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Abstracts

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Chronic obstructive pulmonary disease – biochemical features

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Sadržaj

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Što je kronična opstuktivna plućna bolest?

Sanja Popović-Grle

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Kronična opstuktivna plućna bolest (KOPB) je naziv za bolesti koje smo prije zvali kronični bronhitis i emfizem. Današnja najčešće korištena definicija KOPB-a potiče od inicijative Svjetske zdravstvene organizacije (WHO) i Američkog instituta za srce, pluća i krv (NHLBI), a koja se naziva Global Initiative for Chronic Obstructive Lung Diseases (GOLD). Definicija KOPB-a glasi: "Kronična opstuktivna plućna bolest (KOPB) je bolest koja se može spriječiti i liječiti, povezana sa značajnim izvanplućnim učincima, koje mogu pridonijeti težini bolesti u pojedinog bolesnika. Plućnu komponentu obilježava smanjenje protoka zraka (opstrukcija) kroz dišne putove tijekom višeod 6 mjeseci, koja nije reverzibilna na primjenu bronhodilatatora. To smanjenje protoka zraka je najčešće progresivno i povezano je s neprimjerenim upalnim odgovorom pluća na izlaganje štetnim česticama dimova i plinova".

KOPB je jedna od najslabije prepoznatih bolesti i najmanje dijagnosticiranih bolesti u svijetu, i kod bolesnika i kod liječnika. Oko 75% bolesnika nema postavljenu dijagnozu, najviše njih u blagom stupnju bolesti, ali i oko 4% bolesnika s teškim stupnjem i oko 1% s najtežim stupnjem KOPB-a. Razlog tome je i postupan razvoj tegoba kao kašalj i ubrzano zamaranje u naporu, te pojava u starijoj dobi. KOPB jedna je od bolesti čija prevalencija najbrže raste – tijeko 40 godina porasla je za 163%. Prema mortalitetu KOPB se sada nalazi na četvrtom mjestu, nakon srčanog udara, zloćudnih bolesti i moždanog udara. Danas je u svijetu oko 600 milijuna bolesnika s kroničnom opstuktivnom plućnom bolešću, dvostruko više no bolesnika sa šećernom bolešću.

Općenito je prihvaćeno da bolesnici s KOPB-om imaju snižene spirometrijske pokazatelje, uz forsirani ekspiracijski volumen u prvoj sekundi (FEV1) ispod 80% od referentne vrijednosti te odnos FEV1/ FVC (Tiffeneau-ov indeks) ispod 0,70 (ili 70%). Prema GOLD-u postoje 4 stupnja KOPB-a. Blagi stupanj (GOLD 1) prisutan je u bolesnika koji imaju tegobe, ali još uvijek urednu plućnu funkciju i spirometriju (FEV1 > 80%). Umjereno teški stupanj (GOLD 2) prisutan je u bolesnika s FEV1 između 50-80% od pretpostavljene vrijednosti za tu osobu, teški stupanj (GOLD 3) procjenjuje se u bolesnika s FEV1 između 30 do 50% i vrlo teški KOPB imaju bolesnici s FEV1 ispod 30% uz respiracijsku insuficijenciju

What is chronic obstructive pulmonary disease?

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Chronic obstructive pulmonary disease (COPD) is a term for diseases previously named chronic bronchitis and emphysema. The most commonly used definition today for the COPD is given by the Initiative started by World Health organization (WHO) and National Heart, Blood and Lung Institute (NHLBI) - Global Initiative for Chronic Obstructive Lung Diseases (GOLD): "Chronic obstructive pulmonary disease (COPD) is preventable and treatable disease with some significant extra pulmonary effect that may contribute to the severity in individual patient. Its pulmonary component is characterized by airflow limitation for more than 6 months not fully reversible to bronchodilators. This obstruction is usually progressive and associated with abnormal inflammatory response to noxious particles and gases".

COPD is one of the most under diagnosed diseases in the world, both in patients and doctors. About 75% of patients do not have established diagnosis, most of them in mild degree, but also 4% in severe and 1% in very severe degree of COPD. The reason for that is slow progression of symptoms as cough and excersize intolerance, as well as development of disease in elderly. COPD in one of diseases which prevalence is increasing the most – through 40 years it has increased for + 163%. By mortality the COPD is on 4th place in the world, after myocardial infarction, malignant diseases and cerebrovascular insults. There are around 600 billion patients with COPD today in the world, double than diabetics.

It is usually accepted that COPD patients have decreased values of spirometry parameters, with forced expiratory volume in first second (FEV1) under 80% of reference value, and FEV1/ FVC (index Tiffeneau) under 0,70 (or 70%). According to the GOLD there are 4 degrees of COPD. Mild degree (GOLD 1) is present in smokers who have symptoms, but still have normal lung function (FEV1 > 80%). Moderate degree of COPD (GOLD 2) is present in patients with FEV1 between 50-80% from reference values for that individual, severe degree of COPD (GOLD 3) is present in patients with FEV1 between 30-50%, while very severe degree of COPD (GOLD 4) is present in patients with FEV1 between < 30% with respiratory insufficiency.

Oksidacijski stres i kronična opstrukcijska plućna bolest

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Oksidacijski stres i upalna reakcija glavni su biljezi kronične opstrukcijke plućne bolesti (KOPB). Reaktivni kisikovi spojevi (ROS) i reaktivni dušikovi spojevi (RNS) s jedne strane doprinose normalnoj fiziološkoj funkciji, kao što su stanična diferencijacija, stanična signalizacija, apoptoza i baktericidno djelovanje, a s druge strane, povećane koncentracije oksidansa uzrokuju lipidnu peroksidaciju, oksidaciju proteina te oštećenje DNA. Stanice se brane protiv oksidativnog napada dobro razvijenim enzimskim i neenzimskim sustavom antioksidansa.

U KOPB-u, ROS ili neposredno ili preko stvaranja produkata lipidne peroksidacije, kao što su 4-hidroksi-2-nonenal i F2-izoprostani, mogu igrati ulogu u pojačavanju upale preko aktivacije i fosforilacije proteinskih kinaza aktiviranih mitogenima i redoks osjetlivih transkripcijskih faktora kao što su nuklearni faktor-κB i aktivator protein-1.

Stanice i tkiva neprestano su izloženi oksidansima koji se mogu stvarati ili endogeno (respiracija u mitohondrijima, NADPH oksidaza sustav koji je prisutan u fagocitima i epitelnim stanica, ksantin/ksantin oksidaza) ili egzogeno udisanjem zagađenog zraka ili dima cigarete. S obzirom da dim cigarete sadrži preko 4700 kemjiskih sastojaka, te i katranska i plinovita frakcija sadrže slobodne radikale i druge oksidanse, pušenje cigarete glavni je čimbenik rizika za razvoj KOPB-a. Jedan dim cigarete sadrži preko 1014 slobodnih radikala. ROS kao što su superoksidni anion i hidroksilni radikal su nestabilne molekule s nesparenim elektronima moćni su inicijatori oksidacijskog procesa. Posljedice oksidacijskog stresa u KOPB-u mogu se mjeriti pomoću biomarkera kao što su: a) hidrogen peroksid u izdahnutom zraku, b) otpuštanje ROS-a iz neutrofila, eozinofila i alveolarnih makrofaga u perifernoj krvi, c) aktivnosti MPO-a, EPO-a i ksantin/ksantin oksidaze u bronhoalveolarnom lavatu, te kao d) koncentracije F2-izoprostana, lipidnih peroksida (TBARS), CO i NO u izdahnutom zraku.

Oxidative stress and chronic obstructive pulmonary disease

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Oxidative stress and inflammation are the major hallmarks of chronic obstructive pulmonary disease (COPD). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) contribute to normal physiological functions such as cellular differentiation, cell signalling, apoptosis, and bactericidal activities, but in other hand, the enhanced concentrations of oxidants cause lipid peroxidation, protein oxidation, and DNA damage. Cells are protected against this oxidative attack by well developed enzymatic and non enzymatic antioxidant system.

ROS, either directly or via the formation of lipid peroxidation products, such as 4-hydroxy-2-nonenal and F2-isoprostanes, may play a role in enhancing the inflammation through the activation and phosphorylation of mitogen-activated protein kinases and redox-sensitive transcription factors such as nuclear factor- κB and activator protein-1 in COPD.

Cells and tissues are continuously exposed to oxidants which can be either generated endogenously by metabolic reactions (mitochondrial respiration, the NADPH oxidase system that is present in phagocytes and in epithelial cells, xanthine/xanthine oxidase), or exogenously such as inhaled from air pollutants or cigarette smoke. Whereas, cigarette smoke contains over 4700 chemical compounds, and both the tar and gas phases contain numerous free radicals and other oxidants, thus cigarette smoking becomes the major risk factor of COPD. One cigarette puff contains more than 1014 free radicals. ROS such as superoxide anion and the hydroxyl radical are unstable molecules with unpaired electrons, capable of initiating oxidative processes. Consequences of oxidative stress in COPD can be measured by biomarkers such as: a) hydrogen peroxide in exhaled breath, b) release of ROS from peripheral blood neutrophils, eosinophils and macrophages, c) MPO, EPO and xanthine/xanthine oxidase activities in bronchoalveolar lavage fluid, and d) F2-isoprostane, lipid peroxide (TBARS), CO and NO levels in exhaled breath.

Dim cigarete uzrokuje oksidacijski stres u epitelnim stanicama dišnih putova *in vitro* i u *in vivo* modelu kod miša

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Glavni faktori rizika za razvoj kronične opstrukcijske plućne bolesti (KOPB) su pušenje cigareta i genetska predispozicija. Postavljene su tri hipoteze kao podloga za oštećenje tkiva i kroničnu upalu dišnih puteva karakteristične za bolesnike sa KOPB-om: (i) neravnoteža oksidansa i antioksidansa, (ii) neravnoteža proteaza i antiproteaza, te (iii) prilagodba imunog odgovora karakterizirana CD8+ T-stanicama i B-stanicama. Epitelne stanice dišnih putova su prva linija obrane od štetnih tvari koje udišemo uključujući dim cigareta. Stoga smo proučavali učinke dima cigareta na ljudske epitelne stanice dišnih putova *in vitro*. Pokazalo se da dim cigareta ometa funkciju mitohondrija inhibicijom lanca prijenosa elektrona, što dovodi do sniženja membranskog potencijala mitohondrija, pada potrošnje kisika i produkcije ATP-a, te povećanog stvaranja reaktivnih kisikovih spojeva unutar stanica. Štoviše, gubitak proizvodnje ATP-a vodi apoptotične epitelne stanice dišnih putova u nekrozu. Poznato je da stanice koje umiru nekrozom stvaraju kritične signale koji vode do aktivacije imunih i upalnih stanica. Da bismo primijenili ova opažanja na mišji model *in vivo*, BALB/c miševe smo izložili dimu cigareta ili dimu cigareta sa smanjenom količinom reaktivnih kisikovih spojeva tijekom pet uzastopnih dana. Izloženost dimu cigareta, ali ne i dimu sa smanjenom količinom reaktivnih kisikovih spojeva, uzrokovala je dotok mnoštva neutrofila u dišne putove povezan s povećanjem razine proupalnih citokina i kemokina. Ovaj model biti će korištan za daljnja proučavanja signalnih putova koji vode do akutne upale uzrokovane dimom cigareta.

Cigarette smoke induced oxidative stress in airway epithelial cells *in vitro* and a mouse model *in vivo*

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Cigarette smoking and genetic susceptibility are the main risk factors for the development of Chronic Obstructive Pulmonary Disease (COPD). Three hypotheses have been postulated to underlie the tissue destruction and chronic airway inflammation characteristic for COPD patients: (i) an imbalance between oxidants and anti-oxidants, (ii) an imbalance between proteases and anti-proteases, and (iii) an adaptive immune response characterized by CD8+ T-cells and B-cells. Airway epithelial cells are the first line of defence against harmful inhaled substances including cigarette smoke. Therefore, we studied the effects of cigarette smoke (CS) on human airway epithelial cells *in vitro*. It appeared that CS disturbs mitochondrial function by inhibition of the electron transfer chain, leading to decreased mitochondrial membrane potential, oxygen consumption and ATP production and increased generation of intracellular reactive oxygen species (ROS). Moreover, loss of ATP generation induces airway apoptotic epithelial cells into necrosis. It is well-know that necrotic cell death generates danger signals that lead to activation of immune- and inflammatory cells. In order to translate these observations into an *in vivo* mouse model, BALB/c mice were exposed to CS or ROS-depleted CS for five consecutive days. Exposure to CS, but not ROS-depleted CS, induced massive neutrophil influx into the airways associated with increased levels of pro-inflammatory cyto- and chemokines. This model will be useful to further dissect the pathways leading to acute CS induced inflammation.

Upalna reakcija u kroničnoj opstrukcijskoj plućnoj bolesti

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KOPB je kronična bolest karakterizirana razvojem progresivne opstrukcije malih dišnih putova koja nastaje kao rezultat abnormalnog upalnog odgovora plućnog tkiva na udahnute čestice i plinove. Patogeneza KOPB teme-

Inflammatory mechanisms in chronic obstructive pulmonary disease

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COPD is chronic, slowly progressive disease characterized with accelerated increase in airflow limitation resulting from hiperactive local inflammatory response to inhaled particles and gases. The current paradigm for the patho-

lji se na 1) postojanju lokalne upale u plućnom tkivu, 2) povećanoj tkivnoj razini citokina i kemokina što uzrokuje nakupljanje i aktivaciju neutrofila, makrofaga, monocita i CD8+ citotoksičnih limfocita u respiratornim putovima, 3) sistemskom povećanju koncentracija medijatora upale, posebice TNF-α, i citokina IL-6 i IL-8 u krvi bolesnika u stabilnoj, a naročito u fazi napada bolesti, 4) neravnoteži između aktivnosti proteaza i proteaznih inhibitora koji sudjeluju u procesiranju medijatora upale, 5) razvojem oksidacijskog stresa koji ima lokalnu i sistemsku ulogu, 6) porasta koncentracije redoks-osjetljivih transkripcijskih faktora u plućima te 7) razvojem sistemskih pro-oksidacijskih i upalnih uvjeta koji postepeno dovode do kardiovaskularnih bolesti, promjene koštane mase, te progresivnog gubitka mišićne mase i funkcije mišića. Intenzitet ovih promjena je povezan s progresivnim oštećenjem plućnog parenhima i s gubitkom funkcije pluća.
Poremećena aktivnost i djelovanje proteaza, kao što su metaloproteinaze MMP8 i MMP9, te dipeptidil peptidaze IV koje se oslobađaju iz stanica kao odgovor na upalu dovodi do razgradnje izvanstaničnog matriksa i stvaranja peptidnih fragmenata koji sami djeluju kemotaktično i aktivirajuće na neutrofile i makrofage stimulirajući ih da pojačano luče citokine i tako dalje potenciraju razvoj kronične upale.
Velika heterogenost u brzini progresije, kliničkim manifestacijama i težini bolesti, zahtijeva pouzdan način predviđanja razvoja bolesti i komplikacija kako bi se na tim saznanjima mogla temeljiti racionalna terapija. Ključni biokemijski pokazatelji koji bi specifično pratili razvoj upale do sada nisu definirani.
U našim smo istraživanjima pokušali pronaći biljege koji se mogu ispitivati u uzorcima dobivenim neinvazivnim metodama - serumu, krvnim stanicama (polimorfonuklearima, limfocitima, trombocitima, eritrocitima), u mokraći i eventualno u kondenzatu izdahnutog zraka.

genesis of COPD is involving 1) an abnormall inflammatory response in the lung, 2) increased levels of cytokines and chemokines, 3) accumulation and activation of neutrophils, macrophages, monocytes and CD8+ T lymphocytes in airways, 4) increased levels of inflammatory mediators: TNF-α, cytokines IL-6 and IL-8 in sera of patients, in the stable phase and especially during the exacerbations, 5) deregulation of protease - antiprotease balance resulting in an impaired processing of inflammatory mediators, 6) development of tissue and systemic oxidative stress, 7) increased concentrations of redox-sensitive transcription factors and 8) development of systemic pro-oxidative and inflammatory conditions leading to cardiovascular diseases, changes in the bone masse, progressive muscle waist and loss of muscle function. Intensity of these changes is connected with progressive destruction of pulmonary parenchyma. Deregulation in the activity of proteases, metalloproteinases MMP8 and MMP9 and dipeptidyl peptidase IV released by cells, in response to inflammation, cause breakdown of the extracellular matrix generating peptide fragments still active on leukocytes, which in turn release cytokines and more proteases, leading to further leukocyte infiltration and disease progression.
Great heterogeneity in the rate of progression and clinical manifestations of COPD could not be successfully monitored because of the lack of reliable markers for the severity of the disease.. The specific biochemical markers for the progression of inflammation in COPD have not been proposed. In our work we try to evaluate reliable markers that could be assayed in samples obtained by non-invasive methods – blood serum and cells polymorphonucleares, lymphocytes, erythrocytes, urine and possibly in the expired breath condensate

Genetika i kronična opstrukcijska plućna bolest

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Kronična opstrukcijska plućna bolest (KOPB) značajan je javno-zdravstveni problem, a predstavlja skup poremećaja koji imaju zajedničku osobinu kroničnog ograničenja zračnog protoka.
Temeljni je poremećaj u KOPB, slično astmi, kroničnom bronhitisu i plućnom emfizemu, upalna reakcija pluća na otrovne čestice ili plinove, najčešće duhanski dim. Utvrđe-

Genetics and chronic obstructive pulmonary disease

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Increasing prevalence of chronic obstructive pulmonary disease (COPD) is an important public health concern. COPD is a clinical entity which includes a collection of disorders that share the common physiological features of expiratory airflow limitation. The etiology of COPD involves a complex interplay between genetic and environmental factors.

no je da će se u kavkaskoj populaciji u 10 do 20 % pušača razviti KOPB, ali aktivno pušenje pridonosi razvoju bolesti kod oko 90 % osoba sklonih bolesti, kod kojih genetički čimbenici pridonose njenom nastanku i razvoju.
Etiologija KOPB obuhvaća dakle kompleksnu međusobnu povezanost genetičkih i okolišnih čimbenika. Okolišni su čimbenici poznati, a identificirani su i geni koji djeluju kao genetički čimbenici:

1. geni koji kodiraju enzime za metaboliziranje lijekova (mikrosomalna epoksid hidrolaza, EPHX1; glutation S-transferaza, GST; superoksid dizmutaza, SOD),
2. geni koji kodiraju upalne posrednike (obitelj interleukina 1, IL-1; tumorski nekrotizirajući čimbenik α, TNF-α; transformirajući čimbenik rasta β, TGF-β),
3. geni koji kodiraju proteaze i antiproteaze (inhibitor proteaza, PI; metaloproteinaze matriksa, MMP; elastin, ELN; tkivni inhibitor metaloproteinaze, TIMP),
4. geni koji kodiraju dezintegrin i metaloproteinaze (ADAM)

Osim toga, kod osoba oboljelih od KOPB utvrđena je povećana nestabilnost mikrosatelitne DNA (MSI). MSI je povezana s nefunkcionalnim sustavom popravka pogrešno sparenih baza te je u korelaciji s učestalošću mutacija. MSI je, zapravo, pokazatelj destabilizacije genoma na više lokusa. U stanicama sputuma oboljelih od KOPB analizirana je MSI za nekoliko markera. Pokazano je kako se kod bolesnika pozitivnih za markere G29802, D13S71 i D14S588 češće javljaju pogoršanja bolesti.
Značajna povezanost između MSI i pogoršanja bolesti u KOPB ukazuju na to da su somatske mutacije također važni patogenetski čimbenici.

COPD is associated with an abnormal inflammatory response of the lung to noxious particles or gases, most commonly tobacco smoke. Active cigarette smoking contributes to the origin of COPD in more than 90% of subjects who are susceptible. However, in Caucasians only 10-20% of heavy cigarette smokers develop COPD. This fact suggests that genetic factors are important in developing of COPD.
Several genes that are involved in COPD pathogenesis have been identified and classified into following groups:

1. genes encoding xenobiotic metabolizing enzymes (microsomal epoxide hydrolase, EPHX1; glutathione S-transferase, GST; superoxide dismutase, SOD),
2. genes encoding inflammatory mediators (interleukin-1 family, IL-1; tumor necrosis factorα, TNF-α; transforming growth factorb, TGF-β),
3. genes encoding proteases/antiproteases (protease inhibitor, PI; matrix metalloproteinases, MMPs; elastin, ELN; tissue inhibitor of metalloproteinase, TIMP),
4. novel genes (disintegrin and metalloprotease, ADAM).

Moreover, increased frequency of microsatellite DNA instability (MSI) has been detected in COPD patients. MSI has been correlated with a high somatic mutation rate and is associated with a defective DNA mismatch repair system. MSI indicates destabilisation of the genome at various loci. COPD patients positive for G29802, D13S71 and D14S588 markers presented an increased exacerbation frequency. The significant association between MSI and COPD exacerbations indicates possible involvement of somatic mutations in pathogenesis of disease.

Današnje liječenje kronične opstruktivne plućne bolesti

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Liječenje KOPB-a je kompleksno i uključuje brojne mjere. Kao prvo prestanak pušenja, redovito uzimanje lijekova, ali ostale nefarmakološke mjere (plućnu rehabilitaciju, liječenje kisikom i neke kirurške metode, kao transplantacija pluća).
Osnova liječenja stabilne KOPB-a su bronhodilatatori, lijekovi koji proširuju dišne putove. Dvije osnovne skupine su simpatikomimetici i antikolinergici. Simpatikomimetici su kratkodjelujući β2-agonisti (SABA Short acting β2-agonist) salbutamol-Ventolin®, i dugodjelujući β2-agonisti (LABA

Management of chronic obstructive pulmonary disease

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COPD management is complex and has lot of components. The first measure, is smoking cessation, then regular pharmacologic therapy, but also pulmonary rehabilitation, oxygen therapy and some surgical methods, as lung transplantation.
Basic pharmacologic therapies for the stable COPD are bronchodilators, drugs that increases airways diameter. Two basic groups are simpaticomimetics and anticholinergics. Simpaticomimetics are short-acting β2-agonists (SABA) salbutamol-Ventolin®, and long-acting β2-agonist

Long acting β2-agonist) salmeterol-SereventO, u obliku spreja ili discusa. Što se tiče antikolinergika, također postoje kratko djelujući (ipratropij-Atrovent®) i dugo djelujući lijekovi (tiotropij-Spiriva®).
U grupu bronhodilatatora dolaze i metilksantini, ali oni osim bronhodilatatornog djelovanja imaju i druge učinke, poput protuupalnog, pojačavaju snagu respiracijske muskulature, novije spoznaje pokazuju i da smanjuju rezistenciju steroidnog receptora u pušača. Zbog svoje niske cijene koštanja te simptomatskog učinka uz stanovita protuupalna svojstva, teofilinski pripravci važni su u terapiji KOPB-a. Na tržištu postoje Teolin R® i Teotard R®, a uskoro dolaze i selektivni inhibitori fosfodiesteraze PGE4, kao što je roflumilast-Daxas®.
Prema današnjim stavovima, u terapiji KOPB-a inhalacijski kortikosteroidi indicirani su u osoba s težim oblicima KOPB-a, kada je FEV1 ispod 50% i u osoba koje imaju učestale egzacerbacije (više od tri godišnje). Prednost inhalacijskih lijekova jest u tome da se u bolesni organ (dišne putove) lijek može unijeti izravno, gdje se time postiže visoka koncentracija lijeka, a izbjegavaju se sistemski učinci i nuspojave. Dokazano je da inhalacijski kortikosteroidi najbolje djeluju kada se kombiniraju s bronhodilatatorima, te su u Hrvatskoj dostupni moćni lijekovi Seretide® i Symbicort®. Primjena navedenih lijekova podiže kvalitetu življenja bolesnika s KOPB, te smanjuje mortalitet, osobito zato jer prorjeđuje egzacerbacije KOPB. Egzacerbacije teškog KOPB po život su opasna stanja, gdje je mortalitet jednako visok kao u akutnom koronarnom incidentu. Zbog toga se egzacerbacije KOPB energično liječe antibitiocima, najčešće iz grupe beta-laktama ili kinolona, uz pojačane doze bronhodilatatora i oralnih ili parenteralnih kortikosteoida uz oksigenoterapiju. Najtežim bolesnicima s KOPB preostaje još transplantacija pluća, najčešće unilateralna, gdje je preživljenje 50% u 5 godina nakon transplantacije.

(LABA) salmeterol-Serevent®, in spray or discus. Anticholinergic also exists as short-acting drug (ipratropium-Atrovent®) and long-acting drug (tiotropium-Spiriva®).
There are also other bronchodilators - methylxanthines. These drugs has also other effects, such as antiinflammatory, increasing the strength of respiratory muscles, and novel data shows that methylxanthines decreases steroid receptor resistance in smokers. Because they have low costs with symptomatic effect, and certain antinflammatory effect, methylxanthines are important in COPD therapy. On our market there are Teolin R® i Teotard R®, and soon we expect selective phosphodiesterase inhibitors PGE4, as roflumilast-Daxas®.
According to current knowledge inhaled corticosteroids are indicated in the therapy with severe and very severe degree, when FEV1 is less than 50% and in COPD patients with frequent exacerbations (more than 3 annually). The advantage of inhaled therapy is that drug could be delivered directly in the affected organ, where it has high concentration, while avoiding systemic adverse effects. It is proven that inhaled steroids acts the best when they are combined with long-acting β2-agonists, in Croatia powerful combine drugs Seretide® i Symbicort® are available. Application of these drugs increases quality of life in patients with COPD, decreases mortality, especialy by decreasing frequency of COPD exacerbations. Acute exacerbation of COPD could be life threating, where the mortality rate is the same as in acute coronary incident. Because of that COPD exacerbations should be energically treated with antibiotics, usually from the beta lactam group or cinolons, with increased bronchodilators doses, parenteral or oral steroids and oxygeotherapy. The most severe COPD patients have the last oppurtunity for improved health status in lung transplantation, usually unilateral, with survival rate 50% in 5 years after lung transplantation.

Kronična opstrukcijska plućna bolest i signalne molekule

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Chronic obstructive pulmonary disease and signalling molecules

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Kroničnu opstrukcijsku plućnu bolest (KOPB) karakterizira kronični lokalni i sistemski upalni proces te pojačan oksidacijski stres.
Pušenje cigareta se smatra glavnim etiološkim faktorom u nastanku i razvoju KOPB. Reaktivne kisikove vrste (ROS), čije stvaranje potiču pušenje i aktivirane stanice pluća i krvne tekućine, mogu pojačati upalnu reakciju na način da aktiviraju signalne molekule osjetljive na oksidacijsko-re-

Chronic obstructive pulmonary disease (COPD) is characterized by chronic local and systemic inflammation, and increased oxidative stress.
Cigarette smoking is the major etiological factor responsible for COPD. Reactive oxygen species (ROS) generated by cigarette smoke and by activated lung and peripheral blood cells, may play a role in enhancing the inflammation through activation of redox-sensitive signalling

dukcijske promjene u stanici, poput proteinskih kinaza aktiviranih mitogenima (MAPK) i proteina toplinskoga šoka (Hsp).
Tri glavne MAPK u sisavaca su kinaza regulirana izvanstaničnim signalima (ERK), kinaza koja fosforilira N-kraj transkripcijskog faktora c-Jun (JNK) i p38. Aktivacija ERK kinaze najčešće potiče staničnu proliferaciju, dok aktivacija JNK i p38 kinaza najčešće potiče apoptozu.
Hsp reguliraju proces apoptoze te djeluju kao molekularni pratitelji potpomažući u pravilnom nabiranju proteina i sprječavajući njihovo nakupljanje. Hsp mogu stimulirati antioksidacijsku obranu stanice na način da smanjuju količinu ROS i neutraliziraju toksična djelovanja oksidiranih proteina.
Ispitali smo ekspresiju i aktivaciju MAPK te ekspresiju Hsp70 i Hsp27 u leukocitima KOPB bolesnika i zdravih osoba.
ERK je aktivirana samo u nepušača, dok su se JNK i p38 aktivirale u KOPB pušača i bivših pušača te u zdravih pušača. Opažena je smanjena ekspresija Hsp70 i Hsp27 u KOPB bivših pušača i zdravih pušača, a osobito u KOPB pušača.
Rezultati ukazuju da KOPB i pušenje djeluju na signalne putove u stanici.
Razumijevanje temeljnih staničnih i molekularnih mehanizama KOPB neophodno je za identifikaciju molekula koje bi mogle postati značajne u dijagnostici i u terapiji ove bolesti.

molecules, including mitogen-activated protein kinases (MAPKs) and heat shock proteins (Hsps).
Three major mammalian MAPKs are extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. While ERK pathway usually induces cell proliferation, JNK and p38 pathways primarily induce apoptosis.
Hsps are implicated in many steps of apoptotic machinery and they act as molecular chaperones by assisting the correct folding of proteins, and by preventing their aggregation. Hsps could stimulate antioxidant defence of cells by decreasing intracellular levels of ROS and by neutralizing toxic effects of oxidized proteins.
We assessed expression and activation of MAPKs and expression of Hsp70 and Hsp27 in leukocytes of COPD patients and healthy individuals.
ERK was activated in non-smokers only. In contrast, strong induction of JNK and p38 phosphorylation was detected in COPD smokers and ex-smokers, but also in healthy smokers. In addition, Hsp70 and Hsp27 were decreased in COPD ex-smokers and healthy smokers, and especially in COPD smokers.
These results show that COPD and smoking affect intracellular signaling pathways. Understanding of the basic cellular and molecular mechanisms in COPD is essential for identification of molecules that may serve as targets for diagnosis and therapeutic interventions.

Uloga proteasoma u degradaciji proteina oštećenih dimom cigareta

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Mehanizam kojim pušenje cigareta vodi k razvoju kronične opstrukcijske plućne bolesti još uvijek je nejasan. Dim cigareta pridonosi oksidativnom oštećenju proteina u plućima. Jedan od glavnih načina zaštite stanica od oštećenih proteina je ubikvitin-proteasomalni put. Razgradnja proteina ovim putem uključuje konjugaciju više molekula ubikvitina i razgradnju tako označenog proteina putem proteasoma na peptide koji se dalje proteazama i aminopeptidazama brzo hidroliziraju na aminokiseline. Dokazano je da dim cigareta dovodi do porasta sadržaja slobodnih aminokiselina unutar A549 plućnih epitelnih stanica. Postavlja se pitanje je li proteasom uključen u razgradnju proteina oštećenih dimom cigareta. Izlaganjem A549 stanica dimu cigareta uz Epoximicin (inhibitor aktivnosti proteasoma) metodom LC-MS pokazali smo da proteasom je uključen u porast sadržaja slobodnih aminokiselina ($P < 0,001$). Western blot analizom smo pokazali da dim cigareta uzrokuje povećanje ubikvitin-proteinskih konjugata. Obilježavanjem aktivnih mjesta proteasoma fluorescen-

Cigarette smoke – induced protein damage: role of the proteasome

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The mechanism by which cigarette smoking leads to development of chronic obstructive pulmonary disease is still unclear. Cigarette smoke contributes to oxidative damage of proteins inside the lung. One of the main systems to protect cells from damaged proteins is ubiquitin-proteasome pathway. Degradation of proteins via this pathway involves conjugation of multiple ubiquitin moieties and degradation of the tagged protein by proteasome into peptides which are rapidly hydrolyzed into amino acids by proteases and aminopeptidases. It was shown that cigarette smoke causes an increase of free amino acids inside the A549 airway epithelial cells. The question is weather the proteasome is involved in degradation of proteins damaged by cigarette smoke. We have exposed A549 cells to cigarette smoke and Epoxomicin (inhibitor of proteasomal activity) and we have shown, using LC-MS method, that proteasome is involved in the increase of free amino acids ($P < 0,001$). Western blot analysis has shown that cigarette smoke causes an increase of ubiquitin-protein conjugates. Proteasomal active sites

tnom bojom MV151 dokazali smo da se aktivna mjesta proteasoma uglavnom ne mijenjaju pod utjecajem dima cigareta. Upotrebom fluorogenih supstrata pokazali smo da dim cigareta mijenja sve tri vrste aktivnosti proteasoma. Ukratko, naši rezultati pokazuju da dim cigareta inducira oštećenje proteina koji se potom ubikvitiniraju i obilježavaju za razgradnju putem proteasoma. Proteasom je uključen u uklanjanje proteina oštećenih dimom cigareta. Iako aktivna mjesta proteasoma ostaju očuvana, dim cigareta značajno mijenja aktivnost proteasoma što može pridonijeti agregaciji oštećenih proteina i povećanoj smrti stanica.

were labelled by using the fluorescent probe MV151 and in general were not disturbed by cigarette smoke. Using the fluorogenic substrates we have shown that cigarette smoke changes all three kinds of proteasomal activities. Briefly, our data suggest that cigarette smoke induces damage to proteins which are subsequently ubiquitinated and tagged for proteasomal degradation. Proteasome is involved in elimination of proteins damaged by cigarette smoke. Although proteasomal active sites remain intact, cigarette smoke significantly disturbs proteasomal activity which may contribute to the aggregation of damaged proteins and enhanced cell death.

Polimorfizimi stresnih proteina i KOPB

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Stresni proteini (Hsp) pripadaju skupini unutarstaničnih proteina koji imaju sposobnost stabilizacije nepravilno strukturiranih proteina i peptida, čime promoviraju preživljenje, te inhibiraju programiranu staničnu smrt. Geni koji kodiraju za članove porodice stresnih proteina (Hsp70), hsp70-1, hsp70-2 i hsp70-hom smješteni su unutar gena klase III sustava tkivne kompatibilnosti (MHC). U skladu s njihovim smještajem u genomu istraživana je povezanost polimorfizama ovih gena s različitim bolestima čija se pojavnost zasniva na poremećenoj funkciji imunološkog sustava. U skladu sa šaperonskom ulogom stresnih proteina istraživana je i povezanost istih polimorfizama s neravnotežom oksidativnih/anti-oksidativnih spojeva u organizmu.
Kako je poznato da u patogenezi kronične opstrukcijske plućne bolesti (KOPB) oksidativno/antioksidatvini putevi imaju važnu ulogu, ispitivali smo polimorfizme duljine restrikcijskih fragmenata gena hsp70-2 i hsp70-hom, izlaganjem fragmenata DNA dobivenih umnažanjem genskog slijeda reakcijom lančane reakcije polimeraze, djelovanju restrikcijskih endonukleaza. Nismo uočili statistički značajnu razliku u raspodjeli A/G(+1267) polimorfizma u kodirajućoj regiji gena hsp70-2, kao ni u raspodjeli T/C (+2437) polimorfizma u kodirajućoj regiji gena hsp70-hom između zdravih ispitanika i ispitanika s KOPB.
Hsp32 gen (HMOX-1) kodira za mali stresni protein 32 (Hsp32 ili hem oksigenaza-1), enzim koji katalizira razgradnju hema do biliverdina u procesu koji osigurava zaštitu stanicama od oštećenja uzrokovanih oksidativnim radikalima. U populaciji se polimorfizam ovog gena pojavljuje u obliku različitog broja GT ponavljanja u promotorskoj regiji gena odogovornoj za ekspresiju gena i moguću indukciju aktivnosti ovog enzima u stanjima oksidativnog stresa. Između zdravih ispitanika i ispitanika s KOPB nismo uočili statistički značajnu razliku u broju GT ponavljanja hsp32 (HMOX-1) gena.

Heat shock proteins polymorphisms and COPD

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Heat shock proteins are intracellular proteins with ability to stabilize missfolded proteins and peptides, thereby promoting cell survival and preventing programmed cell death. Genes encoding members of the heat shock protein (Hsp70) family hsp70-1, hsp70-2 i hsp70-hom lie in the class III region of the human major histocompatibility complex (MHC). Relating to their location, polymorphisms of these genes were analyzed in association with occurence of different diseases with immune component. Relating to their chaperone role polymorphisms of these genes were also analyzed in association with an oxidative/antioxidative imbalance.
In the pathogenesis of chronic obstructive lung disease (COPD) oxidative/antioxidative pathways play important role. Therefore we studied hsp70-2 and hsp70-hom gene polymorphisms using restriction fragment length polymorphism – polymerase chain reaction method (RFLP-PCR). The obtained data showed that there were no statistically significant difference in A/G(+1267) polymorphism distribution in the coding region of hsp70-2, as well as in T/C (+2437) polymorphism distribution in the coding region of hsp70-hom between healthy individuals and patients with COPD.
Hsp32 gene (HMOX-1) encodes for small heat shock protein 32 (Hsp32 or heme oxygenase-1), enzyme that catalyzes the degradation of heme to biliverdin in a reaction which provides cells with protection in oxidative mediated injury. Polymorphism of this gene is presented in the number GT repeats in 5'-flanking region responsible for gene expression and plausible enzyme induction in oxidative stress conditions. The obtained data showed that there was no statistically significant difference in hsp32 gene polymorphism between between healthy individuals and patients with COPD.

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