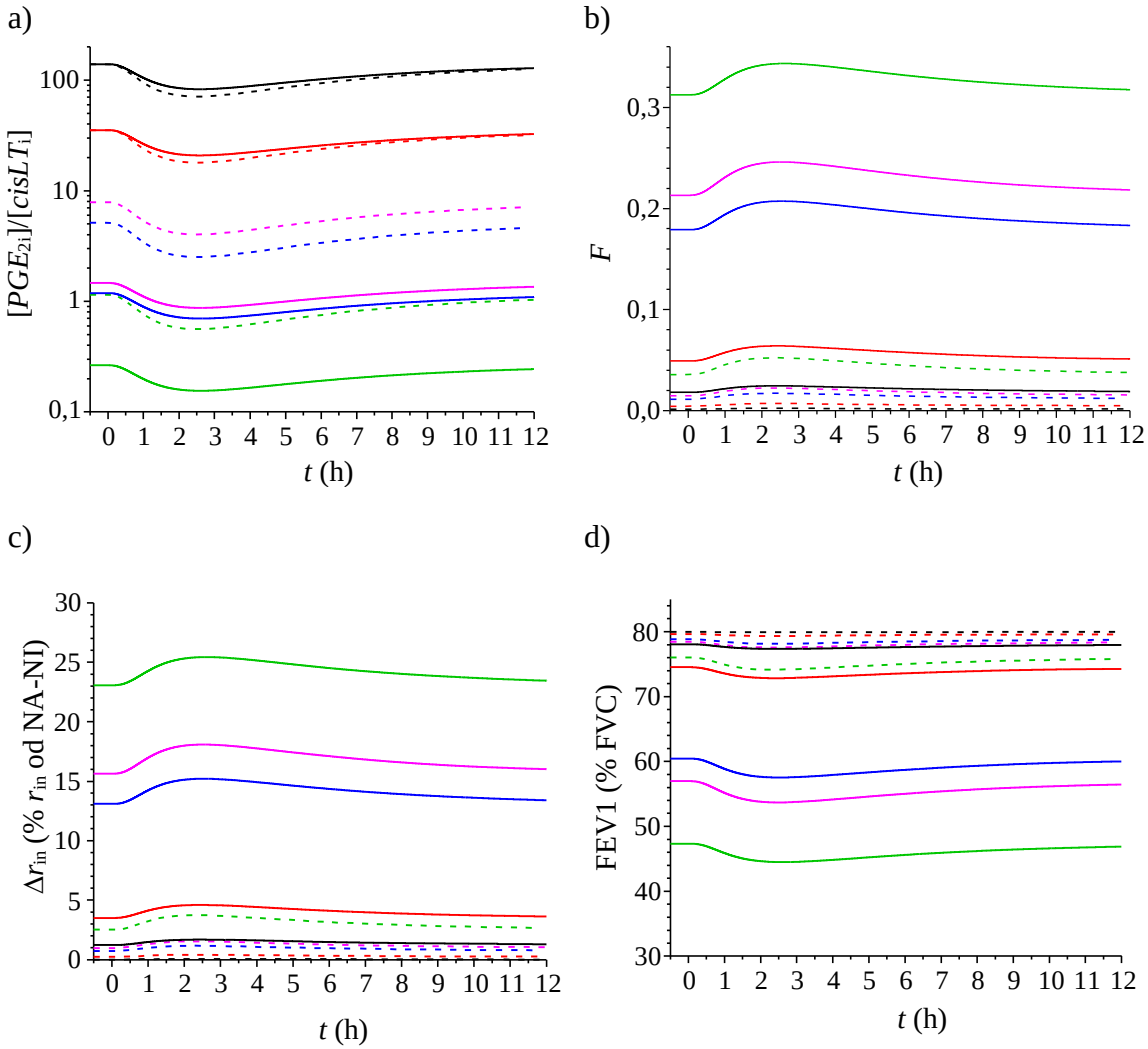
primerljiva s tisto ob astmatičnem napadu. Rezultati na slikah 13b do 13d tako pojasnjujejo, da inflamacija pomeni večje tveganje za bronhokonstrikcijo po zaužitju NSAR. Tega samo na podlagi razmerja med [PGE_{2i}] in [$cisLT_i$] ni bilo mogoče pravilno sklepati.



Slika 13. Modelne napovedi za a) razmerje [PGE_{2i}]/[$cisLT_i$], b) relativno razvito silo F, c) relativno spremembo polmera dihalnih poti Δr_{in} in d) vrednosti FEV1 po oralnem doziranju 25 mg indometacina ob času $t = 0$. Črna krivulja je za NA, rdeča za ATA, modra za AIA$^{(1)}$, vijoličasta za AIA$^{(2)}$ in zelena za AIA$^{(3)}$. Črtkane krivulje predstavljajo stanje neinflamacije, polne krivulje predstavljajo stanje inflamacije.

Za primerjavo z indometacinom, ki se uvršča med močnejše inhibitorje COX, izvedemo simulacijo še za najšibkejši inhibitor med analiziranimi zdravilnimi učinkovinami, tj. za paracetamol, katerega glavna funkcija niti ni inhibicija COX [94]. Z modelom lahko tako

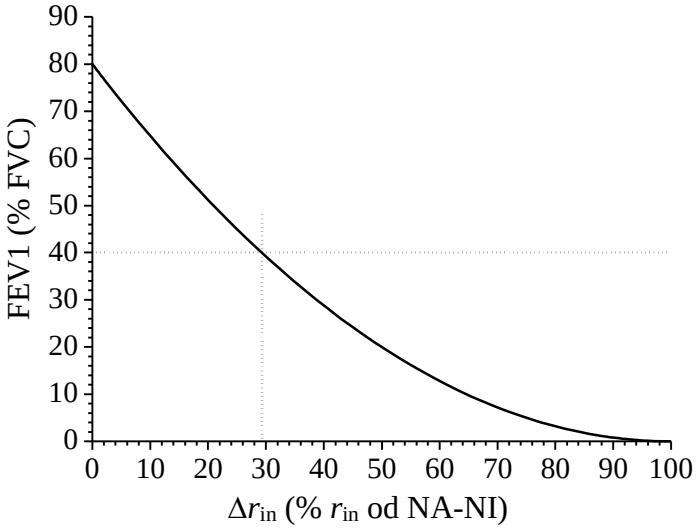
preverimo, ali zaužitje tipične doze paracetamola pomeni tveganje za bronhokonstrikcijo pri aspirinsko intolerantnih astmatikih. Na slikah 14a do 14d so prikazani časovni poteki enakih spremenljivk kot zgoraj na sliki 13. Iz slike 14 lahko razberemo, da se učinek paracetamola na spremembo FEV1 skorajda ne pozna, medtem ko je v stanju inflamacije učinek sicer zaznaven, vendar zelo majhen (sprememba FEV1 je reda nekaj % glede na bazalno vrednost brez zdravila).



Slika 14. Modelne napovedi za a) razmerje $[PGE_{2i}]/[cisLT_i]$, b) relativno razvito silo F, c) relativno spremembo polmera dihalnih poti Δr_{in} in d) vrednosti FEV1 po oralnem doziranju 500 mg paracetamola ob času $t = 0$. Črna krivulja je za NA, rdeča za ATA, modra za AIA$^{(1)}$, vijoličasta za AIA$^{(2)}$ in zelena za AIA$^{(3)}$. Črtkane krivulje predstavljajo stanje neinflamacije, polne krivulje predstavljajo stanje inflamacije.

Na slikah 13 in 14 smo prikazali časovne poteke po oralnem doziranju indometacina kot tipičnega predstavnika NSAR in paracetamola. Časovne odvisnosti za ostale NSAR so podobne, le da so učinki na padec FEV1 različni in so odvisni od jakosti inhibicije posamezne zdravilne učinkovine, njene doze in farmakokinetike. Iz primerjave modelnih rezultatov, prikazanih na slikah 13 in 14, se izkaže, da zaužitje 25 mg indometacina pri modelnih populacijah razvije večjo silo in posledično večjo skrčitev dihalnih poti, kot pa zaužitje 500 mg paracetamola. Maksimalna sila se razvije približno 2–3 ure po zaužitju paracetamola in približno 4–5 ur po zaužitju indometacina. Po zaužitju indometacina je potrebnih približno 24 ur za relaksacijo dihalnih poti v bazalno stanje. Po zaužitju 500 mg paracetamola pa se dihalne poti relaksirajo že po približno 10 urah. Rezultati modela so v skladu s pričakovanimi, saj je indometacin znan kot precej močnejši inhibitor encimov COX-1 in COX-2 kot paracetamol [94]. To pomeni, da lahko oseba doživi v primeru zaužitja indometacina močnejšo bronhokonstrikcijo že pri majhni dozi.

Da bi ovrednotili stopnjo bronhokonstrikcije in jo okarakterizirali kot šibko ali močno, postavimo mejo. Za to izrišemo diagram FEV1 v odvisnosti od relativne spremembe polmera dihalnih poti (Δr_{in}), izražene glede na modelno populacijo neastmatika v stanju neinflamacije (NA-NI). Odvisnost, ki je izračunana z enačbo (26), je prikazana na sliki 15. Prikazuje, da je pri normalnem polmeru dihalnih poti modelnega neastmatika FEV1 enak 80 % FVC. Tipična izmerjena vrednost FEV1 zdrave osebe je med 70–80 % FVC [24]. Predpostavimo, da padec FEV1 pod 40 % FVC pomeni zelo močno bronhokonstrikcijo, ki lahko vodi do astmatičnega napada in v najhujšem primeru postane že življenjsko ogrožajoč. Tak padec je v našem primeru dosežen pri približno za 30-odstotnim zmanjšanjem polmera dihalnih poti (r_{in}) glede na neastmatika v stanju neinflamacije (NA-NI). V primeru popolne obstrukcije dihalnih poti vrednost FEV1 pade na vrednost 0, kar je popolnoma s pričakovanjem in je hkrati dobra potrditev ustreznosti našega modela.



Slika 15. Modelna napoved FEV1 za različne relativne skrčitve polmera dihalnih poti (Δr_{in}) izraženih v % glede na neastmatika v stanju neinflamacije (NA-NI).

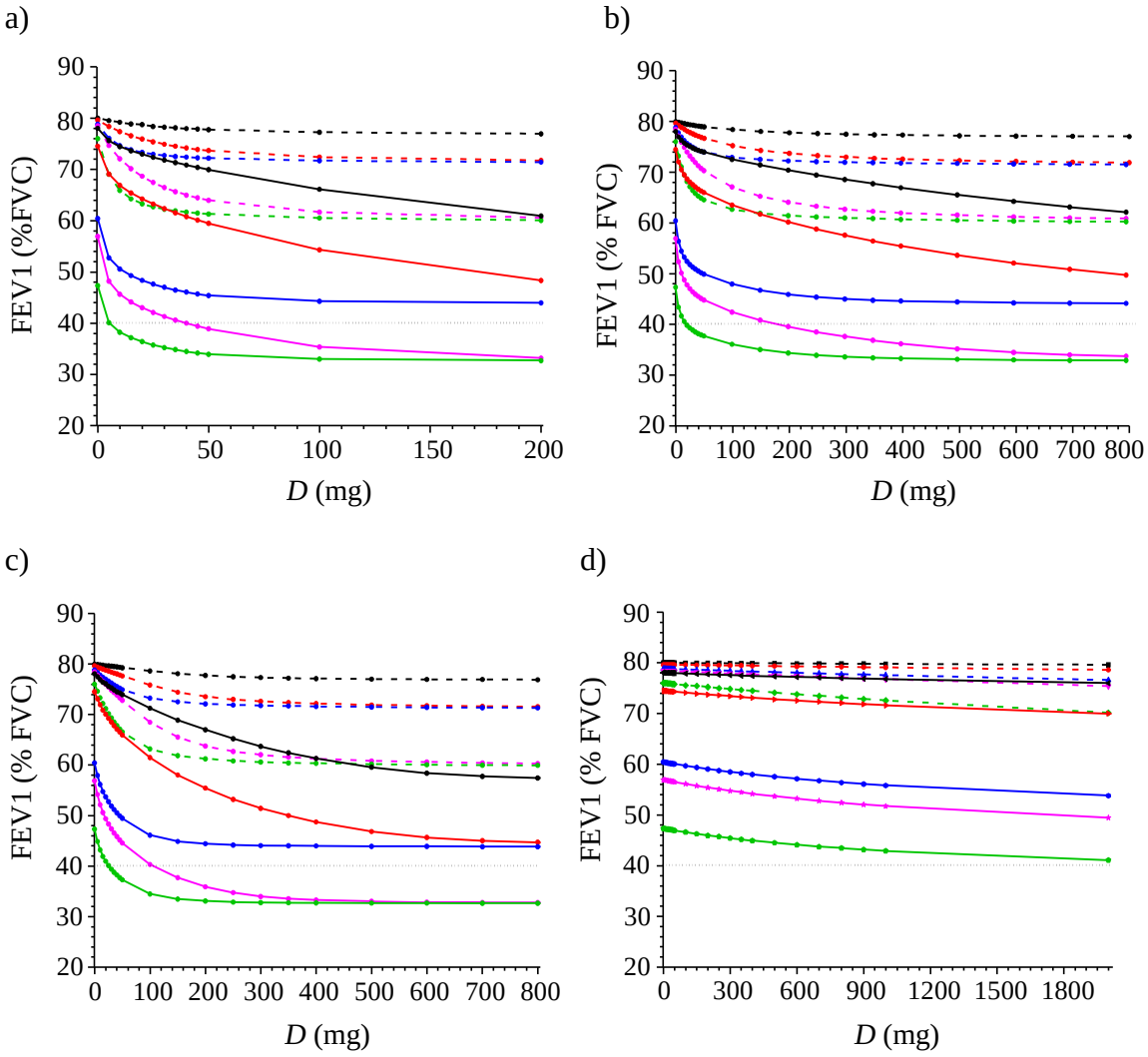
Na tem mestu velja omeniti, da naš model ne upošteva dodatnih razlik v reaktivnosti in senzitivnosti gladkih mišic dihalnih poti, ki so sicer lahko značilnost astmatikov zaradi remodeliranja dihalnih poti in/ali povišanega števila receptorjev na gladkih mišičnih celicah. Prav tako naš model za napoved FEV1 ne upošteva delovanja drugih kontraktilnih agonistov, kot sta npr. histamin in acetilholin. Doseganje teh vrednosti polmera dihalnih poti in FEV1 je v našem modelu zgolj posledica delovanja eikozanoidov PGE_2, PGD_2, LTC_4, LTD_4 in LTE_4. Tudi stanje inflamacije se v našem modelu odraža zgolj na ravni vnetnih celic, ki producirajo eikozanoide, in na ravni ekspresij posameznih encimov, ki so udeleženi v metabolizmu AA, ki poteka v teh celicah.

4.4 MODELNA NAPOVED FEV1 V ODVISNOSTI OD DOZ RAZLIČNIH VRST NSAR

Zaužitje različnih vrst in doz NSAR pri različnih modelnih populacijah povzroči različno zmanjšanje FEV1 in s tem različno tveganje bronhokonstrikcije. Rezultati te analize, ki je izvedena za vse vrste oralnega doziranja študiranih NSAR in paracetamola modelnim populacijam v obeh stanjih, so prikazani na slikah 16a do 16d (zaporedoma za indometacin, ibuprofen, naproksen in paracetamol).

Modelni rezultati na slikah 16a do 16d pokažejo, da nobena izmed modelnih populacij v stanju neinflamacije po zaužitju poljubne doze kateregakoli izmed zdravil ne izkazuje velikega padca v FEV1 in s tem močne bronhokonstrikcije (glej črtkane krivulje). Za indometacin in ibuprofen segajo vrednosti FEV1 najnižje, tja do reda velikosti 60 % FVC. V vseh primerih zdravil so najnižje vrednosti FEV1 dosežene za modelni populaciji $AIA^{(2)}$ in $AIA^{(3)}$. V stanju inflamacije se pri $AIA^{(2)}$ in $AIA^{(3)}$ po zaužitju naproksena, indometacina in ibuprofena FEV1 močno zniža, kar bi lahko pomenilo zelo veliko tveganje za astmatični napad. Modelne simulacije z indometacinom pokažejo, da pade FEV1 pri $AIA^{(2)}$ pod 40 % FVC pri dozi 40 mg, pri $AIA^{(3)}$ pa že pri dozi 5 mg. V primeru ibuprofena sta dozi nekoliko višji – 180 mg pri $AIA^{(2)}$ in 20 mg pri $AIA^{(3)}$. Za naproksen je tak padec pri $AIA^{(2)}$ dosežen pri dozi 100 mg, pri $AIA^{(3)}$ pa pri dozi 20 mg.

Pri nobeni izmed modelnih populacij v stanju neinflamacije in inflamacije po zaužitju paracetamola ne pride do zmanjšanja FEV1 pod 40 % FVC. Iz modelnih simulacij tako sklepamo, da je parcetamol varnejši od NSAR v smislu pojava bronhokonstrikcije pri na aspirin intolerantnih astmatikih. V nasprotju s tem se indometacin, ibuprofen in naproksen izkažejo za zelo močne bronhokonstriktorje, saj se pri dveh modelnih populacijah ($AIA^{(2)}$ in $AIA^{(3)}$) vrednost FEV1 zniža pod 40 % FVC. Pri indometacinu se to zgodi že pri nižjih dozah, pri ibuprofenu in naproksenu pa pri višjih, kar kaže na to, da je indometacin močnejši inhibitor COX. Iz primerjave slik 16a, 16b in 16c je razvidno, da je pri približno 4-krat večji dozi ibuprofena in naproksena kot indometacina učinek v smislu znižanja FEV1 enak. Tudi končne vrednosti pri zelo velikih dozah so v vseh treh primerih približno enake za vse modelne populacije v obeh stanjih. Paracetamol se v modelnih simulacijah izkaže kot zdravilo z nekoliko manjšim tveganjem za bronhokonstrikcijo v primerjavi z NSAR. Vrednosti 40 % FVC se približa zgolj modelna populacija $AIA^{(3)}$, pri dozah, ki predstavljajo nekaj-kratnik tipične terapevtske doze, ki je 500 mg. Vrednosti FEV1 za $AIA^{(2)}$ in $AIA^{(1)}$ sta pri teh dozah približno med 50 in 60 % FVC. To kljub vsemu ni zanemarljiv učinek, je pa res, da je sam padec iz bazalnega stanja (brez zdravila) relativno majhen. Paracetamol bi tako lahko predstavljal tveganje za bronhokonstrikcijo pri najbolj hiperreaktivnih in hipersenzitivnih na aspirin intolerantnih astmatikih v stanju inflamacije ob zaužitju večje doze zdravila od tipične terapevtske.



Slika 16. Modelna napoved minimalne vrednosti FEV1 po zaužitju zdravila za posamezne modelne populacije v stanjih neinflamacije (črtkane krivulje) in inflamacije (polne krivulje) v odvisnosti od doze (*D*) a) indometacina, b) ibuprofena, c) naproksena in d) paracetamola. Črna krivulja je za NA, rdeča za ATA, modra za AIA$^{(1)}$, vijoličasta za AIA$^{(2)}$ in zelena za AIA$^{(3)}$.

V medicini je mera, ki je uveljavljena za ovrednotenje senzitivnosti in odzivnosti dihalnih poti astmatikov na agoniste, 20-odstotni padec v FEV1 glede na referenčno bazalno vrednost FEV1, ki je izmerjena v odsotnosti agonista. Tipičen primer so metaholinski testi, lahko pa se podobni provokacijski testi izvajajo tudi z NSAR ali drugimi zdravili. Bolniki pri testiranju zaužijejo različne doze učinkovin v posameznih časovnih presledkih, pri tem pa se jim s spirometrijo meri FEV1. Ko vrednost FEV1 pade za 20 % oziroma doza preseže maksimalno dovoljeno vrednost v testu, se le-ta prekine. Če je

dosežen padec FEV1 za 20 % ali več, se določi mejna doza, na podlagi katere se ugotovi, kolikšna je senzitivnost osebe na učinkovino. Test se lahko prekine že tudi pri manjši dozi in se z ekstrapolacijskimi metodami določi mejno dozo. Pri kateri dozi zdravila pride pri posamezni modelni populaciji do padca FEV1 za 20 % od bazalne vrednosti (tj. brez zdravila), lahko določimo tudi z našim modelom. Vrednosti modelnih simulacij so prikazane v tabeli 1.

Iz vrednosti v tabeli 1 je razvidno, da pri modelnih populacijah NA, ATA in AIA$^{(1)}$ v stanju neinflamacije po doziranju NSAR sploh ne pride do 20-odstotnega padca FEV1. V primeru paracetamola do 20-odstotnega padca FEV1 v stanju neinflamacije ne pride pri nobeni modelni populaciji, v stanju inflamacije pa zgolj pri AIA$^{(2)}$ in AIA$^{(3)}$ pri dozah, ki so za velikostni razred večje od tipične terapevtske. Po drugi strani pa vse modelne populacije dosežejo padec FEV1 za 20 % v stanju inflamacije. Pri NA je ta doza najvišja, pri ATA pa je približno 3-krat nižja. Pri AIA$^{(1)}$ je mejna doza še za faktor 2 manjša kot pri ATA. Pri AIA$^{(2)}$ in AIA$^{(3)}$ je mejna vrednost približno enaka in je še za faktor 2 manjša kot pri AIA$^{(1)}$. Izmed vseh modelnih populacij se AIA$^{(2)}$ in AIA$^{(3)}$ izkažeta za najbolj senzitivni na NSAR, saj je 20-odstotni padec v FEV1 dosežen že pri zelo majhnih dozah. Senzitivnost posameznih modelnih populacij na zdravila razberemo tudi z grafov na sliki 16, kjer je v primeru večje senzitivnosti na zdravilo naklon krivulje FEV1 v odvisnosti od doze večji. Tudi tokrat se izkaže, da je indometacin učinkovina, ki pri najmanjši dozi v primerjavi z ostalima NSAR izzove padec FEV1 za 20 %. Mejne doze za ibuprofen in naproksen so med seboj primerljive. Razlika je opazna pri modelnih stanjih NA, ATA in AIA$^{(1)}$, saj so doze za naproksen nekoliko nižje kot za ibuprofen. Še posebej velika je razlika pri NA in ATA, kjer je mejna doza za naproksen približno 2-krat nižja. V stanju neinflamacije izkazujeta modelni populaciji AIA$^{(2)}$ in AIA$^{(3)}$ padec FEV1 za 20 % pri tipičnih terapevtskih dozah vseh NSAR – okrog 70 mg za indometacin, okrog 300 mg za ibuprofen in 250 mg za naproksen.

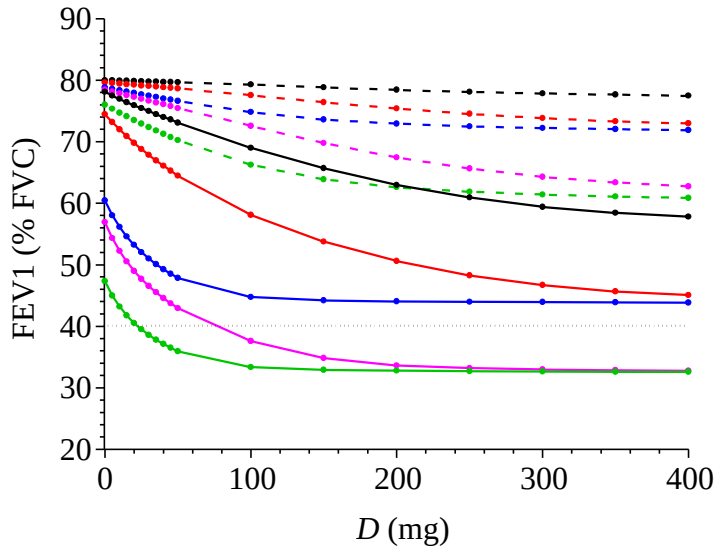
Tabela 1. Z modelom določene mejne doze (D_{20}) različnih zdravil, pri katerih pade vrednost FEV1 za 20 % glede na bazalno vrednost, tj. brez zdravila. Rezultati so prikazani za vse modelne populacije v stanjih neinflamacije (NI) in inflamacije (I).

	NA	**ATA**	**AIA(1)**	**AIA(2)**	**AIA(3)**
			INDOMETACIN		
D_{20} (mg) – NI	/	/	/	67	69
D_{20} (mg) – I	165	49	20	10	12
			IBUPROFEN		
D_{20} (mg) – NI	/	/	/	300	350
D_{20} (mg) – I	780	210	87	40	45
			NAPROKSEN		
D_{20} (mg) – NI	/	/	/	250	250
D_{20} (mg) – I	350	125	62	43	43
			PARACETAMOL		
D_{20} (mg) – NI	/	/	/	/	/
D_{20} (mg) – I	/	/	/	6500	7000

Modelne napovedi za različne NSAR in paracetamol primerjamo še s celecoxibom, ki ga uvrščajo med selektivne inhibitorje encima COX. Ti močneje inhibirajo encim COX-2 kot COX-1. Indometacin, ibuprofen in naproksen se uvrščajo med neselektivne inhibitorje. Selektivni inhibitorji so bili med drugim izdelani tudi z namenom, da bi bili varni za uživanje pri na aspirin intolerantnih astmatikih. Avtorji podajajo različne mejne doze, ki bi povzročile močno bronhokonstrikcijo. V študijah [95, 96] poročajo, da so doze celecoxiba do 400 mg za aspirinsko intolerantne astmatike še varne, medtem ko drugi avtorji poročajo [3, 97], da je to zdravilo (podobno kot tudi ostali selektivni NSAR) varno samo pri nizkih zaužitih dozah. Zaradi nasprotujočih si eksperimentalnih rezultatov z modelom simuliramo zaužitje celecoxiba in ocenimo mejne doze za različne modelne populacije.

Z modelom napovedane minimalne vrednosti FEV1 v odvisnosti od doze celecoxiba, prikazane na sliki 17 za vse modelne populacije v obeh stanjih, imajo dokaj podobne značilnosti kot pri neselektivnih NSAR. Že pri zelo nizkih dozah v modelnih populacijah

AIA$^{(2)}$ in AIA$^{(3)}$ vrednost FEV1 pade pod 40 % FVC, kar kaže na visoko stopnjo tveganja bronhokonstrikcije. Za razliko od neselektivnih NSAR se v modelnih populacijah NA in ATA v stanju inflamacije pri visokih dozah celecoxiba vrednost FEV1 precej bolj zniža. To bi lahko razložili na naslednji način. V stanju inflamacije, za katero je značilna povišana ekspresija encima COX-2, ki ga celecoxib kot selektivni inhibitor močneje inhibira, pride do bolj okrnjene produkcije PGE_2. To vpliva na produkcijo cisLT, ki je v stanju inflamacije že sama po sebi višja. Vse to vodi v večje tveganje bronhokonstrikcije tudi pri NA in ATA. Po drugi strani ima celecoxib v stanju neinflamacije nekoliko manjši vpliv na znižanje FEV1. Kljub temu, da veliko avtorjev poroča o varnosti celecoxiba [95, 96], z modelom pokažemo ravno nasprotno. Modelne simulacije pokažejo, da sta modelni populaciji AIA$^{(2)}$ in AIA$^{(3)}$ v stanju inflamacije že pri zelo nizki dozi celecoxiba izpostavljeni tveganju bronhokonstrikcije. Pri AIA$^{(2)}$ pade vrednost FEV1 pod 40 % FVC pri dozi 80 mg, pri AIA$^{(3)}$ pa že pri dozi 20 mg celecoxiba. Zelo blizu meje 40 % pa sta tudi modelni populaciji AIA$^{(1)}$ in ATA v stanju inflamacije.



Slika 17. Modelna napoved minimalne vrednosti FEV1 po zaužitju različnih doz (*D*) celecoxiba za posamezne modelne populacije v stanjih neinflamacije (črtkane krivulje) in inflamacije (polne krivulje). Črna krivulja je za NA, rdeča za ATA, modra za AIA$^{(1)}$, vijoličasta za AIA$^{(2)}$ in zelena za AIA$^{(3)}$.

Tudi v primeru doziranja celecoxiba modelno določimo doze za doseg 20-odstotnega znižanja FEV1 za različne modelne populacije. Iz tabele 2 je razvidno, da pri modelnih populacijah NA, ATA in AIA$^{(1)}$ v stanju neinflamacije po doziranju celecoxiba ne pride

do 20-odstotnega padca FEV1. V stanju inflamacije vse modelne populacije dosežejo tak padec FEV1, pri čemer z nižjo dozo AIA$^{(2)}$ in AIA$^{(3)}$ ter z višjo dozo NA. Zanimivo je to, da je 20-odstotni padec FEV1 pri NA v stanju inflamacije dosežen z nižjo dozo, kot pa pri AIA$^{(2)}$ in AIA$^{(3)}$ v stanju neinflamacije. V primeru doziranja neselektivnih NSAR model pokaže ravno obratno.

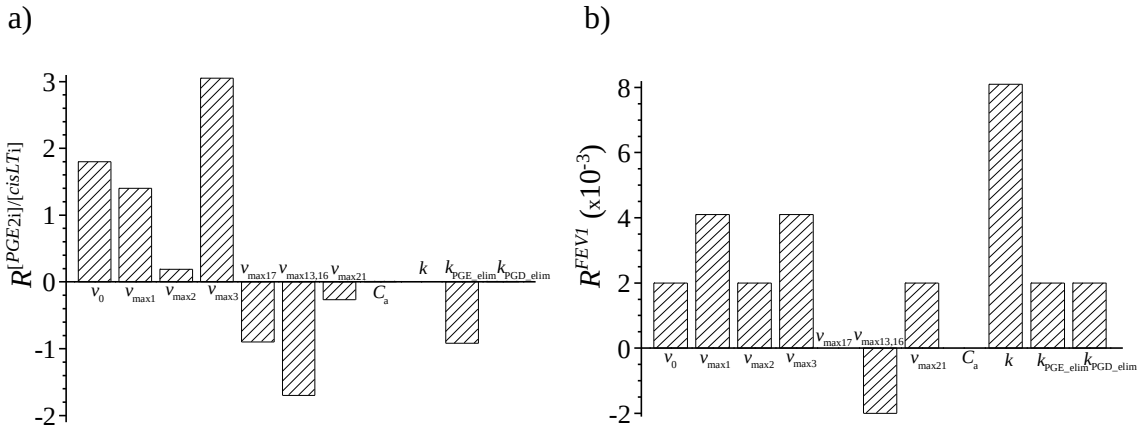
Tabela 2. Z modelom določene mejne doze (D_{20}) celecoxiba, pri katerih pade vrednost FEV1 za 20 % glede na bazalno vrednost, tj. brez zdravila. Rezultati so prikazani za vse modelne populacije v stanjih neinflamacije (NI) in inflamacije (I).

	NA	**ATA**	**AIA$^{(1)}$**	**AIA$^{(2)}$**	**AIA$^{(3)}$**
			CELECOXIB		
D_{20} (mg) – NI	/	/	/	390	400
D_{20} (mg) – I	210	86	45	34	34

4.5 SENZITIVNOSTNA ANALIZA

Senzitivnost dveh ključnih spremenljivk fizikalno-matematičnega modela (razmerja [PGE_{2i}]/[$cisLT_i$] in FEV1) na majhne spremembe posameznih vrednosti modelnih parametrov v referenčnem modelnem stanju neastmatika v stanju neinflamacije (NA-NI) prikazuje slika 18. Izkaže se, da je razmerje [PGE_{2i}]/[$cisLT_i$] veliko bolj občutljivo na spremembo ključnih parametrov kot FEV1. Parameter, ki ima največji vpliv na [PGE_{2i}]/[$cisLT_i$], je v_{max3} , ki določa maksimalno hitrost reakcije encima PGES. Pri 10-odstotnem povišanju v_{max3} se [PGE_{2i}]/[$cisLT_i$] spremeni kar za 30 %. Parametri, kot so koncentracija aktivnih eozinofilcev (C_a), konstanta vzmeti (k) in k_{PGD_elim}, ne vplivajo na omenjeno razmerje. To je v skladu s pričakovanji, saj so to parametri, ki imajo vlogo dol vodno od produkcije PGE_2 in LTC_4. Po drugi strani pa je s slike 18b razvidno, da konstanta vzmeti (k) izmed vseh parametrov najbolj vpliva na vrednost FEV1. Tudi v tem primeru je to v skladu s pričakovanjem, saj je premer dihalne poti direktno odvisen od vrednosti tega parametra. Premer dihalne poti pa seveda bistveno vpliva na vrednost FEV1. Po drugi strani pa FEV1 ni občutljiv na spremembe v vrednosti parametrov, kot sta maksimalna hitrost reakcije encima LTC_4S (v_{max17}) in koncentracija aktivnih eozinofilcev (C_a). Neobčutljivost NA-NI na spremembo v ekspresiji encima LTC_4S je med drugim razvidna tudi z grafa na sliki 11a, ko samo s spremembo parametra v_{max17}

preidemo v modelno populacijo AIA$^{(2)}$ s skoraj nespremenjenim FEV1. Iz rezultatov senzitivnostne analize na sliki 18 lahko tudi razberemo, da je spremenljivka FEV1 veliko manj senzitivna na spremembe vrednosti parametrov, kar naredi sistem bolj robusten. To je pričakovano, saj se razmerje $[PGE_{2i}]/[cisLT_i]$ z večjimi spremembami posameznih parametrov lahko spreminja tudi za celotne rede velikosti.



Slika 18. Koeficient odzivnosti (R) za razmerje $[PGE_{2i}]/[cisLT_i]$ (a) in *FEV1* (b) v odvisnosti od relativnih sprememb posameznih vrednosti parametrov za 10 % za modelno populacijo neastmatika v stanju neinflamacije (NA-NI). v_{max} so maksimalne hitrosti reakcij ustreznih encimov, označenih na sliki 4.

5 ZAKLJUČEK

V magistrski nalogi smo s fizikalno-matematičnim modelom presnove arahidonske kisline v eikozanoide in z modeliranjem njihovega vpliva na zoženje dihalnih poti povezali različne nivoje opazovanja, ključne za opis bronhokonstrikcije, ki je eden najbolj perečih kliničnih znakov aspirinske intolerance. Na ta način smo povezali proteomski in metabolomski nivo na molekularni in celični ravni ter napovedali fiziološki odziv na ravni tkiva in organa. Pri tem smo izhajali iz ugotovitev meritev ekspresij in količin ključnih encimov, ki so udeleženi v presnovi arahidonske kisline. Na podlagi razlik, ki so bile ugotovljene med neastmatiki, ter na aspirin tolerantnimi in intolerantnimi pacienti, smo oblikovali hipotetične modelne populacije, ki bi lahko na proteomskem nivoju ustrezale različnim tipom pacientov. Najprej smo določili referenčno stanje modela, tj. modelna populacija neastmaitkov v stanju neinflamacije. Nato smo na podlagi razlik v ekspresiji encimov LTC_4S in PGES predpostavili 4 modelne populacije astmatikov. Od tega je ena

tolerantna na aspirin, druge tri pa intolerantne. Razlike v ekspresiji encima LTC_4S so bile izmerjene, razlike v encimu PGES pa smo napovedali na osnovi razlik, ki so bile izmerjene na metabolomskem nivoju v eksperimentalnih situacijah, ki so simulirale stanje inflamacije in neinflamacije. Ti dve modelni stanji smo definirali tudi sami na podlagi izmerjenih razlik na metabolomskem nivoju in na podlagi informacij iz različnih virov, ki so nam posredovali odgovor, kako lahko stanje inflamacije vpliva na ekspresijo ključnih encimov v procesu presnove arahidonske kisline. V celoti smo tako obravnavali 10 različnih modelnih stanj – 5 različnih modelnih populacij v dveh stanjih, stanju inflamacije in neinflamacije. Razlike, ki smo jih predpostavili med različnimi modelnimi populacijami astmatikov na proteomskem nivoju, so naslednje: modelna populacija $AIA^{(1)}$ se razlikuje od modelne populacije ATA zgolj po znižani ekspresiji encima PGES, $AIA^{(2)}$ se razlikuje od ATA zgolj po zvišani ekspresiji encima LTC_4S, $AIA^{(3)}$ pa se od ATA razlikuje po zvišani ekspresiji encima LTC_4S in znižani ekspresiji encima PGES. V modelnih izračunih, v katerih simuliramo različno obnašanje modelnih populacij na ravni produkcije eikozanoidov, njihovih razmerij, napovedi sile na celični ravni, skrčitve tipičnih dihalnih poti in napovedi FEV1 v odsotnosti in prisotnosti različnih NSAR, paracetamola in selektivnega inhibitorja COX, se izkaže modelna populacija $AIA^{(3)}$ kot tista, za katero obstaja največje tveganje za bronhokonstrikcijo po zaužitju že nizke doze zdravila. Modelna populacija $AIA^{(2)}$ se izkaže kot malo manj senzitivna, vendar pa pri velikih dozah zdravil kaže enako obnašanje kot $AIA^{(3)}$. Modelni populaciji $AIA^{(1)}$ in ATA kažeta podobno obnašanje pri velikih dozah zdravil, medtem ko pri majhnih dozah izkazuje $AIA^{(1)}$ večjo občutljivost. Očitna razlika med $AIA^{(1)}$ in ATA je še ta, da je v stanju inflamacije pri $AIA^{(1)}$ bazalna stopnja FEV1 (tj. brez zdravila) veliko manjša kot pri ATA. Na metabolomskem nivoju je moč različne modelne populacije razločiti na podlagi razlik v razmerjih med eikozanoidi ($[PGE_{2i}]/[cisLT_i]$ in $[PGE_{2i}]/[PGD_{2i}]$) v bazalnem stanju. Razlike pa so še posebej opazne na ravni absolutnih koncentracij, ki jih tukaj z modelom prvič napovemo za intersticij gladke mišičnine dihalnih poti. Razlike so še posebej očitne med modelnima stanjema inflamacije in neinflamacije. Modelne populacije AIA se med seboj na metabolomskem nivoju razlikujejo na naslednji način: $AIA^{(1)}$ izkazuje v neinflamaciji in v inflamaciji nizko raven $[PGE_{2i}]$ in nizko raven $[cisLT_i]$, $AIA^{(2)}$ izkazuje v neinflamaciji in v inflamaciji visoko raven $[PGE_{2i}]$ ter povišano raven $[cisLT_i]$ v inflamaciji, $AIA^{(3)}$ izkazuje v neinflamaciji in v inflamaciji nizko raven $[PGE_{2i}]$ in v obeh stanjih najvišjo raven $[cisLT_i]$. Vzrok za te razlike na

metabolomskem nivoju so razlike na proteomskem nivoju. Vse to pa ima za posledico različne rezultate na ravni tkiva in organa, tj. na ravni razvoja sile v gladki mišičnini dihalnih poti, njihovega polmera in FEV1. Velike razlike med modelnimi populacijami na tem nivoju obravnave se odražajo predvsem v stanju inflamacije in po simuliranem oralnem zaužitju zdravila. Tudi tokrat se modelna populacija $AIA^{(3)}$ izkaže za najbolj senzitivno. Zelo podoben odziv na zdravila ima tudi $AIA^{(2)}$, medtem ko je pri $AIA^{(1)}$ odziv nekoliko manjši.

Z modelom smo določili mejne doze različnih NSAR, paracetamola in selektivnega inhibitorja COX – celecoxiba, nad katerimi bi pri posamezni modelni populaciji prišlo do tveganja zelo močne bronhokonstrikcije in astmatičnega napada. Določili smo tudi doze zdravil, pri katerih se FEV1 pri določeni astmatični skupini zniža za 20 % od njegove začetne vrednosti brez zdravila (D_{20}). Na tak način se s provokacijskimi testi v klinični praksi določa senzitivnost na NSAR. Izmed vseh simuliranih zdravil, indometacin predstavlja največje tveganje za bronhokonstrikcijo, saj do velikega znižanja FEV1 privede že zelo majhna doza. Po drugi strani imata ibuprofen in naproksen zelo primerljiv vpliv na bronhokonstrikcijo. Za zdravilo z najmanjšim tveganjem za bronhokonstrikcijo se v modelu izkaže paracetamol, ki pa bi pri najbolj senzitivni modelni populaciji $AIA^{(3)}$ ob nekajkratni tipični terapevtski dozi lahko bil potencialno nevaren v smislu bronhokonstrikcije. Celecoxib, ki naj bi po nekaterih študijah veljal za varno zdravilo za aspirinsko intolerantne astmatike, se v modelu izkaže kot enako ali celo bolj nevaren v smislu tveganja bronhokonstrikcije kot ostali neselektivni NSAR. Še posebej to velja v stanju inflamacije, kjer bi lahko tudi pri na aspirin tolerantnih astmatikih izzval močnejšo bronhokonstrikcijo.

Do sedaj smo lahko z modeli ob prisotnosti NSAR napovedali absolutne koncentracije eikozanoidov in njihova razmerja v eni vnetni celici. Na podlagi teh rezultatov smo nato sklepali na verjetnost za pojav bronhokonstrikcije. Mera za to je bilo razmerje $[PGE_2]/[LTC_4]$. Z izračunom absolutnih koncentracij eikozanoidov v intersticiju gladkih mišic dihalnih poti in napovedi razvoja sile v gladki mišičnini dihalnih poti, njihovega polmera in FEV1 pa smo pokazali, da so za napoved bronhokonstrikcije ključne vrednosti absolutnih koncentracij eikozanoidov, ne pa zgolj njihova razmerja. Tako smo z modelnimi simulacijami potrdili našo začetno postavljeno hipotezo, saj se je namreč

izkazalo, da se pri enaki vrednosti razmerja med bronhodilatorjem (PGE_2) in ključnimi bronhokonstriktorji (cisLT) lahko razvije močno različna sila v dihalnih poteh takrat, ko so absolutne koncentracije teh metabolitov večje. Tako stanje ustreza stanju inflamacije, ko so ekspresije posameznih ključnih encimov v procesu presnove arahidonske kisline do eikozanoidov povečane. Rezultat višjih absolutnih koncentracij metabolitov pri ohranjenem razmerju rezultira k povečani zožitvi dihalnih poti in posledičnem znižanju FEV1.

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PRILOGA 1

VREDNOSTI MODELNIH PARAMETROV

Tabela 1. Vrednosti modelnih parametrov podmodela za napoved absolutnih koncentracij eikozanoidov v intersticiju

Parameter	Vrednost in enota	Opis	Referenca
C_a	$1 \cdot 10^{-15}$ M	koncentracija vseh aktivnih eozinofilcev v stanju inflamacije	[1]
V_{AVG}	0,864 pL	povprečni volumen eozinofilca	[2]
V_i	0,21 L	volumen mišičnega intersticija pljuč	[3]
k_{LTC_exp}	$1{,}5 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta pretoka LTC_4 iz celice	[4, 5]
k_{PGE_exp}	$2{,}8 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta pretoka PGE_2 iz celice	[4, 6]
k_{PGD_exp}	$2{,}8 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta pretoka PGD_2 iz celice	[4, 6]
k_{LTC}	$3{,}3 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta pretvorbe LTC_{4i} v LTD_{4i}	[1, 7]
k_{LTD}	$1{,}1 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta pretvorbe LTD_{4i} v LTE_{4i}	[1, 7]
k_{LTE_elim}	$7{,}1 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta eliminacije LTE_{4i} iz mišičnega intersticija pljuč	[1, 8]
k_{PGE_elim}	$2{,}8 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta eliminacije PGE_{2i} iz mišičnega intersticija pljuč	kalibracija modela
k_{PGD_elim}	$2{,}8 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta eliminacije PGD_{2i} iz mišičnega intersticija pljuč	kalibracija modela
V_{d_LTC}	1,54 L	navidezni volumen porazdelitve LTC_{4i} v mišičnem intersticiju pljuč	[1]
V_{d_LTD}	1,54 L	navidezni volumen porazdelitve LTD_{4i} v mišičnem intersticiju pljuč	[1]
V_{d_LTE}	1,54 L	navidezni volumen porazdelitve LTE_{4i} v mišičnem intersticiju pljuč	[1]

V_{d_PGE}	1,54 L	navidezni volumen porazdelitve PGE_{2i} v mišičnem intersticiju pljuč	[1]
V_{d_PGD}	1,54 L	navidezni volumen porazdelitve PGD_{2i} v mišičnem intersticiju pljuč	[1]

Tabela 2. Vrednosti modelnih parametrov podmodela za napoved razvoja sile v gladkih mišicah dihalnih poti

Parameter	Vrednost in enota	Opis	Referenca
		LTC_{4i}	
F_{max}	0,82	maksimalna razvita sila	[9]
K	$9{,}0 \cdot 10^{-4}$ µM	$[LTC_{4i}]$ pri ½ F_{max}	[9]
n	0,81	Hillov koeficient	[9]
		LTD_{4i}	
F_{max}	0,62	maksimalna razvita sila	[9]
K	$2{,}2 \cdot 10^{-3}$ µM	$[LTD_{4i}]$ pri ½ F_{max}	[9]
n	0,76	Hillov koeficient	[9]
		LTE_{4i}	
F_{max}	0,56	maksimalna razvita sila	[9]
K	0,11 µM	$[LTE_{4i}]$ pri ½ F_{max}	[9]
n	0,64	Hillov koeficient	[9]
		PGD_{2i}	
F_{max}	0,60	maksimalna razvita sila	[10]
K	5,4 µM	$[PGD_{2i}]$ pri ½ F_{max}	[10]
n	0,60	Hillov koeficient	[10]
		PGE_{2i}	
F_{start}	1,00	razvita sila pri začetni koncentraciji	[11]
F_{end}	0,14	razvita sila pri končni koncentraciji	[11]
K	$2{,}6 \cdot 10^{-2}$ µM	$[PGE_{2i}]$ pri ½ F_{max}	[11]
n	0,81	Hillov koeficient	[11]

Tabela 3. Vrednosti modelnih parametrov podmodela za izračun FEV1

Parameter	Vrednost in enota	Opis	Referenca
R	3,007 mm	polmer hrustančnega obročka	[1, 12]
R_{in}	2,073 mm	polmer notranjega roba gladke mišičnine pri popolnoma relaksiranem stanju	[1, 12]
k	4,8 mm^{-1}	konstanta vzmeti	kalibracija modela
$FEV1_{MAX}$	4,94 L	maksimalna vrednost FEV1	[1, 13]

Tabela 4. Vrednosti farmakokinetičnih parametrov zdravil in njihove inhibitorne konstante na encima COX-1 in COX-2

Parameter	Vrednost in enota	Opis	Referenca
		indometacin	
k_a	$1{,}0 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta absorpcije	[14]
k_e	$9{,}5 \cdot 10^{-5}$ s^{-1}	hitrostna konstanta eliminacije	[14]
V	45 L	navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila	[14]
K_{I1}	$3{,}2 \cdot 10^{-2}$ µM	ravnotežna disociacijska konstanta vezave inhibitorja (indometacina) s COX-1	[15]
K_{I2}	1,02 µM	ravnotežna disociacijska konstanta vezave inhibitorja (indometacina) s COX-2	[15]
		ibuprofen	
k_a	$2{,}8 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta absorpcije	[16]
k_e	$1{,}0 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta eliminacije	[16]
V	8,4 L	navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila	[16]

K_{I1}	1,3 μM	ravnotežna disociacijska konstanta vezave inhibitorja (ibuprofena) s COX-1	[17]
K_{I2}	60 μM	ravnotežna disociacijska konstanta vezave inhibitorja (ibuprofena) s COX-2	[17]
		naproksen	
k_a	$1{,}3 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta absorpcije	[18]
k_e	$1{,}8 \cdot 10^{-5}$ s^{-1}	hitrostna konstanta eliminacije	[18]
V	6,9 L	navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila	[18]
K_{I1}	5,5 μM	ravnotežna disociacijska konstanta vezave inhibitorja (naproksena) s COX-1	[19]
K_{I2}	41,8 μM	ravnotežna disociacijska konstanta vezave inhibitorja (naproksena) s COX-2	[19]
		paracetamol	
k_a	$1{,}1 \cdot 10^{-3}$ s^{-1}	hitrostna konstanta absorpcije	[20]
k_e	$6{,}9 \cdot 10^{-5}$ s^{-1}	hitrostna konstanta eliminacije	[20]
V	63 L	navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila	[20]
K_{I1}	49,9 μM	ravnotežna disociacijska konstanta vezave inhibitorja (paracetamola) s COX-1	[21]
K_{I2}	1962 μM	ravnotežna disociacijska konstanta vezave inhibitorja (paracetamola) s COX-2	[21]
		celecoxib	
k_a	$2{,}0 \cdot 10^{-4}$ s^{-1}	hitrostna konstanta absorpcije	[22]
k_e	$4{,}4 \cdot 10^{-5}$ s^{-1}	hitrostna konstanta eliminacije	[22]
V	242 L	navidezni volumen porazdelitve zdravila normaliziran z deležem v kri absorbiranega zdravila	[22]
K_{I1}	0,2 μM	ravnotežna disociacijska konstanta vezave inhibitorja (celecoxiba) s COX-1	[23]
K_{I2}	0,07 μM	ravnotežna disociacijska konstanta vezave inhibitorja (celecoxiba) s COX-2	[23]

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PRILOGA 2

IZVIRNI ZNANSTVENI ČLANEK

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Dynamic model of eicosanoid production with special reference to non-steroidal anti-inflammatory drug-triggered hypersensitivity

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Abstract: The authors developed a mathematical model of arachidonic acid (AA) degradation to prostaglandins (PGs) and leukotrienes (LTs), which are implicated in the processes of inflammation and hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs). The model focuses on two PGs (PGE_2 and PGD_2) and one LT (LTC_4), their % increases and their ratios. Results are compared with experimental studies obtained from non-asthmatics (NAs), and asthmatics tolerant (ATA) or intolerant (AIA) to aspirin. Simulations are carried out for predefined model populations NA, ATA and three AIA, based on the differences of two enzymes, PG E synthase and/or LTC_4-synthase in two states, that is, no-inflammation and inflammation. Their model reveals that the model population with concomitant malfunctions in both enzymes is the most sensitive to NSAIDs, since the duration and the capacity for bronchoconstriction risk are highest after simulated oral dosing of indomethacin. Furthermore, inflammation prolongs the duration of the bronchoconstriction risk in all AIA model populations, and the sensitivity analysis reveals multiple possible scenarios leading to hypersensitivity, especially if inflammatory processes affect the expression of multiple enzymes of the AA metabolic pathway. Their model estimates the expected fold-changes in enzyme activities and gives valuable information for further targeted transcriptomic/proteomic and metabolomic studies.

1 Introduction

Eicosanoids are chemical compounds with essential functions for maintaining cellular activities in living organisms. They operate as paracrine and autocrine mediators. Therefore eicosanoids are also implicated in many inflammatory processes and diseases. Thus the production and signalling pathways of eicosanoids are the targets of several pharmacological interventions. For all mammalian, arachidonic acid (AA) is the essential precursor of eicosanoids. The metabolism of AA is a complex network of non-enzymatic and enzyme-driven steps, comprising a multitude of different enzymes, receptors and additional compounds. These metabolic pathways are also the targets of multiple endogenous as well as exogenous modulators, eventually causing up- and down-regulation of eicosanoid production, accompanied by various physiological effects [1–4].

On the basis of an enzymatic view, metabolism of AA can be roughly subdivided into the cyclooxygenase (COX) pathway and the lipoxygenase (LOX) pathway revealing prostanoids and leukotrienes (LTs) as metabolites, respectively [5]. Representative bioactive metabolites of the COX-pathway are prostaglandin (PG) E_2 (PGE_2) and PG D_2 (PGD_2), and main metabolites of LOX pathway are cysteinyl-LTs (cys-LTs, i.e. LTC4, LTD4, LTE4) [5]. The biological effects of eicosanoids are pleiotropic. Some effects and related diseases of the above mentioned eicosanoids are selected to give a general idea of their pivotal biological relevance. To begin with the LOX-pathway: cys-LTs cause bronchoconstriction [6], recruit inflammatory cells [7], increase swelling of the nasal mucosa and increase mucus secretion [5]. They are approximately 30–3000-times more potent bronchoconstrictors than histamine in healthy humans in-vivo [8]. Their main systemic sources are leukocytes like eosinophils and basophils, as well as mast cells and epithelial cells [9–12]. Overproduction of cys-LTs is a hallmark of the aspirin-exacerbated respiratory disease (AERD), which is characterised by chronic rhinosinusitis, nasal polyps and severe bronchial asthma because of the hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs) [13]. The effects of the metabolites of the COX-pathway are more diverse. For instance, PG D_2 (PGD_2) induces bronchoconstriction in allergic asthma [14], whereas PG E_2 (PGE_2) prevents bronchoconstriction [15, 16], protects airways against inflammation, reduces LT production through the inhibition of the 5-LOX pathway [12, 16–19], inhibits mast cell degranulation, and promotes normal airway function [20–23]. Underproduction of PGE_2 strongly diminishes the inhibitory effects of PGE_2 on the 5-LOX pathway, thus enhances cys-LT production [12] and it reduces the inhibitory effect of PGE_2 on mast cells [13, 22, 24]. On the basis of the thereon, it was hypothesised that decreased levels of PGE_2 might induce bronchoconstriction by underproduction of cAMP [11, 12, 19]. On the contrary, locally increased levels of PGE_2, for example, by inhalation, might induce bronchodilation and could potentially protect aspirin-precipitated attacks of asthma [16, 23, 25]. In human airways PGE_2 is produced by many cells including epithelium, fibroblasts, smooth muscle cells, alveolar cells, macrophages, phagocytes and lymphocytes [2, 26]. The COX pathway comprises of two isozymes, COX-1 and COX-2. Both pathways are inhibited by NSAIDs, comprising non-selective and

selective COX-inhibitors. The LOX pathway is most often blocked by LOX-inhibitors or it is down-regulated by the action of cys-LT-receptor antagonists. These substances are well known and prominent drugs for many diseases like tumour or asthma [27–29]. NSAIDs play a crucial role in tilting AA metabolism in favour of the LOX pathway causing cys-LT overproduction [4, 9, 11, 12, 18, 19, 30, 31]. There is increasing evidence that NSAID-triggered hypersensitivity, like AERD [also known as aspirin induced asthma (AIA)], partly originates from the imbalance of eicosanoids, secreted by activated cells. These imbalances, in concert with others, result in bronchial hyper-reactivity – a characteristic symptom of asthma.

Patients suffering from AIA demonstrate a characteristic pattern of multiple defects, observed either as hyper-inflammation or underproduction of anti-inflammatory mediators, but also detected as overproduction of bronchoconstrictors and pro-inflammatory mediators [9–13, 19, 25, 32–38]. A strict separation of eicosanoid profiles of un-inflamed and inflamed states had not been completely accomplished by metabolomic studies because of the considerably overlapping metabolomic profiles of these entities. Nevertheless, there have been several promising attempts during the recent years [10–13, 19, 25, 30, 32–38].

In one of these studies, Pierzchalska *et al.* [32] systematically studied and measured eicosanoid production in response to proinflammatory stimuli in different asthmatic patient groups and controls. Large deviations of cellular eicosanoid production were noted between the subgroups of asthmatic patients that are tolerant or intolerant to aspirin as well as between the latter two subgroups and a group of non-asthmatics (NAs) in their experiments [32]. We performed this theoretical study to find out with the help of mathematical modelling whether the differences in eicosanoid profiles of different patient groups might be predicted from the differences in their enzyme-expression profiles within the cells responsible for eicosanoid production. We focused on the maximal velocities of the enzymes PG E synthase (PGES) and LT C_4-synthase (LTC_4S). The selection of these two parameters was encouraged by experimental findings that different patient groups exhibited different expressions of LTC_4S [10, 39], and, moreover, that they differently responded to inflammatory stimuli in terms of PGES up-regulation [32]. We investigated whether maximal velocities of enzymes PGES and LTC_4S characterise and distinguish different patient groups [i.e. NA, asthmatics tolerant to aspirin (ATA) and asthmatics intolerant to aspirin (AIA)] at the level of eicosanoid production. The effect of inflammation on eicosanoid production was also modelled, both at rest in equilibrium conditions without the drug as well as after simulated oral dosing of indomethacin. The impact of the state of inflammation on the model results (concerning absolute and relative values of $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ as well as the ratios $[PGE_2]/[LTC_4]$ and $[PGE_2]/[PGD_2]$) was analysed especially with regard to sensitivity of different model populations to indomethacin – a typical exemplar of NSAIDs.

2 Materials and methods

2.1 Kinetic scheme and mathematical model

The kinetic scheme of AA metabolism and its interaction with an NSAID, as considered for mathematical modelling, is presented in Fig. 1. In brief, AA with constant influx is first transformed via a LOX pathway into either 5- or 15-hydroperoxyeicosatetraenoic acid (5- or 15-HPETE). Enzymes involved in this step are 5-LOX for the conversion of AA into 5-HPETE, and, 15-LOX for the conversion of AA into 15-HPETE. 15-HPETE is further transformed into 15-HETE by phospholipid-hydroperoxy-glutathione-peroxidase (PHGPx), whereas 5-HPETE is further transformed either into LT A_4 (LTA_4) by 5-LOX or into 5-HETE by PHGPx. LTA_4 is then transformed either by LTC_4S into LT C_4 (LTC_4) or by LTA_4 hydrolase (LTA_4H) into LT B_4 (LTB_4), which is exported from the cells via ATP-binding cassette transporter C4 (ABCC4). Via COX pathway, AA is transformed into PG H_2 (PGH_2) by two isozymes, COX-1 and COX-2, acting in parallel. PGH_2 is the precursor of all prostanoids, that is, PGs (PGE_2, PGD_2 and $PGF_{2\alpha}$), prostacyclin (PGI_2) and thromboxane (TXB_2). The synthases PGES, PGDS, PGFS, PGIS and TXS are the corresponding enzymes of the subsequent catalytic reactions generating the above mentioned prostanoids. An efflux of all modelled eicosanoids is considered. Their degradation rate might be interpreted either by their export or by further metabolisation. The inhibitory effect of PGE_2 on 5-LOX, and of NSAIDs on COX-1 and COX-2, plus the auto-regulation of LTC_4S is in Fig. 1 denoted by dashed lines.

The mathematical model consists of a set of fourteen first-order non-linear ordinary differential equations, describing the time evolution of system variables, which are concentrations of fourteen metabolites (M), that is, [AA], $[PGH_2]$, $[PGE_2]$, $[PGD_2]$, $[PGI_2]$, $[PGF_{2\alpha}]$, $[TXB_2]$, [5-HPETE], [5-HETE], $[LTA_4]$, $[LTC_4]$, $[LTB_4]$,

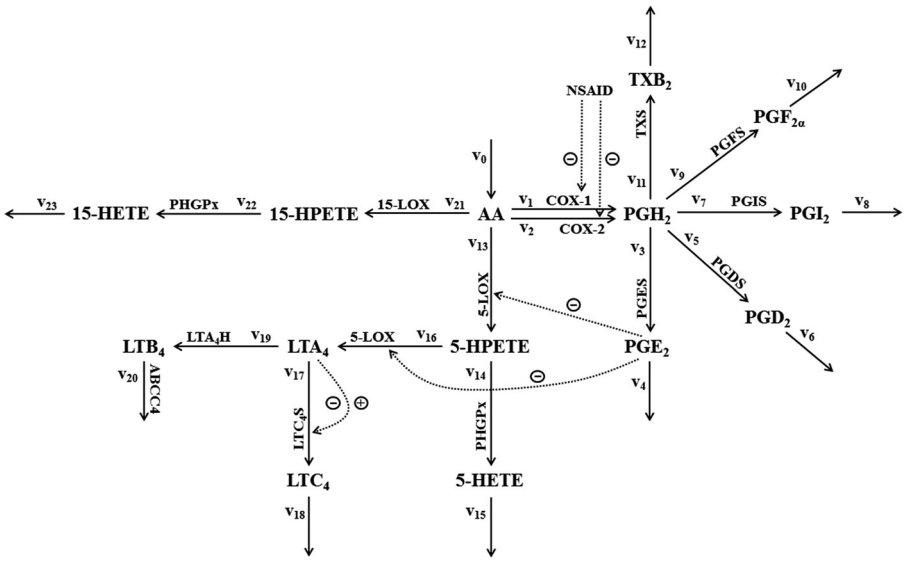

Fig. 1 *Kinetic scheme of AA metabolism considered for mathematical modelling*

Metabolic degradation of AA via COX and LOX pathway yields eicosanoids: PGs, thromboxane (TX), LTs and hydro(pero)xyeicosatetraenoic acids
Full lines with the corresponding enzymes depict the direction of metabolic fluxes, dotted lines depict either inhibitory or activating effects on enzymes
Numeration of the fluxes reflects the reaction rates of the mathematical model

[15-HPETE] and [15-HETE]. The time derivative of a selected system variable (d[M]/dt) equals the difference between the sum of all incoming ($\sum v_{in}$) and the sum of all outgoing ($\sum v_{out}$) metabolic fluxes, all with respect to numeration and the direction of arrows in the kinetic scheme in Fig. 1

$$\frac{d[M]}{dt} = \sum v_{in} - \sum v_{out} \quad (1)$$

Metabolic fluxes or reaction velocities (v_i) are defined as

$$v_i = \text{const.}$$

for $i = 0$;

$$v_i = \frac{v_{max\,i}[S]}{K_i + [S]} \quad (2)$$

for $i = 3$, 5, 7, 9, 11, 14, 19, 20, 21, 22 (decoding enzymes PGES, PGDS, PGIS, PGFS, TXS, PHGPx, LTA_4H, ABCC4, 15-LOX and PHGPx, respectively), where $v_{max\,i}$ is the maximal reaction velocity, K_i is the Michaelis–Menten constant and S is either PGH_2 (for $i = 3$, 5, 7, 9, 11), AA (for $i = 21$), 15-HPETE (for $i = 22$), 5-HPETE (for $i = 14$), LTA_4 (for $i = 19$) or LTB_4 (for $i = 20$)

$$v_i = \frac{v_{max\,i}[S]}{K_i\alpha_i + [S]}, \text{ where } \alpha_i = \left(1 + \frac{[I]}{K_{Ii}}\right) \quad (3)$$

for $i = 1$, 2, 13, 16, (decoding enzymes COX-1, COX-2 and 5-LOX (2 ×), respectively), where $v_{max\,i}$ is the maximal reaction velocity, K_i is the Michaelis–Menten constant, K_{Ii} is the equilibrium dissociation constant of the inhibitor I from an enzyme in case of reversible competitive enzyme inhibition, S is either AA (for $i = 1$, 2, 13) or 5-HPETE (for $i = 16$) and I is either NSAID (for $i = 1$, 2) or PGE_2 (for $i = 13$, 16)

$$v_i = \frac{v_{max\,i}[S]}{A + B[S] + C[S]^2} \quad (4)$$

for $i = 17$ (decoding enzyme LTC_4S), where A, B and C are the corresponding parameters, and S is LTA_4

$$v_i = k_i[S] \quad (5)$$

for $i = 4$, 6, 8, 10, 12, 15, 18, 23, S is either PGE_2 (for $i = 4$), PGD_2 (for $i = 6$), PGI_2 (for $i = 8$), $PGF_{2\alpha}$ (for $i = 10$), TXB_2 (for $i = 12$), 5-HETE (for $i = 15$), LTC_4 (for $i = 18$) or 15-HETE (for $i = 23$) and k_i is the rate constant.

Equation (2) known as steady-state Michaelis–Menten kinetics, was used in all enzyme-driven reaction steps, which corresponded well with this type of enzyme kinetics. In the cases in which the effect of an inhibitor on an enzyme was simulated a reversible competitive inhibition, described by (3), was used. This was the case for the modelling of the inhibitory effect of NSAIDs. Indomethacin is known as a reversible competitive inhibitor of COX-1 and COX-2. We considered also the inhibitory effect of PGE_2 on 5-LOX [12, 16–19] in the model. Since the entire mechanism of this inhibition is not completely elucidated [9, 11, 18, 40], a reversible competitive enzyme inhibition was considered here as in our previous [41–43] and the other models [44] of eicosanoid production. Equation (4) was used in the case of reaction step 17, in which enzyme LTC_4S catalyses conversion of substrate LTA_4 into the product LTC_4. Kinetics of this particular enzyme and the substrate involves substrate auto-inhibition [45], which is characterised by a bell-shaped velocity-substrate relationship. In other words, this is the enzyme kinetics, in which LTA_4 auto-regulates the velocity of the enzyme LTC_4S. Equation (5), known as linear mass-action law, was used in those cases in which enzymes were either not involved or not identified. This was primarily the case for eicosanoid effluxes except for the efflux of LTB_4. Similar approaches in modelling were used also in other models of eicosanoid production [29, 44, 46–48].

In the dynamic simulations, time dependent drug plasma concentration [NSAID] is modelled with the standard two-store pharmacokinetic model for oral drug dosing, taking into account absorption and elimination phases

$$[\text{NSAID}] = \frac{Dk_a}{MV(k_a - k_e)}\left(e^{-k_e t} - e^{-k_a t}\right) \quad (6)$$

where D is the drug dose, M is the molecular mass of the drug, V is the apparent volume of drug distribution normalised with the fraction of the absorbed drug, and, k_a and k_e are the first-order absorption and elimination rate constants, respectively. These pharmacokinetic parameters are presented in Supplementary Table 2 along with other kinetic parameters of the model.

For the purpose of sensitivity analysis, the response coefficients, R, defined as the ratio between the relative change of the selected system variable with respect to small alterations of a single parameter value, and the relative change of this particular model parameter, were calculated according to the equation

$$R = \frac{(\Delta X/X)}{(\Delta P/P)} \quad (7)$$

where X is the model variable and P is the model parameter. For instance, response coefficient −2 means that system variable decreased for 20%, if one parameter value increased for 10%.

Software used for all calculations was Berkeley Madonna 8.0.1 (R. Macey and G. Oster, University of California at Berkeley). The system of fourteen differential equations (explicitly presented in Supplemental information), evolved from (1) by considering all definitions of the reaction velocities (2)–(5) and in dynamic simulations considering also the time-dependent plasma drug concentration (6), was numerically integrated from the initial stationary state, which was determined separately for each model variable in the absence of NSAID for each particular simulation (see Supplementary Table 5 for the complete set of initial conditions used in the modelling). The numerical integration method with variable integration step was used. The complete set of parameters for a reference state, that is, NA model population in the state of no-inflammation (NI), is presented in Supplementary Table 2. Some parameter values are subject to changes. These values depend on the model population and the model state and are separately presented in Tables 1–3, and they are discussed in the following sections.

Table 1 Values of model parameters that define model states of NI and I in all model populations

Parameter	Enzyme	NI	I
v_{max2}, μM s^{-1}	COX-2	0.055	0.28
$v_{max13,\ 16}$, μM s^{-1}	5-LOX	2.53	86
v_{max21}, μM s^{-1}	15-LOX	2.5	25
v_0, μM s^{-1}	PLA_2	0.07	0.7

Table 2 Values of maximal velocities of the enzyme LTC_4S (v_{max17}) for: NA model population, aspirin-tolerant asthmatic model population (ATA) and three different aspirin-intolerant-asthmatic model populations ($AIA^{(1)}$, $AIA^{(2)}$, $AIA^{(3)}$)

Parameter	Enzyme	NA	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
v_{max17}, μM s^{-1}	LTC_4S	0.057	0.23	0.23	1.15	1.15

Table 3 Values of maximal velocities of the enzyme PGES (v_{max3}) for: NA model population, aspirin-tolerant asthmatic model population (ATA) and three different aspirin-intolerant-asthmatic model populations ($AIA^{(1)}$, $AIA^{(2)}$, $AIA^{(3)}$) either in model states NI or inflammation (I)

Model state	Parameter	Enzyme	NA	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
NI	v_{max3}, μM s^{-1}	PGES	5.0	5.0	2.5	5.0	2.5
I	v_{max3}, μM s^{-1}	PGES	20	20	5.0	10	5.0

2.2 Reference points for model simulations

In the process of constructing the model, we first determined the set of parameter values simulating NA model population in state of NI (NA-NI), which first served as a reference for the definition of the model state of inflammation (I), and also later for the definition of all other model populations. In addition to NA, we defined four hypothetical model populations simulating asthmatic patients. One of them a simulated patient group of ATA and three of them simulated patient group of asthmatics intolerant to aspirin ($AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$). A similar approach was used in our previous, simpler model of eicosanoid production [41, 43, 49]. One of the differences among both models is that in the previous one, the populations were defined differently and the model states of inflammation and NI were not considered for modelling. Since each of the model population in the present study could exist in either state of NI or inflammation we will analyse and compare ten model cases, in total. The following paragraphs will present the parameter estimation procedures for the model state of inflammation (I) and the model populations that are all derived from the reference model state – NA in state of NI (NA-NI). The complete description of the definition of the reference model state is presented in the Supplementary Information.

Here we first focus on the definition of transition from the model state of NI to inflammation (I) for the model population NA. The modelling of two different states, inflammation and NI, was motivated by the publication of Pierzchalska *et al.* [32], who investigated the impact of proinflammatory stimuli on the cellular production of eicosanoids and on the expression of enzymes in cells obtained from patient groups NA, ATA and AIA. Their results were obtained from cultured fibroblasts gained from human airways. Fibroblasts were cultured with or without addition of cytomix and the supernatants were analysed by gas chromatography/mass spectrometry. Furthermore, they estimated COX-1 and COX-2 expression by RT-PCR and immunoblotting. The addition of cytomix imitated cytokine- and bacterial-driven inflammation, whereby cytomix used in experiments was a mixture of human recombinant interleukin (IL-1β), tumour necrosis factor (TNF-β) and lipopolysaccharide from *Pseudomonas aeruginosa* [32]. This experiment revealed several-fold higher concentrations of PGE_2 and PGD_2 in sample cells obtained from NA after the addition of cytomix. These results are depicted in Figs. 2*a* and *b* as grey columns with error bars for NA, where % increases of $[PGE_2]$ and $[PGD_2]$ are presented for all patient groups (NA, ATA and AIA). In the NA group more than twenty-five-fold increase in $[PGE_2]$ and approximately four-fold increase in $[PGD_2]$ was observed after the addition of cytomix. Such extensive increases of the model variables $[PGE_2]$ and $[PGD_2]$ had to be predicted in the transition from the model state of NI to inflammation via changes in particular model parameters. Therefore we first carried out the sensitivity analysis for our reference model state (NA-NI) to identify the parameters with the highest impact on the model variables. Response coefficients for the variables $[PGE_2]$ and $[PGD_2]$ as well as of the ratios $[PGE_2]/[LTC_4]$ and $[PGE_2]/[PGD_2]$, with respect to small alterations in maximal velocities of the enzymes 5-LOX ($v_{max13,\ 16}$), COX-1 (v_{max1}), COX-2 (v_{max2}), PGES (v_{max3}), PGDS (v_{max5}), 15-LOX (v_{max21}) and LTC_4S (v_{max17}) as well as of the inflow of AA (v_0) were calculated according to (7). Maximal velocities of enzymes (v_{max}) were selected for the sensitivity analysis, since they reflect the differences in total concentrations of the enzymes, which in turn depend also on their gene expression. Specifically, v_{max} is defined in all expressions for the enzyme kinetics used here (2)–(4) as a product of total enzyme concentration ($[E]_{tot}$) and the catalytic rate constant (k_{cat}) ($v_{max} = k_{cat}\ [E]_{tot}$). The sensitivity analysis revealed (for details see Supplemental Figures 2–5):

(i) variables $[PGE_2]$ and $[PGD_2]$ are both equally sensitive to changes in v_0 and v_{max} of both COX enzymes, whereby in both cases response coefficients for COX-2 (v_{max2}) are four-fold smaller than those for COX-1 (v_{max1}),
(ii) with regards to enzymes in the prostanoid production pathway downstream of PGH_2, variables $[PGE_2]$ and $[PGD_2]$ are sensitive exclusively to changes of the enzyme of their own production

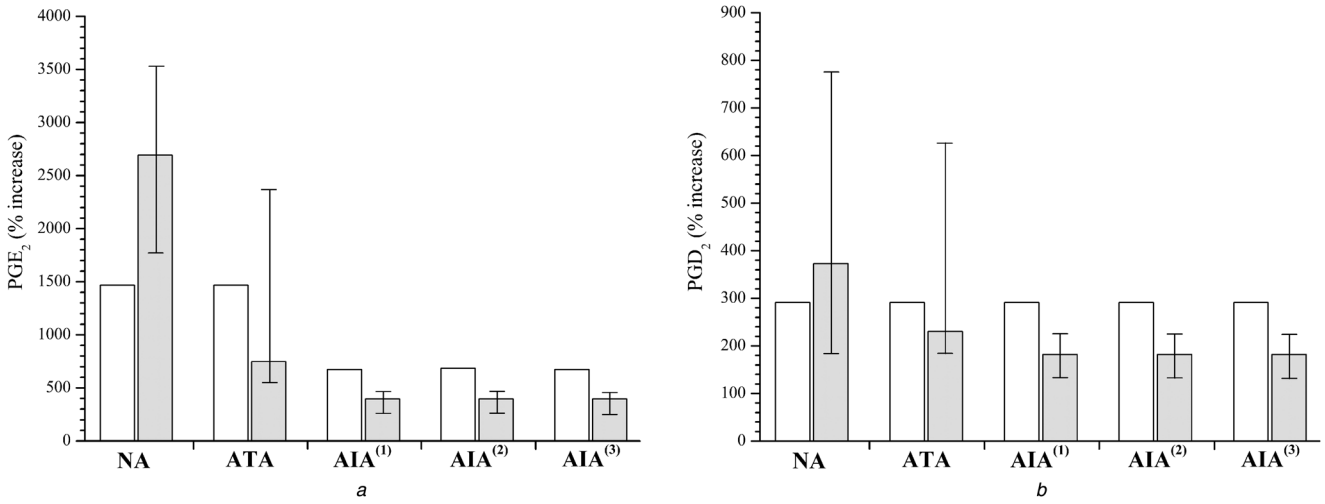

Fig. 2 *Percentage increases (% increase) of PGs in the transition from NI to I*

a $[PGE_2]$
b $[PGD_2]$
Model simulations (open columns) and experimentally induced values upon exposure to cytomix, mimicking inflammation (grey columns with error bars)
Experimental data are obtained from [32] by digitalisation of their Fig. 2

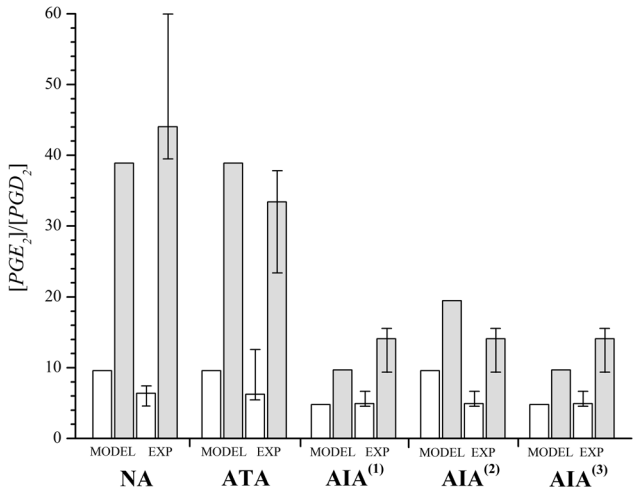

Fig. 3 *[PGE_2]/[PGD_2] ratio in the model states (MODEL) of NI (open columns) and I (grey columns) compared to the experimental setup (EXP) without cytomix (open columns with error bars) and with cytomix (grey columns with error bars)*

Experimental data are obtained from [32] by digitalisation of their Fig. 3 (without indomethacin)

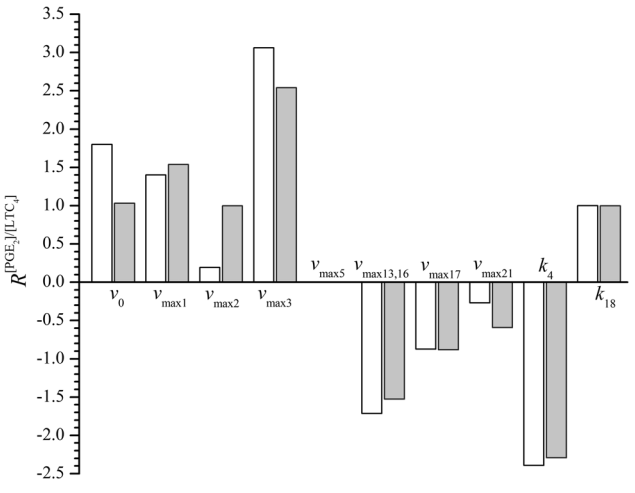

Fig. 5 *Response coefficients (R) of the model variable [PGE_2]/[LTC_4] in model population $AIA^{(3)}$ with respect to variations in parameter values v_{maxi} (i = 0, 1, 2, 3, 5, 13(16), 17, 21) and k_i (i = 4, 18) by 10% in both model states NI (open columns) and I (grey columns)*

pathway (e.g. PGE_2 is sensitive exclusively to changes in PGES) and not to the changes of the others,
(iii) variable [PGE_2]/[PGD_2] is sensitive exclusively (and almost equally with the opposite signs) to changes in parameters of the enzymes PGES (v_{max3}) and PGDS (v_{max5}),
(iv) variable [PGE_2]/[LTC_4] is not sensitive to any of the v_{max} in the prostanoid production pathway downstream of PGH_2 except of v_{max} for PGES (v_{max3}),
(v) increases in parameter values concerning the enzymes COX-1 (v_{max1}), COX-2 (v_{max2}), PGES (v_{max3}) and PLA_2 (v_0) result in positive response coefficients for [PGE_2]/[LTC_4] with a maximal value for v_{max3},
(vi) increases in parameter values concerning the enzymes 5-LOX ($v_{max13,16}$), 15-LOX (v_{max21}) and LTC_4S (v_{max17}) result in negative response coefficients for [PGE_2]/[LTC_4] with the most negative value for $v_{max13,\ 16}$,
(vii) in order to simulate several-fold-increases of the system variables under study almost the same fold-changes of parameter values, to which the variables are most sensitive, will be necessary.

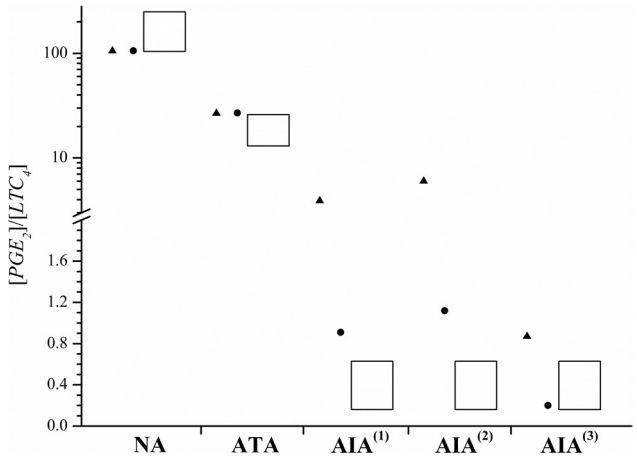

Fig. 4 *[PGE_2]/[LTC_4] ratio simulated by the model in the states of NI (black up-pointing triangle) and I (black circle)*

Measured values (*Rf*) presented with columns are from [19]
Note: the scale on the ordinate is broken, whereby the upper part has logarithmic scale and the lower part has linear one
Logarithmic scaling was chosen for displaying all results

Furthermore, published results of experimental studies considering the expressions of enzymes involved in the pathway of eicosanoid production under inflammatory conditions demonstrated:

(i) increased expression of COX-2 in cultured human fibroblasts after addition of cytomix, mimicking inflammation [32],
(ii) increased expression of 15-LOX in chronic asthma [50], in mild atopic asthma after allergen challenge [51], and as a result of different interleukins actions in various types of cells [51–55],
(iii) increased activation and gene expression of 5-LOX in response to action of various cytokines produced under inflammatory conditions [56–58],
(iv) substantially increased expression of X phospholipase A_2 ($sPLA_2$-X) in acute and chronic mouse asthma models [59],
(v) higher fold-increase in microsomal PGES expression in control than in asthmatic mice in response to IL-1β stimulation [60].

In order to simulate the transition of our reference model state (NA-NI) into the state of inflammation (NA-I), and, moreover, to be in a position to compare our model predictions to measured cytomix-provoked results [32] as well as the others [19], explicit constraints and postulates had to be considered:

(i) based on measured data for NA [32], [PGE_2] has to increase approximately 15–35-fold in inflammation (see Fig. 2*a*); NA – grey column with error bars),
(ii) based on measured data for NA [32], [PGD_2] has to increase approximately 3–8-fold in inflammation (see Fig. 2*b*); NA – grey column with error bars),
(iii) based on measured data for NA [32], [PGE_2]/[PGD_2] ratio has to increase approximately four-fold in inflammation (see Fig. 3; compare the values of open and grey columns with error bars for NA),
(iv) based on measured data for NA [19], the [PGE_2]/[LTC_4] ratio has to be between 100 and 200 (see Fig. 4; column for NA),
(v) postulate of the model is that the [PGE_2]/[LTC_4] ratio has to be conserved in the transition from NI to inflammation in the model population NA.

Note that the latter postulate serves purely as a forecast, if this conservation holds also for the other model populations or not. Based on these five constraints, we determined the values of five model parameters for the state of inflammation in the model population NA. The model parameters were v_{max} of those enzymes, for which experimental evidence was found concerning their up- or down-regulation under different experimental inflammatory conditions as described above [32, 50–60]. These

enzymes and their corresponding parameters given in parentheses are: PLA_2 (v_0), COX-2 (v_{max2}), 5-LOX ($v_{max13,16}$), 15-LOX (v_{max21}) and PGES (v_{max3}). Parameter estimation advanced step-by-step with constant rechecking if the results fulfil the proposed constraints. First, v_{max3} was increased four-fold, which increased the $[PGE_2]/[PGD_2]$ ratio in inflammation for four-fold with respect to NI. Then, v_0 and v_{max2} were arbitrarily increased for ten-fold and five-fold, respectively, which resulted in an increase of $[PGE_2]$ and $[PGD_2]$ as well as in a decrease of $[LTC_4]$, and therefore in a drastic increase of $[PGE_2]/[LTC_4]$. Finally, by keeping all other parameters, that is, v_0, v_{max2} and v_{max3} fixed, the $v_{max13,\ 16}$ and v_{max21}, were determined such that:

(i) absolute concentration of PGD_2 $[PGD_2]$ increased four-fold. Note: this is a typical % increase for NA group, determined in experiment [32] (see Fig. 2*b*); NA – grey column with error bars),
(ii) absolute concentration of PGE_2 $[PGE_2]$ increased four-times as much as that of PGD_2; that is, 16-fold. Note: this is the lowest margin of % increase for NA group, determined in experiment [32] (see Fig. 2*a*); NA – grey column with error bars),
(iii) the ratio $[PGE_2]/[LTC_4]$ was conserved in the transition from NI to inflammation.

Thirty-four-fold and ten-fold larger values of parameters $v_{max13,\ 16}$ and v_{max21}, respectively, reasonably fulfilled all above mentioned constraints. In this way, we have defined a set of parameter values that encodes the model state of inflammation (see Table 1), which will be applied to all other model populations. Parameter value of v_{max3} was intentionally left out, since changes in this parameter value will be considered also in the definition of the model populations.

From this point on, four different sets of parameters for four hypothetical asthmatic model populations were proposed comprising ATA and three types of asthmatics intolerant to aspirin specified as $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$. The AIA model populations differ in their model parameter of maximal enzyme velocities of PGES and LTC_4S. Using this approach, we intended to find out, which of the predicted model populations agrees most consistently with measured results from patient groups. In return, we reveal information which parameters of our model populations might reproduce most likely findings of the published patient groups. Up- and/or down-regulations of PGES and LTC_4S were either measured [10, 39] or hypothesised [32] in experiments related to NSAID-triggered hypersensitivity and inflammation in humans. PGES up-regulation was found also in mouse asthma models [60]. In this respect, v_{max} of PGES and LTC_4S are evidently implicated in the occurrence of NSAID-triggered hypersensitivity. Our model states NA and ATA differ among each other only in the value of maximal velocity for LTC_4S. On the other hand, model-predicted AIA populations differ from ATA in the maximal velocity of either PGES or LTC_4S or in maximal velocities of both. Model population $AIA^{(1)}$ differs from model population ATA only in one parameter value, that is, v_{max} of PGES, whereas model population $AIA^{(3)}$ differs from model population ATA in two parameters, that is, v_{max} of PGES and LTC_4S. Model population $AIA^{(2)}$ in state of NI is characterised by the same v_{max} of PGES as model population ATA. Model populations NA, ATA and AIA have been introduced already in our previous model [41, 43, 49]. That model was simpler, as it did not consider the conversion of AA to PGH_2 and its further conversions to other prostanoids (particularly not to PGE_2). Therefore our previous model did not enable the explicit simulation of the differences in v_{max} of PGES. It also did not consider conversions of AA to 5- and 15-HETE. In the previous model, we defined model populations with differences in v_{max} of COX-1, COX-2 or LTC_4S. COX-2 is known as an inducible enzyme in inflammation [32, 61]. Pierzchalska *et al.* [32] reported no statistically significant differences in the expression of COX-2, when comparing the patient ATA and AIA or NAs after cytomix-induced inflammation. Therefore we considered in our present model the differences in v_{max} of COX-2 for the characterisation of the model states of NI and of inflammation. By assuming diminished v_{max} of LTC_4S our previous model [41, 43, 49] described AIA patients most consistently. Based thereon, we considered the same assumptions for the definition of model populations based on LTC_4S, applying v_{max} of LTC_4S and fold-ratios as used in [41, 43, 49]. Accordingly, the measured five-fold higher expression of LTC_4S in patient group AIA compared to ATA [10, 39] is reflected in our recent model by a five-fold higher value of v_{max} of LTC_4S in the model populations $AIA^{(2)}$ and $AIA^{(3)}$ compared to ATA. In addition, measured four-fold lower expression of LTC_4S in group NA compared to patient group ATA [10, 39] is reflected by a four-fold lower value of v_{max} of LTC_4S in the model population NA compared to model population ATA. The explicit parameter values are presented in Table 2.

Our previous model [41, 43, 49] could not consider differences in v_{max} of PGES. On the other hand, our present model considers differences in v_{max} of PGES twice; first, in the definitions of the model states inflammation and NI, and, second, in the definition of the model populations. The latter consideration was again motivated by observations of Pierzchalska *et al.* They reported the discrepancies in the production of PGE_2 in the cultured samples of bronchial fibroblasts, obtained from NA, ATA and AIA patient groups, which were analysed under two conditions – with or without addition of cytomix in vitro. They concluded [32]:

(i) measured $[PGE_2]/[PGD_2]$ ratio in experimental groups NA and ATA was approximately four-fold larger in case of added cytomix than without cytomix,
(ii) measured $[PGE_2]/[PGD_2]$ ratio in AIA patient group was approximately two-fold larger in case of added cytomix than without cytomix,
(iii) measured $[PGE_2]/[PGD_2]$ ratio was in patient groups NA and ATA not significantly different neither in case of added cytomix nor in case without cytomix,
(iv) measured $[PGE_2]$ increased in patient group ATA approximately two-fold more compared with AIA after addition of cytomix to sample cells.

Pierzchalska *et al.* suggested that cytomix-caused changes in the $[PGE_2]/[PGD_2]$ ratio in different patient groups arise from the different impact of cytokines on the expression of PGES in cells, which were taken from each particular patient group. A different expression of PGES, however, has not been explicitly confirmed by their experiments, since they did not measure it. According to these experimental findings and in relation to the previously defined reference model state – NA-NI, we implemented the following v_{max} of PGES for our model populations for each model state, inflammation or NI:

(i) in NI, in ATA and $AIA^{(2)}$ v_{max3} is the same as in NA,
(ii) for ATA (and in NA), v_{max3} is four-fold higher in inflammation than in NI,
(iii) for $AIA^{(2)}$, v_{max3} is two-fold higher in inflammation than in NI,
(iv) in inflammation, in $AIA^{(1)}$ and $AIA^{(3)}$ v_{max3} is four-fold lower than in ATA,
(v) for $AIA^{(1)}$ and $AIA^{(3)}$, v_{max3} is two-fold lower in NI than in inflammation.

Explicit values of v_{max3} derived from these postulates are presented in Table 3.

2.3 Aims of the investigation

For each model population, that is, NA, ATA and all three AIA, the state of NI and of inflammation (I) was investigated. The absolute concentrations of the main system variables $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ were determined. In addition, their percentage increases (% increases) in the transition from the state of NI to inflammation were analysed and compared to the data measured on patients. Moreover, the $[PGE_2]/[LTC_4]$ ratio and $[PGE_2]/[PGD_2]$ ratio were predicted in the basal state (i.e. at rest without drug) and after

simulated model-based oral dosing of indomethacin. The latter resulted in a dynamical response of the system to the drug. In this concern, we tested the simulated model populations with respect to sensitivity to NSADs. We investigated whether our model results, based on the proposed model populations, agree with the published eicosanoid profiles from asthmatic patient groups. In addition, we aimed to find out whether or not our model might give some rationale for bio-pharmacological-based explanations of the differences observed among patient groups with respect to the metabolomic level of eicosanoid production from the proteomic point of view. Pure prediction of our dynamic model of eicosanoid production depicts the simulation of oral drug dosing for each of our model population, either in model state of NI or in a state of inflammation. A rough estimation for the bronchoconstriction which does not provide any information of the airway smooth muscle reactivity of a patient group or an individual patient will here be predicted purely on the basis of calculated the ratio $[PGE_2]/[LTC_4]$ after oral indomethacin dosing. Finally, the sensitivity analysis of the model population $AIA^{(3)}$, which exhibited the most sensitive response to simulated oral indomethacin dosing, was worked out in terms of response coefficient (R) for the model variable $[PGE_2]/[LTC_4]$.

Throughout Section 3, the results of our model are compared with two independently performed and published experiments, which are closely related with NSAID-triggered hypersensitivity in humans [19, 32]. First, both experimental references accounted for the proper definition of our model's reference state, and partially also for the definition of the model populations. Later, their results served as a strong reference when comparing our model results with those obtained from asthmatic patient groups. Furthermore, these publications served as references for adequacy testing of our predicted kinetic scheme, the methods of mathematical modelling and the consistency of our proposed asthmatic model populations in the model states NI and inflammation.

3 Results and discussion

3.1 Experimental results from patients against results from model populations

Cultured cells, obtained from different patient groups, pre-incubated for 18 h with cytomix, mimicking inflammation, dramatically increased the release of both types of PGs, PGE_2 and PGD_2 [32]. As presented in Fig. 2*a*, significant differences in % increases of $[PGE_2]$ were observed among the patient groups after exposure to cytomix in vitro [32]. However, no significant differences were observed in % increase of $[PGD_2]$ among these groups [32] (see Fig. 2*b*). The in vitro addition of cytomix resulted in the approximately 2500% (26-fold), approximately 1000% (11-fold) and 500% (6-fold) increase in $[PGE_2]$ in patient groups NA, ATA and AIA, respectively [32]. Our model populations, analysed at rest (i.e. without simulated drug dosing) revealed the following increases of $[PGE_2]$ in the transition from our model state of NI to inflammation: 1500% (16-fold) for NA and ATA, and 750% (8.5-fold) for all three AIA model populations (see Fig. 2*a*). Measured % increases of $[PGD_2]$ ranged between 200% (three-fold) and 400% (five-fold), whereas model predicted values were approximately 300% (four-fold) and were the same for all model populations (see Fig. 2*b*). According to their results, Pierzchalska *et al.* [32] hypothesised that cytomix, mimicking inflammation, had no effect on the expression of PGDS but had varying effects on the expression of PGES in different patient groups. Our model results agree reasonably well with these measured % increases of [32] which speaks in favour of the consistency of our definitions of the model states and accounts for the definition of populations based on the differences in PGES.

Pierzchalska *et al.* did not measure increases in LTC_4 but our model predicted them: The % increases of $[LTC_4]$ and $[LTB_4]$ were both equal and were approximately 1500% (16-fold) for ATA and NA, approximately 3000% (31-fold) for $AIA^{(1)}$ and $AIA^{(3)}$, and, approximately 4000% (41-fold) for $AIA^{(2)}$. We compared these model results to those measured after ovalbumin-triggered inflammation in BAL fluid obtained from mouse asthma models of Henderson *et al.* [59]. They measured increases of [cys-LT] by approximately 1100% (12-fold), $[PGE_2]$ by approximately 300% (4-fold), $[PGD_2]$ by approximately 100% (2-fold) and $[LTB_4]$ by approximately 300% (4-fold) [59]. A general comparison of results from the asthma mouse model and from our model revealed that our model populations AIA in a state of inflammation consistently predicted approximately four-fold higher increases for all studied eicosanoids as reported by Henderson *et al.* [59] for the mouse asthma model. This outcome indicates and speaks in favour of the validity of the definition of our model states of NI and inflammation.

The next comparison focused on the $[PGE_2]/[PGD_2]$ ratio, which was measured by Pierzchalska *et al.* [32]. They analysed cellular samples of patient groups AIA, ATA and NA, which were exposed to cytomix in vitro. They revealed significantly smaller increases of $[PGE_2]/[PGD_2]$ ratio in patient group AIA than in that of patient groups ATA and NA (see Fig. 3 for reproduced data). On the basis of their results they concluded that the activity of PGES in cells of patients suffering from AIA is lower than in those of ATA and NA. They also hypothesised that cytomix, mimicking inflammation in vitro, would up-regulate PGES in cells of the AIA patient group to a smaller extent than those from NA and ATA patient groups. Our model-predicted increases of $[PGE_2]/[PGD_2]$ in transition from the model state of NI to that of inflammation were in all our AIA model populations obviously lower than in NA and ATA. These findings of our model are the consequence of smaller fold-differences in v_{max} for PGES in AIA than in ATA and NA model populations, when comparing the values in the model states of NI and inflammation (see Table 3 for comparison). The results of our model predictions for ATA and all AIA model populations revealed good agreement with measured results of [32]. For $AIA^{(1)}$ and $AIA^{(3)}$, the agreement was better than for $AIA^{(2)}$, since $AIA^{(2)}$ was characterised by the same v_{max} of PGES (v_{max3}) as the model population ATA in state of NI. On the other hand, the increase in v_{max3} from NI to inflammation was two-fold in all of our AIA model populations. Model simulations thus revealed that the fold-changes of v_{max} for PGES (v_{max3}) are almost directly reflected in the fold-changes of the ratio $[PGE_2]/[PGD_2]$.

Good agreement between our model results and the experimental results [19] was achieved also when looking at our model-predicted ratio $[PGE_2]/[LTC_4]$ and measured *Rf*, defined as $[PGE_2]/$[cys-LT] in [19]. Our results are outlined in Fig. 4. The columns in Fig. 4 represent the intervals of measured *Rf* obtained from patients [19], whereas dots (black circle) and triangles (black up-pointing triangle) represent the results of mathematical model in states of inflammation (I) and NI, respectively. Note that results of mathematical model for ATA of both model states (i.e. NI and I) fall fully within the measured intervals of [19]. This is due to the fact that parameter values for the model population NA in the model state of inflammation (NA-I) were deduced from the reference model population (NA-NI) and the values were chosen so that the $[PGE_2]/[LTC_4]$ ratio did not change in the transition from NI to inflammation. It turned out that model population ATA also possesses the same property as NA. On the other hand, $[PGE_2]/[LTC_4]$ ratios for all AIA model populations were significantly smaller in states of inflammation than in states of NI (see Fig. 4 and Table 4 for exact model-predicted values).

Published *Rf* for AIA patient group was around or below 1.0, ranging from 0.16 to 0.63 [19]. In our model state of NI (black up-pointing triangle), the model predicted values were slightly larger than 1.0 for $AIA^{(1)}$ and $AIA^{(2)}$ (4.1 and 6.2, respectively) and lower than one for $AIA^{(3)}$ (0.91). In the model state of inflammation (black circle), the values were: 0.94, 1.2 and 0.21 for $AIA^{(1)}$, $AIA^{(2)}$ and $AIA^{(3)}$, respectively. These results were in good agreement with reported intervals of 0.16–0.63 [19]. According to this criterion, our model population $AIA^{(3)}$ described a patient group suffering from AIA slightly better than model populations $AIA^{(1)}$ and $AIA^{(2)}$, although, both of them roughly matched the properties of experimentally revealed results from

Table 4 Values of $[PGE_2]/[LTC_4]$ ratio at rest and minimal values after dosing of 25 mg indomethacin (~at $t = 4$ h) for all model populations in states of NI and I

Condition	Model state	NA	ATA	$AIA^{(1)}$	$AIA^{(2)}$	$AIA^{(3)}$
basal (without drug)	NI	110.4	28.0	4.1	6.2	0.91
	I	110.4	28.0	0.94	1.2	0.21
minimum after indomethacin (25 mg)	NI	1.1	0.28	0.045	0.063	0.010
	I	7.0	1.8	0.044	0.073	0.0099

AIA patients. Based on our model results for $AIA^{(3)}$ (i.e. model population with altered PGES and LTC_4S maximal velocities with respect to ATA), these results suggest that differences in maximal velocity of PGES obviously largely contribute to a lower $[PGE_2]/[LTC_4]$ ratio. This property could not be recognised solely from the data concerning $[PGE_2]$ and $[PGD_2]$ as described above (see Figs. 2*a* and *b*). The results of the model simulations thus suggest that differences in maximal velocities of each particular enzyme, that is, LTC_4S or PGES, might lead to NSAID-triggered hypersensitivity. On the basis of our model, the worst case of hypersensitivity is expected, if maximal velocities of both enzymes, PGES and LTC_4S, are affected (with a decrease in PGES and an increase in LTC_4S maximal velocity) at the same time. The same property was revealed by the sensitivity analysis and by the dynamic simulation of eicosanoid production after indomethacin dosing, which is presented in the following sections.

3.2 Sensitivity analysis

Sensitivity analysis of the model variable $[PGE_2]/[LTC_4]$ for the model population $AIA^{(3)}$ was carried out. This was performed with respect to small alterations in maximal velocities of the enzymes 5-LOX ($v_{max13,\ 16}$), COX-1 (v_{max1}), COX-2 (v_{max2}), PGES (v_{max3}), PGDS (v_{max5}), 15-LOX (v_{max21}) and LTC4S (v_{max17}), the inflow of AA (v_0), plus the parameter values concerning PGE_2 and LTC_4 elimination rates (k_4 and k_{18}, respectively), as described in Sections 2.1 and 2.2. The response coefficients (R) are presented in Fig. 5. In both states of NI and inflammation, the $[PGE_2]/[LTC_4]$ ratios were most sensitive to changes in the parameter v_{max} of PGES (v_{max3}). Almost the same but negative response coefficient was because of changes in parameter value k_4, which describes the PGE_2 elimination rate. Response coefficients for 5-LOX and 15-LOX were negative, whereas those for COX-1 and COX-2, which indirectly increase $[PGE_2]$ levels in the model, were positive. Unexpectedly, the $[PGE_2]/[LTC_4]$ ratio was somewhat less sensitive to changes in v_{max} of LTC_4S (v_{max17}) than in v_{max} of 5-LOX ($v_{max13,16}$). The opposite would be expected, since 5-LOX is more upstream of the production of LTC_4 than LTC_4S. This is probably because of the kinetics of LTC_4S used for modelling, which is not simple Michaelis–Menten kinetics but implements the enzyme kinetics with the substrate auto-inhibition [see (4)]. In the COX pathway, the sensitivity of $[PGE_2]/[LTC_4]$ to changes in v_{max} of PGES (v_{max3}), which is less upstream of the PGE_2 production, was larger than the sensitivity to changes in v_{max} of COX-1 (v_{max1}) and COX-2 (v_{max2}), which are more upstream (see Fig. 5 for comparison). The system was also almost equally sensitive (albeit with different signs) to changes in the parameters implicating inflow and elimination rates of LTC_4, that is, v_{max17} and k_{18}, respectively, plus inflow and elimination rates of PGE_2, that is, v_{max3} and k_4, respectively. The same effects on the production of either LTC_4 or PGE_2 could thus be achieved either by enhancing the inflow rates or reducing the corresponding elimination rates for the same factor. Changes in v_{max} of PGDS (v_{max5}) or in v_{max} of any other of the second level enzymes within the COX pathway, except PGES, did not have any effect on the $[PGE_2]/[LTC_4]$ ratio, neither in a state of inflammation nor in the state of NI. Fig. 5 presents the results of modifications for PGDS, solely. Furthermore, there were no major differences in the sensitivity analysis comparing the model states of NI and of inflammation for each particular model population, apart from one exception. When focusing on v_{max} of COX-2 (v_{max2}), the sensitivity was much lower in state of NI than in that of inflammation. Finally, it is worth noting that although response coefficients are rather high, our model is robust to small perturbations. This means that small changes of parameter values do not cause the transitions from one model population to another. For such changes substantial changes of parameter values, that is, fold-increases/decreases, are necessary.

3.3 Dynamic simulations

The effect of NSAIDs on $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ as well as on their ratios was studied with our dynamic model simulations using indomethacin. All parameters and initial conditions for the dynamic simulations are presented in the Supplementary Information. Supplementary Table 2 summarises all kinetic and pharmacokinetic parameters for the reference model state (NA-NI), and Supplementary Table 5 summarises initial conditions for simulation of all model populations in both model states. For the simulation of the time evolution of the system variables from their initial concentrations the time-dependent NSAID plasma drug concentration, described by (6), was applied. Equation (6) was derived from a standard two-store pharmacokinetic model with absorption and elimination phases. With the use of pharmacokinetic parameters: $k_a = 1.0 \cdot 10^{-4}\ s^{-1}$, $k_e = 9.5 \cdot 10^{-5}\ s^{-1}$ and $V = 45$ L this model consistently described measured indomethacin plasma concentration after oral dosing in [62]. Indomethacin was used for simulations since Pierzchalska *et al.* [32] also used this drug in their experiments. The time-evolution of all above mentioned absolute concentrations is presented in Fig. 6 for our model population $AIA^{(3)}$ in state of inflammation after simulated oral dosing of 25 mg indomethacin. This is a typical oral therapeutic dose. The dose of 25 mg indomethacin resulted in maximal blood plasma concentration of indomethacin yielding 0.59 µmol/L [NSAID] approximately 3 h after simulated application time (at $t = 0$).

NSAID dosing causes decreased synthesis of PGD_2 and PGE_2 [12, 63]. The dynamic simulations of NSAID dosing revealed decreased values of PGD_2 and PGE_2, accompanied by increased synthesis of LTC_4. This indicates a tilt of the AA metabolic pathway in favour of the LTC_4 production because of NSAIDs. For indomethacin, the extremes of $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ were achieved approximately 3–4 h after the moment of dosage, which almost coincided with the maximal indomethacin plasma concentration. The recovery of $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ back to their initial values was slow and lasted up to 24 h (see Fig. 6).

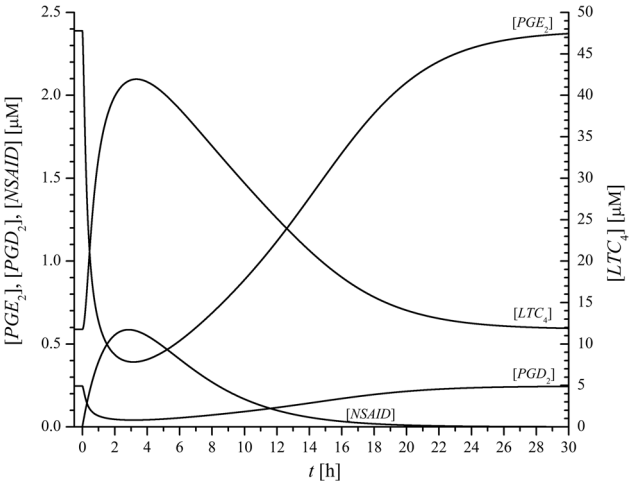

Fig. 6 *Time evolution of the absolute concentrations $[PGE_2]$, $[PGD_2]$ and $[LTC_4]$ as well as of plasma indomethacin concentration [NSAID] in model population $AIA^{(3)}$ in state of inflammation after simulated oral dosing of 25 mg indomethacin*

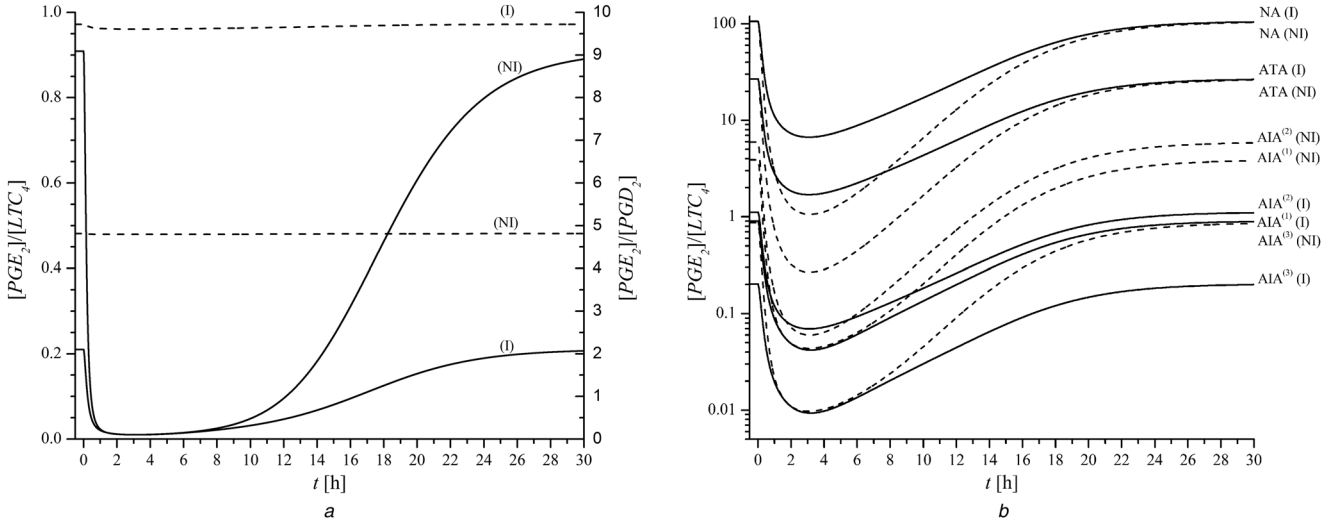

Fig. 7 *Simulated time dependencies of the*

a $[PGE_2]/[LTC_4]$ (full line) and $[PGE_2]/[PGD_2]$ (dashed line) ratios for the model population $AIA^{(3)}$ in states of NI and I for indomethacin dose 25 mg
b $[PGE_2]/[LTC_4]$ ratios for all model populations in both model states, NI (dashed lines) and I (full lines), for indomethacin dose 25 mg
Note: Scale of ordinate in Fig. 7*b* is logarithmic and thus minima look apparently narrower
Drug is in all cases applied at time 0

The ratios of $[PGE_2]/[PGD_2]$ (full line) and $[PGE_2]/[LTC_4]$ (dashed line) after indomethacin dosing are presented in Fig. 7*a* for $AIA^{(3)}$ model states of NI and of inflammation (I). Evidently, indomethacin revealed no effect on the $[PGE_2]/[PGD_2]$ ratio. The same was observed for all other cases. These results are in complete accordance with the experiment of Pierzchalska *et al.*, where no significant differences in $[PGE_2]/[PGD_2]$ ratio were found after addition of 10 μmol/L indomethacin to samples with and without cytomix [32]. In our model, maximal plasma concentration of indomethacin 10 μmol/L was achieved with the dosing of 430 mg indomethacin. Even this high dose of indomethacin did not change the $[PGE_2]/[PGD_2]$ ratio of any of the model populations. On the other hand, after simulated indomethacin dosing the $[PGE_2]/[LTC_4]$ ratio dropped significantly, for approximately 20-fold in a state of inflammation and approximately 90-fold in state of NI. The time interval of extremely low $[PGE_2]/[LTC_4]$ ratios (e.g. values lower than 0.16, the minimal values measured in AIA patients without drug) represents the duration of the largest risk for bronchoconstriction in our model simulation. Note, it is assumed that extended time interval in combination with the drop to the lowest absolute values of the $[PGE_2]/[LTC_4]$ ratio goes along with increasing risk of bronchoconstriction. Among all AIA model populations simulated with the dosing of 25 mg indomethacin, $AIA^{(3)}$ exhibited the broadest time interval and the lowest $[PGE_2]/[LTC_4]$ ratio (i.e. below 0.1 for $\sim$12 h) (see Fig. 7*b*). Thus, model population $AIA^{(3)}$ was recognised as the most sensitive among the predicted three AIA model populations. For NA and ATA model populations, the minimal $[PGE_2]/[LTC_4]$ ratios were much higher (several orders of magnitude) after 25 mg of indomethacin than those for AIA model populations (see Table 4 for the exact values). Furthermore, our dynamic model simulations with indomethacin dosing revealed smaller minimal $[PGE_2]/[LTC_4]$ ratios for NA and ATA in state of NI than in state inflammation, whereas in AIA model populations no significant differences were calculated. However, our dynamic simulations revealed that those time intervals indicating risk of bronchoconstriction (with $[PGE_2]/[LTC_4]$ ratios below 0.16) were in all AIA model populations significantly more extended in inflammation than in NI (see Fig. 7*b*) for comparison). Note: the ordinate is drawn in the logarithmic scale, in order to present all model populations in a single diagram; therefore minima look apparently narrower.

The results of our model thus indicate that the state of inflammation of the underlying model population prolongs the duration of the risk for bronchoconstriction. This was demonstrated for all three investigated AIA model populations. Among them, model population $AIA^{(3)}$, which is characterised by concomitantly increased v_{max} of LTC_4S and decreased v_{max} of PGES, exhibited the highest sensitivity to NSAIDs. It demonstrated the lowest $[PGE_2]/[LTC_4]$ ratio and the longest duration of extremely low ratio (below 0.1). PGE_2 is attributed to bronchodilation [15, 16] and LTC_4 to bronchoconstriction [6]. Therefore a rough estimation of bronchoconstriction risk might be defined and predicted from their ratio. However, this value does not provide any information to the patient's individual bronchial hyper-reactivity and hypersensitivity. With this in mind, our model population $AIA^{(3)}$ demonstrated the highest and the most long-lasting risk for bronchoconstriction. In addition, our model results also suggest that differences in v_{max} of either enzyme, LTC_4S or PGES, might also lead to NSAID hypersensitivity, as demonstrated for model populations $AIA^{(1)}$ and $AIA^{(2)}$, albeit, their capacity and duration of bronchoconstriction risk were weaker and shorter than in $AIA^{(3)}$.

3.4 Explanatory impact of the model

The results of our mathematical model correspond to the following experimental patient-based observations and might explain the reported imbalances of eicosanoids in NSAID-triggered hypersensitivity:

(i) deficiency in the production of PGE_2 in cells obtained from patient group suffering from AIA after addition of cytomix [32],
(ii) increased $[PGE_2]/[PGD_2]$ ratio after addition of cytomix to cells obtained from NA, ATA and AIA patient groups [32]
(iii) significantly lower increase of $[PGE_2]/[PGD_2]$ ratio after addition of cytomix to cells obtained from AIA than in those obtained from ATA and NA patient groups [32],
(iv) no significant impact of NSAID on $[PGE_2]/[PGD_2]$ ratio in any of the patient groups [32],
(v) order of 1000-fold lower $[PGE_2]/[LTC_4]$ ratio in AIA than in NA patient groups after NSAID dosing [19],
(vi) the ratio $[PGE_2]/[LTC_4]$ around 1.0 in AIA patient group and approximately 100-times larger value in NA group in the basal state (without drug) [19],
(vii) higher basal concentrations of LTC_4 in AIA than in ATA and NA patient groups [4, 9, 18, 30, 31].

Our model predicts and gives some hints on imbalances of different eicosanoid ratios, which arise from differences in the maximal velocities of particular enzymes. This endorses the idea that inflammatory stimuli might have a different impact on the protein levels and/or their expressions in the cells obtained from different patient groups. The model predicts which enzymes might be affected and what fold-changes might occur because of the inflammatory stimuli. However, it has to be noted that the fold-changes of parameter values which are implicated in the model are only estimates. These estimates might offer preliminary results on the expected orders of magnitude, additionally, they might give some clues as to the parameter values which are more and those which are less affected by inflammation. The experiment of [32] leads to the suggestion that PGES and COX-2 were affected by inflammation; thereby inflammation was simulated in vitro by cytomix, a mixture of proinflammatory agents. Changes in COX-2 were experimentally confirmed, whereas the changes in PGES were only hypothesised by this ex vivo/in vitro approach [32]. On the basis of our reference model state (i.e. model population NA-NI), only the changes of two parameter values, that is, the v_{max} of PGES and COX-2, could not reflect the results of experimentally induced inflammation, gained from samples of the NA patient group. The reported approximately 20-fold increase of $[PGE_2]$ in patient group NA after cytomix was in our model achieved by a series of fold-changes of parameter values. An influx of AA was increased, two parameters of the LOX pathway, the v_{max} of 5-LOX and 15-LOX, were increased in addition in order to obtain an agreement with the results obtained experimentally for the NA patient group in [19, 32]. Moreover, specific fold-changes for v_{max} of PGES were applied for the definition of model populations ATA and three AIA as well as for the simulation of the transition from state of NI to inflammation in these model populations (see Tables 2 and 3). Fold-changes in parameter values used in our model are deduced from the metabolomic studies of eicosanoid profiles [19, 32] and could be used as a starting point for further proteomic or transcriptomic studies.

In addition, we analysed which parameter values of the system variables have the highest impact on the $[PGE_2]/[LTC_4]$ ratio, known as a sensitive indicator of NSAID-triggered hypersensitivity [19]. Our study revealed parameters implicated in the PGE_2 production and elimination pathway. Therefore patient group AIA might differ from ATA and NA in this particular property. It was hypothesised in [32], that cytomix increased PGES expression in the AIA patient group in a smaller extent than in ATA and NA patient groups. Another possible explanation might be that inflammatory processes differently affected the mechanism of conversion of PGE_2 into biologically inactive metabolite(s) of PGE_2 – PGE-M, or, that inflammatory processes might affect both mechanisms. Recent results obtained from exhaled breathe condensates, quantified by highly sensitive gas chromatography/mass spectrometry, revealed higher levels of PGE-M in AIA than in ATA, and NA patient groups [64, 65]. However, the fact that the $[PGE_2]/[LTC_4]$ ratio, which became a benchmark for distinguishing AIA from ATA in recent years [11, 12, 19, 33], is up to 1000-fold lower in patient group AIA than in patient group NA. This in turn might then only be explained by the assumption that the process of PGE_2 degradation is faster in AIA than in NA and ATA, which up to date, was not proved.

Sensitivity analysis of our model system revealed that elevation of the parameters v_{max3} and k_4 (implicated in the production and degradation of PGE_2, respectively) by the same factor affects neither the $[PGE_2]/[LTC_4]$ nor the $[PGE_2]/[PGD_2]$ ratios (see Fig. 5). In addition, the overall flux directed to the metabolite of PGE_2 would increase. These results of our model system might thus explain low levels of PGE_2 and high levels of PGE-M at the same time in patients suffering from AIA.

According to the results of our presented model the worst case of NSAID-triggered hypersensitivity would be expected from concomitantly increased expression of LTC_4S and decreased expression of PGES, although, many other combinations are also possible. This would be the case, if parameters with positive response coefficients with respect to $[PGE_2]/[LTC_4]$ ratio (e.g. v_{max} of enzymes PLA_2, COX-1, COX-2 and PGES plus the rate of LTC_4 degradation) are down regulated, and/or, if parameters with negative response coefficients (e.g. v_{max} of enzymes 5-LOX, 15-LOX and LTC4S, plus the rate of PGE_2 degradation), are up regulated at the same time. Various combinations of increased/decreased parameter values provide rationales for a variety of clinical symptoms like bronchoconstriction, nasal polyps, rhinosinusitis, urticaria, conjunctivitis and so on, as well as their acuteness and strength, ranging from acute to chronic and from mild to severe. For a more complete description of such diseases by modelling, additional experimental and theoretical studies will have to be performed, integrating metabolomic and proteomic studies. Our current mathematical modelling approach is one of the first systematical attempts in this regard.

Dynamic model simulations after oral indomethacin dosing endorsed the results of sensitivity analysis. They both reveal the model population $AIA^{(3)}$ as the most sensitive model population with respect to the duration and capacity of the risk for bronchoconstriction. $AIA^{(3)}$ is characterised by concomitantly increased v_{max} of LTC_4S and decreased v_{max} of PGES. Moreover, our dynamic simulations revealed that in all AIA model populations the state of inflammation prolonged the duration of bronchoconstriction risk. This property is again most evident in the model population $AIA^{(3)}$. The latter simulations might help to reveal differences in NSAID-triggered hypersensitivity of different patient groups. Accordingly, this might facilitate estimations on the outcome of oral drug dosing in different patient populations, depending on the anamnesis of their inflammatory state. Future proteomic and transcriptomic studies, combined with metabolomic studies, will contribute to improvements of the models such as ours, and, at least in our opinion, might be of high relevance in discovering more insights on the intrinsic factors involved in AERD.

4 Conclusions

In spite of accumulating experimental and clinical results the molecular and cellular mechanisms, which induce and initiate processes in AERD, are still not completely elucidated and understood. In the past, experimental and clinical research dealing with AERD was primarily focused on a single level, either on concerning the gene, molecular, cellular or tissue/organ level. Theoretical analyses and predictions attained from mathematical models, such as ours, integrate the accumulated knowledge of these different levels of research. Therefore the mathematical model analysis might serve as a suitable tool aiding to figure out and test new hypotheses, check molecular mechanisms, analyse experimental results, guide novel experiments as well as give ideas for new methods and tools for diagnosing and treatment diseases, such as AERD in that case, on a multiscale level.

Our dynamic mathematical model represents a step forward towards multiscale models as described for the lung [46, 66]. In such multi-cellular models airway smooth muscle cells will be coupled with cells involved in inflammation via inflammatory mediators acting on airway smooth muscle cells by contraction/relaxation mechanisms [67, 68]. These multiscale models might elucidate the ‘functional signature’ of AERD [33], that is, the underlying functional and molecular abnormalities, which result from complex cellular alterations. Those complex alterations are expressed in terms of inter- and intra-cellular signalling of metabolism because of over- and under-expression of genes, gene products, consider possible input stimuli which finally integrate and reveal a unique message in response to external and internal events, for example, an inflammatory disease [11].

Ambitious efforts will be to extentd our recent model to facilitate meaningful estimations for an individual risk of bronchoconstriction regarding asthmatic patients, supposed to be intolerant to aspirin, ideally with regard to the type and dose of the NSAID as well as his/her acute original condition of inflammation and bronchial hyper-responsiveness.

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6 References

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PRILOGA 3

PRILOGA IZVIRNEGA ZNANSTVENEGA ČLANKA – Supplementary material

A. Fajmut, T. Emeršič, A. Dobovisek, N. Antić, D. Schafer, and M. Brumen, "Dynamic model of eicosanoid production with special reference to non-steroidal anti-inflammatory drug-triggered hypersensitivity," *IET Systems Biology,* 2015.

Supplementary Information

Dynamic model of eicosanoid production with special reference to NSAID-triggered hypersensitivity

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Introduction to Supplementary Information

In the section Parameter estimation of this Supplementary Information, we describe the procedure of parameter estimation of those parameter values that are essential for the determination of the reference model state – model population non-asthmatics (NA) in model state of no-inflammation (NI) (NA-NI). In Supplementary Table 2 we present the complete set of parameter values for the reference model state (NA-NI), their description, units and the corresponding experimental and/or theoretical references. The sensitivity analysis, to which we are referring in the main text, is presented here. The complete mathematical modelling of the kinetic scheme, including all mathematical expressions for the reaction rates along with the parameter values for each expression (see Supplementary Table 3) as well as the first-order non-linear ordinary differential equations and the initial conditions for all simulations (see Supplementary Table 4), and, moreover, the model code in ASCII format, are presented at the end of the document.

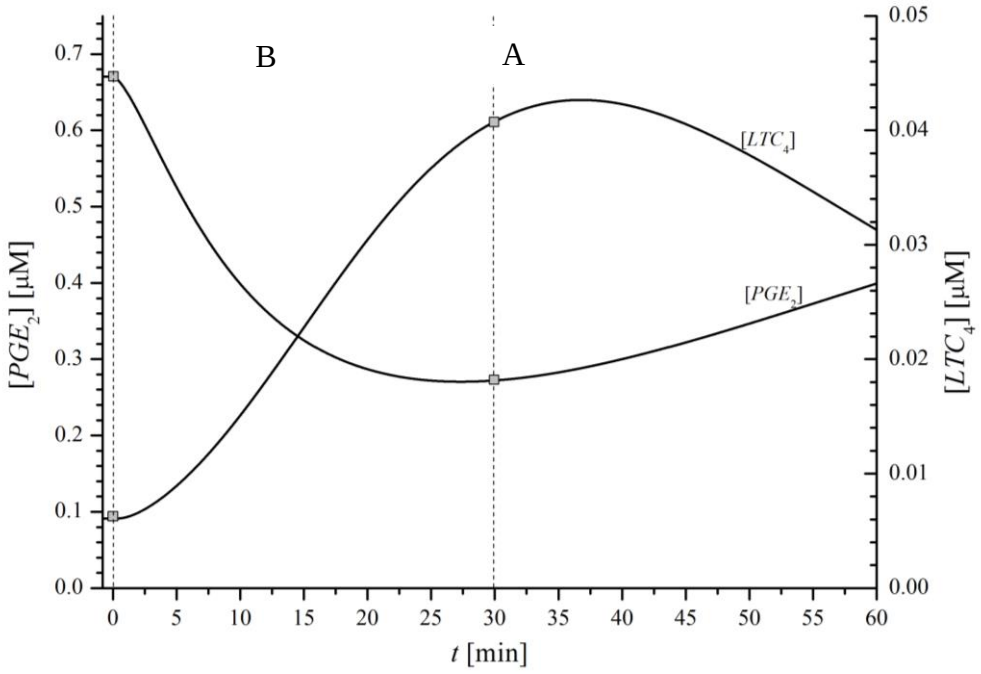
Parameter estimation for the reference model state – non-asthmatic model population in the model state of no-inflammation (NA-NI)

The reference model parameters presented in Supplementary Table 2 were first determined for non-asthmatic model population (NA) in state of no-inflammation (NI) (NA-NI), which served as a reference for the definition of the model state of inflammation as well as for the definition of different asthmatic model populations (ATA and three different AIA).

Values of all Michaelis-Menten constants (K_i) and all equilibrium dissociation constants (K_{Ii}) as well as parameters *A*, *B* and *C*, representing 21 out of 45 kinetic model parameters, were

taken directly from experimental and/or theoretical references. The same was done for the selected v_{max} of enzymes: COX-1, LTC_4S, LTA_4H, ABCC4, PHGPx(2×), which were all taken from the same theoretical reference [1]. Another 6 parameters were determined in this way. Due to lack of information on the elimination processes of particular eicosanoids we considered two constraints for the determination of the rate constants (k_i). Another reason for these constraints was that we did not want to prefer any of the eicosanoid elimination pathways since our focus of the study was pointed to the enzymes upstream of eicosanoid production pathway. These two constraints were: no differences between the elimination rates of all eicosanoids, except of PGE_2, PGD_2 and LTC_4, and no differences between PGE_2 and PGD_2 elimination rates. The same assumptions were used elsewhere [1]. The parameter values were taken from references [2-4]. In this way we determined another 8 parameter values. Due to lack of explicit information on the v_{max} of prostanoid production pathway (v_{max7}, v_{max9} and v_{max11}) we attributed half of the maximal eicosanoid production rate 37 µM/s [5] to them and split it equally among them. 6 parameters remained undetermined, whereby two of them are describing the same enzyme. Thus, 5 parameters: v_0, v_{max2}, v_{max3}, v_{max5}, $v_{max13,16}$ and v_{max21} had to be further determined for NA. We already had estimations of these parameter values, since some of them have been used elsewhere [1-3], and therefore we were able to start the calculations. In the following process of parameter estimation we have been manually iterating the values of these parameters and checking instantly if the results fulfil certain constraints presented below. In the process of determination of values for these five parameters we fixed two ratios, $[PGE_2]/[LTC_4]=110$, which was a minimal value, measured by [6] and $[PGE_2]/[PGD_2]=10$, which was determined by [7]. Both ratios were measured on NA. These two fixed ratios were essential for the later comparison with the measured results for asthmatic patient populations dealt within these two references [6, 7]. Ratio $[PGE_2]/[PGD_2]$ determined well the ratio of the eicosanoids within COX pathway and ratio $[PGE_2]/[LTC_4]$ determined well the relationship

between the two most important members of COX and LOX pathway. As a reference for the ratios of eicosanoids within the LOX pathway we took the ratios of four eicosanoids measured by Sanak et al. [8] in breath condensates of NA test group by means of gas chromatography and mass spectrometry. The recommended ratios for comparison with the model predictions were: [LTB_4]/[LTC_4]~6, [*5-HETE*]/[*15-HETE*]~1 [8]. In order to well determine the response of the system to NSAIDs, we considered certain constraints also in terms of the ratios for the single variable determined before and after NSAID. As a reference we took the ratios for LTC_4 and PGE_2, both measured after (A) and before (B) inhalative aspirin challenge in NA test group [6]. Measured ratios ranged for $[LTC_4]_A/[LTC_4]_B$ between 3.1 and 8.3, for $[PGE_2]_A/[PGE_2]_B$ between 0.10 and 0.26, and for $[PGE_2]_A/[LTC_4]_A$ between 3.2 and 8.7 [6]. In order to compare measured results with the results of model simulation, we simulated dynamic model response to an oral aspirin dose of 650 mg (typical therapeutic dose). Pharmacokinetic parameters used for the simulation of oral aspirin dosing were: $k_e=5.8\cdot10^{-4}$ s^{-1}, $k_a=0.023$ s^{-1}, $V=74$ L [9] and $M=180.157$ g/mol. Equilibrium dissociation constant considered for aspirin were $K_{I1}=85$ µM in case of COX-1 and $K_{I2}=4$ µM in case of COX-2 [10]. Values of [PGE_2] and [LTC_4] determined by the model were taken in equilibrium (B) and after 30 minutes (A) (see Supplementary Figure 1).



Supplementary Figure 1. Time courses of [PGE_2] and [LTC_4] after aspirin dosing. Point A and B denote times before and after aspirin, respectively. The ratio $[PGE_2]/[LTC_4]$ before aspirin was considered as fixed constraint and the ratios $[LTC_4]_A/[LTC_4]_B$, $[PGE_2]_A/[PGE_2]_B$, $[PGE_2]_A/[LTC_4]_A$ were considered as non-fixed constraints.

Finally, manual iteration of the abovementioned 5 parameter values starting from the estimated values used elsewhere [1, 2] under particular fixed constraints, was worked out, until the model predicted ratios of non-fixed constraints did not fit within an order of magnitude with the measured constraints. Let us note that all these constraints were obtained from measurements specific to NA test group [6, 7, 11], thus with this set of parameter non-asthmatic model population (NA) in the model state of no-inflammation (NI) (NA-NI) was determined. Supplementary Table 1 presents, how the final set of parameter values (see Supplementary Table 2), considered as the reference model state, fulfilled the proposed measured fixed and non-fixed constraints.

Supplementary Table 1. Comparison of the constraints based on the measurements of eicosanoids in non-asthmatic test groups with those predicted by the model in the reference model state – non-asthmatic model population (NA) in state of no-inflammation (NA-NI).

Constraint	Fixed/non-fixed	Predicted	Measured	Ref.
$[PGE_2]/[LTC_4]$	fixed	110	110	[6]
$[PGE_2]/[PGD_2]$	fixed	10	10	[7]
$[LTB_4]/[LTC_4]$	non-fixed	7	6	[8]
[*5-HETE*]/[*15-HETE*]	non-fixed	1	0.5	[8]
$[LTC_4]_A/[LTC_4]_B$	non-fixed	6.7	3.1 – 8.3	[6]
$[PGE_2]_A/[PGE_2]_B$	non-fixed	0.41	0.10 – 0.26	[6]
$[PGE_2]_A/[LTC_4]_A$	non-fixed	6.7	3.2 – 8.7	[6]

The absolute concentration of PGE_2 and LTC_4 for the reference model state agreed within an order of magnitude with the measured value by Schäfer et al. [6]. They reported concentration 236 pg/mL for PGE_2 and 1.5 pg/mL, per 10^5 cells in the solution of 1 mL for the NA test group [6]. By considering the normal mean cell volume of neutrophils 468 fL [12] and the molecular mass of PGE_2 (352.5 g/mol) the value 236 pg/mL per 10^5 cells/mL was recalculated into the estimated concentration per 1 isolated cell, which yielded 13 µM. Similar recalculation (by considering molecular mass of LTC_4 625.8 g/mol) yielded value 0.05 µM for the concentration of LTC_4. The predicted absolute concentrations in the reference state of the model were 0.67 µM and 0.0061 µM for PGE_2 and LTC_4, respectively.

Pharmacokinetic parameters for indomethacin (rates of absorption (k_a) and elimination (k_e) as well as of the apparent volume normalized with fraction of absorbed drug (V) were determined by fitting the standard two-store pharmacokinetic model for oral dosing (E.q. (6) in the main text) of the same indomethacin dose, as used in experiment [13] (90 mg) to measured data. Pharmacokinetic parameters are presented at the end of the Supplementary Table 2.

Supplementary Table 2. A complete set of model parameters and their values used for the model simulation of the model reference state - non-asthmatic model population (NA) in the model state of no-inflammation (NI).

Parameter	Value and units	Description	References
v_0	0.070 μM s^{-1} *	Influx of AA	[2, 3, 14, 15]
v_{max1}	0.48 μM s^{-1} *	Maximal velocity of COX-1	[1, 2, 16]
K_1	3.0 μM	Michaelis–Menten constant for COX-1	[2, 14]
K_{I1}	0.0032 μM	Equilibrium dissociation constant for inhibitor (indomethacin) and COX-1	[16]
v_{max2}	0.055 μM s^{-1} *	Maximal velocity of COX-2	[2]
K_2	2.5 μM	Michaelis–Menten constant for COX-2	[2, 14]
K_{I2}	1.02 μM	Equilibrium dissociation constant for inhibitor (indomethacin) and COX-2	[16]
v_{max3}	5.0 μM s^{-1} *	Maximal velocity of PGES	[17]
K_3	160.0 μM	Michaelis–Menten constant for PGES	[5, 17, 18]
k_4	0.0028 s^{-1} *	Rate constant of PGE_2 efflux	[2, 3]
v_{max5}	0.045 μM s^{-1}	Maximal velocity of PGDS	[1]

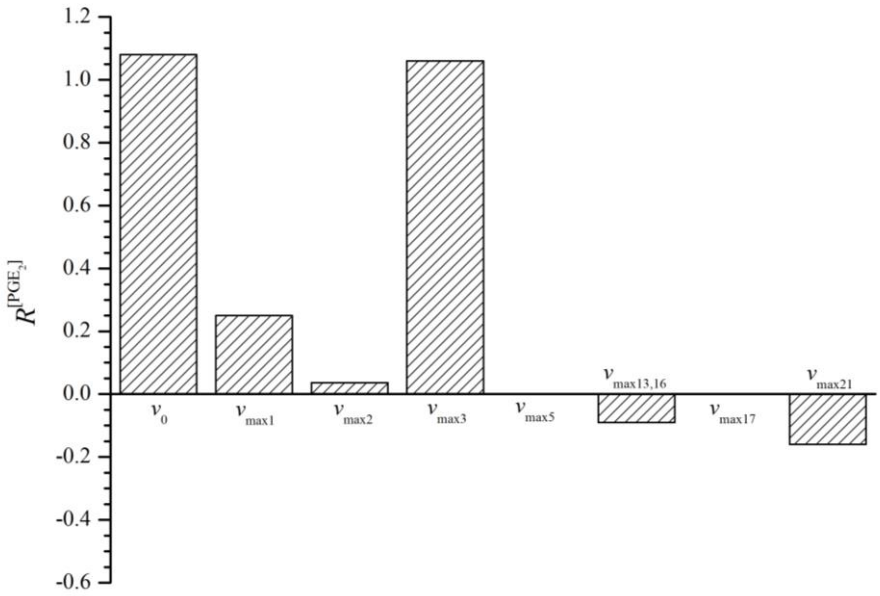
K_5	13.8 µM	Michaelis–Menten constant for PGDS	[19]
k_6	0.0028 s^{-1}	Rate constant of PGD_2 efflux	[2, 3]
$v_{max\,7}$	6.2 µM s^{-1}	Maximal velocity of PGIS	[5]
K_7	30.0 µM	Michaelis–Menten constant for PGIS	[20]
k_8	0.028 s^{-1}	Rate constant of PGI_2 efflux	[2, 3]
$v_{max\,9}$	6.2 µM s^{-1}	Maximal velocity of PGFS	[5]
K_9	18.0 µM	Michaelis–Menten constant for PGFS	[21]
k_{10}	0.028 s^{-1}	Rate constant of $PGF_{2\alpha}$ efflux	[2, 3]
v_{max11}	6.2 µM s^{-1}	Maximal velocity of TXS	[5]
K_{11}	22.0 µM	Michaelis–Menten constant for TXS	[22]
k_{12}	0.028 s^{-1}	Rate constant of TXB_2 efflux	[2, 3]
v_{max13}	2.5 µM s^{-1} *	Maximal velocity of 5-LOX	[2, 23]
K_{13}	5.0 µM	Michaelis–Menten constant for 5-LOX	[5, 24]
K_{I13}	0.03 µM	Equilibrium dissociation constant for inhibitor (PGE_2) and 5-LOX	[2, 15]
v_{max14}	6.7 µM s^{-1}	Maximal velocity of PHGPx	[5]
K_{14}	70.0 µM	Michaelis–Menten constant for PHGPx	[5]
k_{15}	0.028 s^{-1}	Rate constant of 5-HETE efflux	[2]
v_{max16}	2.5 µM s^{-1} *	Maximal velocity of 5-LOX	[2, 23]
K_{16}	5.0 µM	Michaelis–Menten constant for 5-LOX	[5, 24]
K_{I16}	0.03 µM	Equilibrium dissociation constant for inhibitor (PGE_2) and 5-LOX	[2, 15]

v_{max17}	0.057 µM s^{-1} *	Maximal velocity of LTC_4S	[2, 25]
A	56.0 µM	Constant	[2, 25]
B	1,4	Constant	[2, 25]
C	0.17 μM^{-1}	Constant	[2, 25]
k_{18}	0.0015 s^{-1}	Rate constant of LTC_4 efflux	[2, 4]
v_{max19}	3.7 µM s^{-1}	Maximal velocity of LTA_4H	[2, 26]
K_{19}	27.0 µM	Michaelis–Menten constant for LTA_4H	[2, 26]
v_{max20}	5.74 s^{-1}	Maximal velocity of LTB_4 efflux	[2, 27]
K_{20}	239 µM	Half-saturation constant for LTB_4 efflux	[2, 27]
v_{max21}	2.5 µM s^{-1} *	Maximal velocity of 15-LOX	[5]
K_{21}	70.0 µM	Michaelis–Menten constant for 15-LOX	[5]
v_{max22}	6.6 µM s^{-1}	Maximal velocity of PHGPx	[5]
K_{22}	70.0 µM	Michaelis–Menten constant for PHGPx	[5]
k_{23}	0.028 s^{-1}	Rate constant of 15-HETE efflux	[2]
k_a	$1.0 \cdot 10^{-4}$ s^{-1}	Absorption rate constant for indomethacin	[13]
k_e	$9.5 \cdot 10^{-5}$ s^{-1}	Elimination rate constant for indomethacin	[13]
D	$25 \cdot 10^3$ µg	Dose of indomethacin	
V	45 L	Apparent volume of drug distribution normalized with the fraction of the absorbed drug	[13]
M	357.8 g mol^{-1}	Molecular mass of indomethacin	

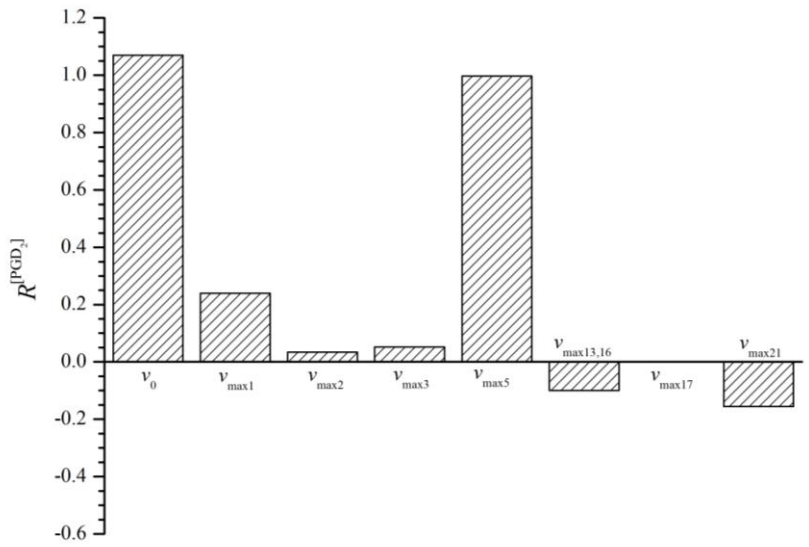
* Parameter values that are subject to changes. Values depend on the selected model population and the model state.

Sensitivity analysis for the reference model state NA-NI

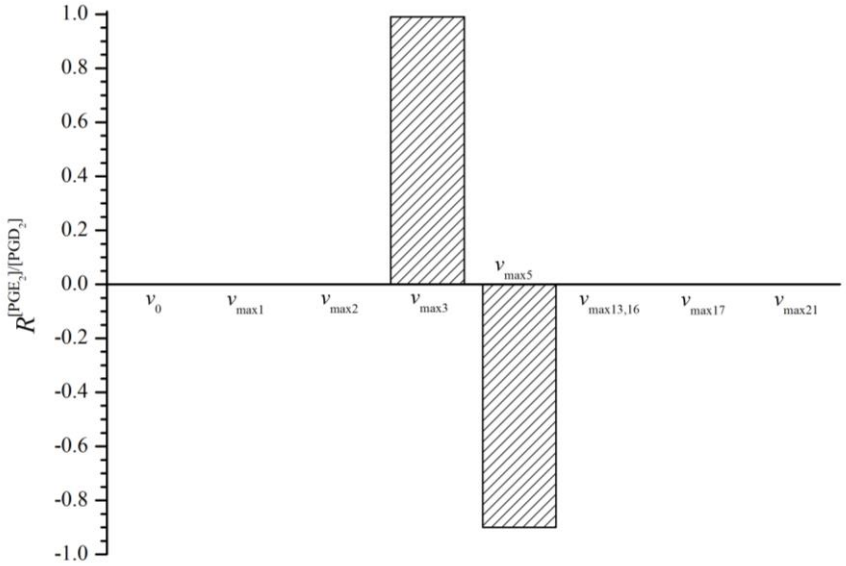
In the process of derivation and definition of the model state inflammation, evolved from the reference model state of non-asthmatic in state of no-inflammation (NA-NI), we referred also to the sensitivity analysis of particular variables to alterations in parameter values. Response coefficients for the variables [PGE_2], [PGD_2], [PGE_2]/[LTC_4] and [PGE_2]/[PGD_2], with respect to small alterations in maximal velocities of parameter values v_0, v_{max1}, v_{max2}, v_{max3}, v_{max5}, $v_{max13,16}$, v_{max17} and v_{max21} are presented in Supplementary Figures 2-5.



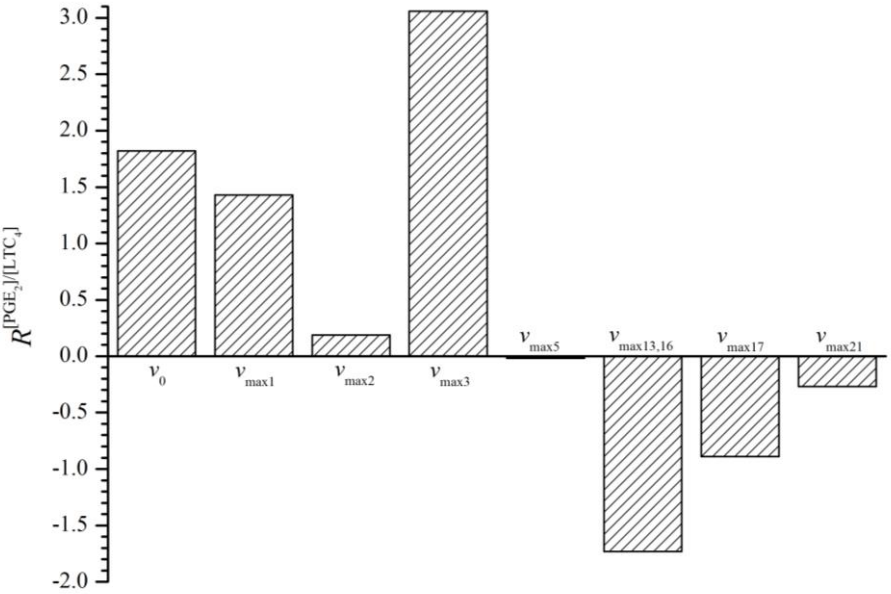
Supplementary Figure 2: Response coefficients (R) of the model variable [PGE_2] in case of reference model state (NA-NI) with respect to alterations in parameter values v_{maxi}.



Supplementary Figure 3: Response coefficients (*R*) of the model variable [PGD_2] in case of reference model state (NA-NI) with respect to alterations in parameter values v_{maxi}.

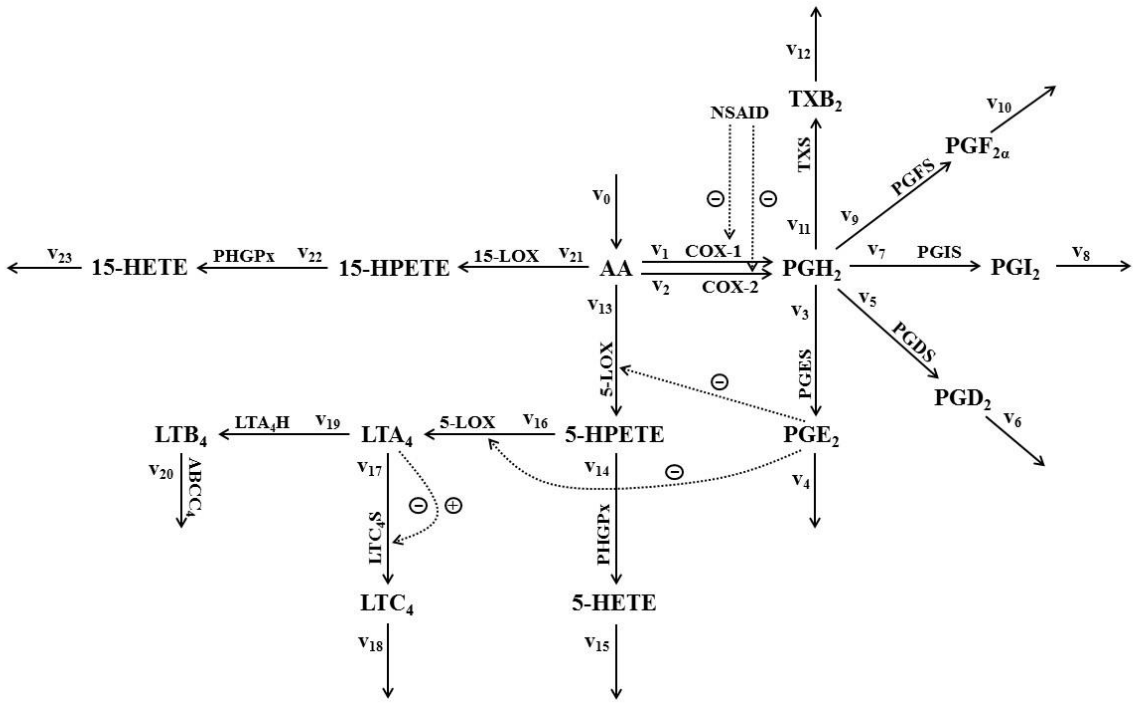


Supplementary Figure 4: Response coefficients (*R*) of the model variable [PGE_2]/[PGD_2] in case of reference model state (NA-NI) with respect to alterations in parameter values v_{maxi}.



Supplementary Figure 5: Response coefficients (*R*) of the model variable [PGE_2]/[LTC_4] in case of reference model state (NA-NI) with respect to alterations in parameter values v_{maxi}.

Kinetic scheme and the complete mathematical model



Supplementary Table 3. Reactions, reaction rates and the parameter values for the reference model state – non-asthmatic model population (NA) in the model state of no-inflammation (NI).

No.	Reaction	Reaction rate	Parameter values
0	Ø→AA	$v_0 = v_0$	v_0 = 0.070
1	AA→PGH2	$v_1 = \frac{v_{max1} \cdot [AA]}{K_1 \cdot (1 + [NSAID] / K_{I1}) + [AA]}$	v_{max1} = 0.48, K_1 = 3.0, K_{I1} = 0.032
2	AA→PGH2	$v_2 = \frac{v_{max2} \cdot [AA]}{K_2 \cdot (1 + [NSAID] / K_{I2}) + [AA]}$	v_{max2} = 0.055, K_2 = 2.5, K_{I2} = 1.02

3	PGH2→PGE2	$v_3 = \frac{v_{max3} \cdot [PGH_2]}{K_3 + [PGH_2]}$	v_{max3} = 5.0, K_3=160.0
4	PGE2→ Ø	$v_4 = k_4 \cdot [PGE_2]$	k_4 = 0.0028
5	PGH2→PGD2	$v_5 = \frac{v_{max5} \cdot [PGH_2]}{K_5 + [PGH_2]}$	v_{max5} = 0.045, K_5= 13.8
6	PGD2→ Ø	$v_6 = k_6 \cdot [PGD_2]$	k_6 = 0.0028
7	PGH2→PGI2	$v_7 = \frac{v_{max7} \cdot [PGH_2]}{K_7 + [PGH_2]}$	v_{max7} = 6.2, K_7= 30.0
8	PGI2→ Ø	$v_8 = k_8 \cdot [PGI_2]$	k_8 = 0.028
9	PGH2→PGF2α	$v_9 = \frac{v_{max9} \cdot [PGH_2]}{K_9 + [PGH_2]}$	v_{max9} = 6.2, K_9= 18.0
10	PGF2α→ Ø	$v_{10} = k_{10} \cdot [PGF_{2\alpha}]$	k_{10} = 0.028
11	PGH2→TXB2	$v_{11} = \frac{v_{max11} \cdot [PGH_2]}{K_{11} + [PGH_2]}$	v_{max11} = 6.2, K_{11}= 22.0
12	TXB2→ Ø	$v_{12} = k_{12} \cdot [TXB_2]$	k_{12} = 0.028
13	AA→5-HPETE	$v_{13} = \frac{v_{max13} \cdot [PGH_2]}{K_{13} \cdot (1 + [PGE_2] / K_{I13}) + [PGH_2]}$	v_{max13} = 2.5, K_{13} = 5.0, K_{I13} = 0.03
14	5-HPETE→5-HETE	$v_{14} = \frac{v_{max14} \cdot [5-HPETE]}{K_{14} + [5-HPETE]}$	v_{max14} = 6.7, K_{14}= 70.0
15	5-HETE→ Ø	$v_{15} = k_{15} \cdot [5-HETE]$	k_{15} = 0.028

16	5-HPETE→LTA4	$v_{16} = \frac{v_{max16} \cdot [5 - HPETE]}{K_{16} \cdot (1 + [PGE_2] / K_{I16}) + [5 - HPETE]}$	v_{max16} = 2.5, K_{16} = 5.0, K_{I16} = 0.03
17	LTA4→LTC4	$v_{17} = \frac{v_{max17} \cdot [LTA_4]}{A + B \cdot [LTA_4] + C \cdot [LTA_4]^2}$	v_{max17} = 6.2, A = 56.0, B = 1.4, C = 0.17
18	LTC4→ Ø	$v_{18} = k_{18} . [LTC_4]$	k_{18} = 0.0015
19	LTA4→LTB4	$v_{19} = \frac{v_{max19} \cdot [LTA_4]}{K_{19} + [LTA_4]}$	v_{max19} = 3.7, K_{19}= 27.0
20	LTB4→ Ø	$v_{20} = \frac{v_{max20} \cdot [LTB_4]}{K_{20} + [LTB_4]}$	v_{max20} = 5.74, K_{20} = 239.0
21	AA→15-HPETE	$v_{21} = \frac{v_{max21} \cdot [AA]}{K_{21} + [AA]}$	v_{max21} = 2.5, K_{21} = 70.0
22	15-HPETE→15-HETE	$v_{22} = \frac{v_{max22} \cdot [15 - HPETE]}{K_{22} + [15 - HPETE]}$	v_{max22} = 6.7, K_{22} = 70.0
23	15-HETE→ Ø	$v_{23} = k_{23} \cdot [15 - HETE]$	k_{23} = 0.028
	NSAID	$[NSAID] = \frac{D \cdot k_a}{M \cdot V \cdot (k_a - k_e)} \cdot (e^{-k_e \cdot t} - e^{-k_a \cdot t})$	k_a = 10^{-4}, k_e = 9.5 10^{-5}, V = 45.0, D = 25 10^3, M = 357.8

Supplementary Table 4. Dynamical system of the first-order non-linear ordinary differential equations for all fourteen system variables considered in the kinetic scheme. Initial values are the concentrations of each particular variable in a stationary state in the absence of drug (NSAID). Initial conditions are presented for the reference model population NA-NI.

Left-hand sides	**Right-hand sides**	**Initial concentrations [μM]**
d[AA]/dt	$v_0 - v_1 - v_2 - v_{13} - v_{21}$	0.32
d[PGH_2]/dt	$v_1 + v_2 - v_3 - v_5 - v_7 - v_9 - v_{11}$	0.060
d[PGE_2]/dt	$v_3 - v_4$	0.67
d[PGD_2]/dt	$v_5 - v_6$	0.070
d[PGI_2]/dt	$v_7 - v_8$	0.44
d[$PGF_{2\alpha}$]/dt	$v_9 - v_{10}$	0.74
d[TXB_2]/dt	$v_{11} - v_{12}$	0.60
d[5-HPETE]/dt	$v_{13} - v_{14} - v_{16}$	0.058
d[5-HETE]/dt	$v_{14} - v_{15}$	0.20
d[LTA_4]/dt	$v_{16} - v_{17} - v_{19}$	0.0090
d[LTC_4]/dt	$v_{17} - v_{18}$	0.0061
d[LTB_4]/dt	$v_{19} - v_{20}$	0.051
d[15-HPETE]/dt	$v_{21} - v_{22}$	0.12
d[15-HETE]/dt	$v_{22} - v_{23}$	0.40

Supplementary Table 5: Initial concentrations [in μM] for each model population (NA, ATA, $AIA^{(1)}$, $AIA^{(2)}$,$AIA^{(3)}$) either in model state of inflammation (I) or no-inflammation (NI).

Variable	**NA (NI)**	**NA (I)**	**ATA (NI)**	**ATA (I)**	**$AIA^{(1)}$ (NI)**	**$AIA^{(1)}$ (I)**	**$AIA^{(2)}$ (NI)**	**$AIA^{(2)}$ (I)**	**$AIA^{(3)}$ (NI)**	**$AIA^{(3)}$ (I)**
AA	0.32	1.2	0.32	1.2	0.29	0.91	0.32	1.1	0.29	0.91
PGH_2	0.060	0.24	0.060	0.24	0.056	0.22	0.060	0.24	0.056	0.22
PGE_2	0.67	10.6	0.67	10.6	0.31	2.4	0.67	5.3	0.31	2.4
PGD_2	0.070	0.27	0.070	0.27	0.065	0.25	0.070	0.27	0.065	0.25
PGI_2	0.44	1.7	0.44	1.7	0.41	1.6	0.44	1.8	0.41	1.6
$PGF_{2\alpha}$	0.74	2.9	0.74	2.9	0.68	2.6	0.74	2.9	0.68	2.6
TXB_2	0.60	2.4	0.60	2.4	0.56	2.2	0.60	2.4	0.56	2.2
5-HPETE	0.058	0.40	0.058	0.40	0.090	0.63	0.058	0.55	0.090	0.63
5-HETE	0.20	1.4	0.20	1.4	0.31	2.1	0.20	1.9	0.31	2.1
LTA_4	0.0090	0.14	0.0088	0.14	0.028	0.97	0.0078	0.34	0.025	0.86
LTC_4	0.0061	0.096	0.024	0.38	0.076	2.6	0.11	4.6	0.34	11.5
LTB_4	0.051	0.81	0.050	0.79	0.16	5.5	0.045	1.9	0.14	4.9
15-HPETE	0.12	4.6	0.12	4.6	0.11	3.5	0.12	4.2	0.11	3.5
15-HETE	0.40	14.9	0.40	14.9	0.36	11.5	0.40	13.7	0.36	11.5

Mathematical model in ASCII format

begin name

dynamic model of eicosanoid production (reference model state NA-NI)

end name

begin reactions

v[0] {1}x = {1}AA

v[1] {1}AA = {1}PGH2

v[2] {1}AA = {1}PGH2

v[3] {1}PGH2 = {1}PGE2

v[4] {1}PGE2 = {1}PGE2M

v[5] {1}PGH2 = {1}PGD2

v[6] {1}PGD2 = {1}PGD2M

v[7] {1}PGH2 = {1}PGI2

v[8] {1}PGI2 = {1}PGI2M

v[9] {1}PGH2 = {1}PGF2a

v[10] {1}PGF2a = {1}PGF2aM

v[11] {1}PGH2 = {1}TXB2

v[12] {1}TXB2 = {1}TXB2M

v[13] {1}AA = {1}HPETE5

v[14] {1}HPETE5 = {1}HETE5

v[15] {1}HETE5 = {1}HETE5M

v[16] {1}HPETE5 = {1}LTA4

v[17] {1}LTA4 = {1}LTC4

v[18] {1}LTC4 = {1}LTC4M

v[19] {1}LTA4 = {1}LTB4

v[20] {1}LTB4 = {1}LTB4M

v[21] {1}AA = {1}HPETE15

v[22] {1}HPETE15 = {1}HETE15

v[23] {1}HETE15 = {1}HETE15M

end reactions

begin rate equations

v[0] = v0

v[1] = vmax1 AA[t]/(K1 (1 + NSAID[t]/KI1) + AA[t])

v[2] = vmax2 AA[t]/(K2 (1 + NSAID[t]/KI2) + AA[t])

v[3] = vmax3 PGH2[t]/(K3 + PGH2[t])

v[4] = k4 PGE2[t]

v[5] = vmax5 PGH2[t]/(K5 + PGH2[t])

v[6] = k6 PGD2[t]

v[7] = vmax7 PGH2[t]/(K7 + PGH2[t])

v[8] = k8 PGI2[t]

v[9] = vmax9 PGH2[t]/(K9 + PGH2[t])

v[10] = k10 PGF2a[t]

v[11] = vmax11 PGH2[t]/(K11 + PGH2[t])

v[12] = k12 TXB2[t]

v[13] = vmax13 AA[t]/(K13 (1 + PGE2[t]/KI13) + AA[t])

v[14] = vmax14 HPETE5[t]/(K14 + HPETE5[t])

v[15] = k15 HETE5[t]

v[16] = vmax16 HPETE5[t]/(K16 (1 + PGE2[t]/KI16) + HPETE5[t])

v[17] = vmax17 LTA4[t]/(A + B LTA4[t] + C LTA4[t] LTA4[t])

v[18] = kout18 LTC4[t]

v[19] = vmax19 LTA4[t]/(K19 + LTA4[t])

v[20] = vmax20 LTB4[t]/(K20 + LTB4[t])

v[21] = vmax21 AA[t]/(K21 + AA[t])

v[22] = vmax22 HPETE15[t]/(K22 + HPETE15[t])

v[23] = k23 HETE15[t]

NSAID[t]=(D ka)/(M V (ka-ke)) (exp(-ke t)-exp(-ka t))

end rate equations

begin parameters

K1 = 3.0 (uM)

K2 = 2.5 (uM)

K3 = 160.0 (uM)

K5 = 13.8 (uM)

K7 = 30.0 (uM)

K9 = 18.0 (uM)

K11 = 22.0 (uM)

K13 = 5.0 (uM)

K14 = 70.0 (uM)

K16 = 5.0 (uM)

K19 = 27.0 (uM)

K20 = 239.0 (uM)

K21 = 70.0 (uM)

K22 = 70.0 (uM)

v0 = 0.070 (uM/s)

vmax1 = 0.48 (uM/s)

vmax2 = 0.055 (uM/s)

vmax3 = 5.0 (uM/s)

vmax5 = 0.045 (uM/s)

vmax7 = 6.2 (uM/s)

vmax9 = 6.2 (uM/s)

vmax11 = 6.2 (uM/s)

vmax13 = 2.5 (uM/s)

vmax14 = 6.7 (uM/s)

vmax16 = Vmax13

vmax17 = 0.057 (uM/s)

vmax19 = 3.7 (uM/s)

vmax20 = 5.74 (uM/s)

vmax21 = 2.5 (uM/s)

vmax22 = 6.7 (uM/s)

k4 = 0.0028 (1/s)

k6 = 0.0028 (1/s)

k8 = 0.028 (1/s)

k10 = 0.028 (1/s)

k12 = 0.028 (1/s)

k15 = 0.028 (1/s)

k18 = 0.0015 (1/s)

k23 = 0.028 (1/s)

KI1 = 0.032 (uM)

KI2 = 1.02 (uM)

KI13 = 0.03 (uM)

KI16 = 0.03 (uM)

A = 56.0 (uM)

B = 1.4

C = 0.17 (1/uM)

ka = 10^-4 (1/s)

ke = 9.5 10^-5 (1/s)

V = 45.0 (L)

D = 25000 (ug)

M = 357.8 (g/mol)

end parameters

begin initial conditions

AA[0] = 0.32 (uM)

PGH2[0] = 0.060 (uM)

PGE2[0] = 0.67 (uM)

PGD2[0] = 0.070 (uM)

PGI2[0] = 0.44 (uM)

PGF2a[0] = 0.74 (uM)

TXB2[0] = 0.60 (uM)

HPETE5[0] = 0.058 (uM)

HETE5[0] = 0.20 (uM)

LTA4[0] = 0.0090 (uM)

LTC4[0] = 0.0061 (uM)

LTB4[0] = 0.051 (uM)

HPETE15[0] = 0.12 (uM)

HETE15[0] = 0.40 (uM)

end initial conditions

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