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ULOGA METFORMINA U TERAPIJI NESITNOĆELIJSKOG KARCINOMA PLUĆA

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SAŽETAK

Nesitnoćelijski karcinom pluća (NSCLC) sačinjava 80-85% svih dijagnostikovanih formi karcinoma pluća. Konvencionalni terapijski modaliteti pokazuju malu uspešnost u lečenju uznapredovalih formi bolesti, što dovodi do razvoja novih lekova koji bi zajedno sa klasičnim hemioteraputicima pobošljali odgovor na postojeću terapiju. Jedan od takvih terapeutika je i antidijabetik metformin koji je pokazao obećavajuće rezultate tokom pretkliničkih i retrospektivnih studija. U ovom radu analizirane su prospektivne kliničke studije koje su ispitivale efekat lečenja karcinoma pluća pomoću metformina i konvencionalnih terapijskih pristupa, kao i pretkliničke studije koje opisuju mogući mehanizam dejstva metformina objavljene u *PubMed* bazi podataka u prethodnih 10 godina. U pojedinim prospektivnim kliničkim studijama uočene su naznake da terapija metforminom dovodi do poboljšanja opšte stope preživljavanja i produžetka perioda bez progresije bolesti. Međutim, ovakve studije su malobrojne i karakteriše ih nedovoljan broj ispitanika kao i smanjena komplijantnost prema terapiji metforminom. Pretkliničke studije ukazuju na citotoksični efekat metformina prema ćelijama NSCLC, aktivaciju apoptoze, kao i sinergizam sa radioterapijom, hemioterapeuticima i biološkom terapijom koja se primenjuje, ali su pokazani mehanizmi dejstva upitni uzimajući u obzir visoke koncentracije metformina koje se primenjuju *in vitro*. Na osnovu dostupnih podataka nije moguće sa sigurnošću proceniti da li metformin dovodi do poboljšanja efekta lečenja u poređenju sa konvencionalnim terapijskim pristupima, niti doneti jasan zaključak o ćelijskim mehanizmima kojima bi se ovakav efekat ostvario. Stoga je neophodno da buduća pretklinička istraživanja budu bolje dizajnirana u smislu mogućnosti translacije rezultata na *in vivo* okolnosti, a kliničke studije bolje kontrolisane kao i da obuhvate veći broj precizno odabranih ispitanika.

Ključne reči: karcinom pluća, metformin, klinička studija, molekularni mehanizam

Uvod

Karcinom pluća predstavlja vodeći uzrok mortaliteta u svetu, sačinjavajući 18% ukupnih smrtinih ishoda nastalih kao posledica maligniteta. Prema podacima Svetske zdravstvene organizacije (engl. *World Health Organization* - WHO) iz 2020. godine, sa 1,2 miliona slučajeva karcinom pluća je i najčešće dijagnostikovani tumor muškaraca preko 55 godina starosti (1). Najčešći faktor rizika i dalje predstavlja pušenje duvana, ali se kao etiološki faktori sa sve većom incidencijom pominju i izloženost industrijskim kancerogenima poput azbesta, arsena kao i policikličnih aromatičnih ugljovodonika (2). Ovakvim činjenicama u prilog ide i podatak da u proseku 20% osoba preminulih od karcinoma pluća predstavljaju nepušači (1).

Patohistološki, najčešći tipovi karcinoma pluća se mogu klasifikovati kao nesitnoćelijski (engl. *Non-small Cell Lung Cancer* – NSCLC) koji sačinjava 80-85% i ređe dijagnostikovani, sitnoćelijski karcinom pluća (engl. *Small Cell Lung Cancer* – SCLC) (3). Terapija izbora u ranim stadijumima bolesti (I-IIIa) je hirurška sa adjuvantnom hemioterapi-

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THE ROLE OF METFORMIN IN THE TREATMENT OF NON-SMALL CELL LUNG CANCER

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SUMMARY

Out of the all newly diagnosed lung cancers, non-small cell lung carcinoma (NSCLC) comprises 80-85%. When treating advanced stages of the disease, conventional therapy shows poor results, which implies that there is a need for new drugs that will improve the response to current therapy. Metformin, drug used to threat Diabetes mellitus showed promising results in preclinical and retrospective clinical studies. We have analyzed prospective clinical trials investigating the combined effect of conventional therapy and metformin in treating lung cancer, as well as preclinical studies investigating its possible mechanisms of action published in PubMed database in the last 10 years. Several studies indicated that combination therapy with metformin led to the improvement in categories like overal survival (OS) and progression-free survival (PFS). However, the number of studies is limited and is characterized by a low number of subjects, as well as by a reduced compliance in subjects using metformin. Preclinical studies suggest cytotoxic effects of metformin, activation of apoptosis, as well as synergistic effect with chemotherapeutics, radiotherapy and biological agents used. The relevance of determined results is questionable, taking into account high metformin concentrations used in vitro. Based on the clinical studies published in the last ten years, there is insufficient data to conclude whether metformin improves prognostic factors in comparison to the conventional therapy. It is also not clear which mechanisms are responsible for possible beneficial effects of metformin. Future preclinical studies thus have to be better designed in order to increase their translational potential, while clinical studies have to be better controlled with improved selection and higher number of subjects enrolled.

Keywords: lung cancer, metformin, clinical study, molecular mechanism

Introduction

Lung cancer is the leading cause of death worldwide, making up 18% of all deaths caused by malignancies. According to the data of the World Health Organization (WHO) for 2020, lung cancer was the most frequently diagnosed cancer in men older than 55 years, with 1.2 million cases occuring annualy (1). Tobacco use is still the most common risk factor, while etiological factors identified as risk factors include exposure to the industrial carcinogens such as asbestos, arsenic, as well as polycyclic aromatic hydrocarbons (2). This is supported by the fact that, on average, 20% of people who die of lung cancer are non-smokers (1).

Pathohistologically, the most common lung cancers are classified as NSCLC (Non-small Cell Lung Cancer), comprising 80-85% of all cases, while SCLC (Small Cell Lung Cancer) is more rarely diagnosed (3). The treatment of choice for early stages of NSCLC (I-IIIa) is surgical treatment with adjuvant chemotherapy, aimed at eliminating potential micrometastases. In advanced disease (stages IIIb-IV), standard treatment includes concomitant chemoradiotherapy, which is most frequently based on the application of platinum-based doublets in combination with radiotherapy (4,5). The specificities of treatment modalities

jom u cilju eliminacije potencijalnih mikrometastaza. Kod uznapredovale bolesti (stadijumi IIIb-IV) standardnu terapiju sačinjava konkomitantna hemioradioterapija, koja se najčešće zasniva na primeni platinskih dubleta u kombinaciji sa zračnom terapijom (4,5). Pojedinosti modaliteta lečenja zavise ne samo od stadijuma proširenosti, već i od histološke forme tumora, kao i od opšteg stanja samog pacijenta. U okolnostima kada ne postoje kontraindikacije, prednost se daje cisplatinu koji se najčešće kombinuje sa inhibitorima topoizomeraze II (etopozid), vinka alkaloidima (vinorelbin), antifolatima (pemetreksed) i nukleozidnim analozima (gemcitabin). Primena karboplatina je najčešća u kombinaciji sa lekovima koji inhibiraju polimeraziciju mikrotubula - paklitakselom u solubilnoj formi ili u obliku tzv. nab-paklitaksela koji je vezan za albumine. Radioterapija se primenjuje u ukupnoj dozi 60-65 Gy frakcionisano u 30-35 dnevnih zračenja, a prema preporukama u periodu ne dužem od 6-7 meseci (6). Pacijenti koji se nalaze u stadijumu metastatske bolesti (IV stadijum) se testiraju na prisustvo molekularnih markera na tumorskim ćelijama, u cilju potencijalne primene ciljane terapije (engl. *targeted therapy*) ili imunoterapije (5,6). Od najveće važnosti je inicijalna procena ekspresije PD-L1 proteina na tumorskim ćelijama, gde u slučaju pozitivnog nalaza u više od 50% analiziranog tkiva terapiju izbora kod ovih pacijenata predstavlja imunoterapija. U te svrhe primenjuje se anti-PD-1 agens pembrolizumab koji predstavlja humanizovano antitelo koje vezuje PD-1 protein eksprimiran na brojnim ćelijama imunskog sistema, blokirajući na taj način inhibitorno dejstvo PD-L1 tumorskih ćelija. Na ovaj način se povećava specifičan antitumorski imunski odgovor (7). Radi moguće primene ciljane terapije pacijenti se analiziraju na prisustvo tzv. onkogenih „*driver*" mutacija i u većini evropskih zemalja obavezno je testiranje gena EGFR, ALK, ROS1. U slučaju potvrde neke od mutacija u genu za receptor za epidermalni faktor rasta, EGFR, terapiju izbora predstavlja specifični EGFR inhibitor tirozin kinaze (TKI) poput erlotiniba, gefitiniba, ili afatiniba (8). Kod pacijenata sa prisustvom rearanžmana, tj. fuzije gena ALK koji kodira receptorsku tirozin kinazu primenjuje se neki od ALK inhibitora (krizotinib, ceritinib, alektinib) (9), dok je inhibitor izbora kod pacijenata sa rearanžmanom ROS1 gena (10), takođe inhibitor receptorske tirozin kinaze, krizotinib. Pored ovih mutacija, sve češće se ispituje i prisustvo mutacija u BRAF genu zbog mogućnosti primene specifičnih TKI, dabrafeniba ili trametiniba, lekova koji deluju sinergistički inhibirajući BRAF i MEK protein kinaze u protoonkogenom signalnom putu. Ciljana i imunoterapija je često praćena prethodno pomenutom platinskom dublet terapijom, dok se radioterapija kod ovih pacijenata primenjuje kao palijativna, tj. u cilju kontrole simptoma poput opstrukcije disajnih puteva, koštanih metastaza i slično (6).

Brojni mehanizmi dovode do stvaranja rezistencije na primenjenu hemio-radioterapiju što za posledicu ima veći stepen progresije bolesti i nastanak recidiva, čime se objašnjava i relativno loš uspeh terapije i pored primene različitih modaliteta, kao i relativno loša prognoza bolesti. Tako je petogodišnje preživljavanje pacijenata sa uznapredovalom formom karcinoma pluća svega 6-8 % (11). Upravo iz tog razloga sve se češće u lečenju maligniteta, uključujući NSCLC, ispituju lekovi čija primarna indikacija nije maligna bolest, ali koji bi potencijalno mogli da pokažu sinergistički efekat sa primenjenom hemioradioterapijom ili da povećaju osetljivost tumorskih ćelija na istu. U ovu grupu lekova se ubraja i antidijabetik metformin. Metformin predstavlja lek iz grupe bigvanida koji se primenjuje kao terapeutik izbora u lečenju *Diabetes mellitus*-a tipa II (DM tip II). Euglikemijski efekat ovog leka posledica je smanjenja apsorpcije glukoze na nivou intestinalnog trakta, inhibicije glukoneogeneze kao i povećanja osetljivosti insulinskog receptora (3). Prethodno sprovedene retrospektivne opservacione studije pokazale su da kod pacijenata koji boluju od NSCLC na kombinovanoj terapiji metforminom i hemioterapeuticima dolazi do značajnog poboljšanja efekta lečenja posmatrano kroz ukupno preživljavanje (engl. *overall survival* – OS), preživljavanje bez progresije bolesti (engl. *progression-free survival* – PFS) i objektivnu stopu odgovora na terapiju (engl. *objective response rate* – ORR) (12-17). Takođe, retrospektivno je pokazano i da metformin dovodi do poboljšanja 2-godišnjeg i 5-godišnjeg preživljavanja pacijenata nakon radioterapije NSCLC (18). Kako su pacijenti u ovim studijama bolovali od dijabetesa i uzimajući u obzir da su retrospektivne studije sklone pristrasnostima (19), u toku je veliki broj prospektivnih randomizovanih kontrolisanih kliničkih studija koje ispituju potencijalne benefite metformina kod pacijenata sa uznapredovalim NSCLC. Molekularni mehanizmi dejstva metformina kojima bi se mogao objasniti

depend not only on the stage of the disease development, but also on the histological form of the tumor, as well as on the patient's performance status. When there are no contraindications, an advantage is given to cisplatin, which is most frequently combined with topoisomerase II inhibitors (etoposide), vinca alkaloids (vinorelbine), antifolates (pemetrexed) and nucleoside analogs (gemcitabine). The application of carboplatine is most frequent in combination with drugs that inhibit the polymerization of microtubules – paclitaxel in a soluble form or in the form of nab-paclitaxel which is albumin-bound. Radiotherapy is applied in a total dose of 60-65 Gy divided into 30-35 daily fractions, and, according to the recommendations, no longer than 6-7 months (6). If the metastasis are present (stage IV), patients are tested for the presence of molecular markers on tumor cells, in order to potentially administer targeted therapy or immunotherapy (5,6). The initial estimation of expression of PD-L1 protein on tumor cells is of the greatest significance. If more than 50% of analyzed tissue sample is positive for PD-L1, the treatment of choice is immunotherapy – anti-PD-1 agent pembrolizumab. This humanized antibody binds lymphocytic PD-1 protein and by doing so blocks the inhibiting effect of tumor cells, PD-L1.

Thus, specific antitumor immune response is increased (7). In order to possibly administer targeted therapy, patients are analyzed for the presence of the so called oncogenic "driver" mutations and in most European countries EGFR, ALK, ROS1 testing is mandatory. If any of the mutations in the gene for the epidermal growth factor receptor, EGFR, are confirmed, the treatment of choice is specific inhibitor of EGFR tyrosine kinase (TKI) such as erlotinib, gefitinib, or afatinib (8). In patients with rearrangement (fusion) of ALK gene that encodes a receptor tyrosine kinase, ALK inhibitors are administered (crizotinib, ceritinib, alectinib) (9), while the inhibitor of choice in patients with the rearrangement of ROS1 gene (10) is also the inhibitor of a receptor tyrosine kinase, crizotinib. Besides these mutations, the presence of mutations in BRAF gene can also be examined due to the possibility of application of specific TKI, dabrafenib or trametinib. The targeted therapy and immunotherapy are often followed by the previously mentioned platinum-based doublets, while radiotherapy in these patients is applied as palliative, to control symptoms such as the airflow obstruction, bone metastasis etc. (6).

Numerous mechanisms lead to resistance to applied chemoradiotherapy, resulting in greater disease progression and the occurrence of relapse, and can be the explanation for relatively poor success of treatment and relatively poor prognosis although different modalities are used. Thus, five-year survival of patients with advanced stages of lung cancer is only 6-8% (11). Therefore, drugs whose primary indications do not include malignant diseases, but which could show synergism with chemoradiotherapy or increase the sensitivity of tumor cells to therapy, are examined more and more in the treatment of malignancies. The antidiabetic metformin belongs to this group of drugs. Metformin is a drug from the group of biguanides that is used as the treatment of choice in *Diabetes mellitus* type II (DM type II). The euglycemic effect of this drug is the consequence of decrease of glucose absorption in the intestinal tract, inhibition of gluconeogenesis, as well as the increase of insulin sensitivity (3). Previously conducted retrospective observational studies have shown that patients with NSCLC who use metformin in combination with chemotherapy, have significant improvement in terms of overall survival (OS), progression-free survival (PFS) and objective response rate (ORR) (12-17). Also, it has been shown retrospectively that metformin leads to the improvement of 2-year and 5-year survival of patients with NSCLC after radiotherapy (18). Since patients in these studies had diabetes and considering the fact that retrospective studies are prone to bias (19), many prospective randomized controlled studies that examine the potential benefits of metformin in patients with advanced NSCLC are currently underway. Molecular mechanisms of metformin effects, which could explain the favorable effect on NSCLC, have not been completely explained. Therefore, the aim of this study is the analysis of the results of prospective clinical studies that investigated the role of metformin in the treatment of NSCLC during the last 10 years, as well as the analysis of available literature that explains the possible mechanisms of antitumor effects of metformin *in vitro*.

povoljni efekat na NSCLC još uvek nisu u potpunosti razjašnjeni. Stoga je cilj ovog rada analiza rezultata prospektivnih kliničkih studija koje su se bavile ulogom metformina u terapiji NSCLC u proteklih 10 godina, kao i analiza dostupne literature koja objašnjava moguće mehanizme antitumorskog efekta metformina *in vitro*.

Metode

U cilju sumiranja aktuelnih podataka o ulozi metformina u terapiji pacijenata sa NSCLC u zavisnosti od primenjenih terapijskih modaliteta korišćena je literatura dobijena pretragom *PubMed* baze podataka poštujući pravila MeSH (engl. *Medical Subject Headings*) indeksiranja. Obuhvaćena su sva klinička ispitivanja koja su se bavila datom temom objavljena u periodu od januara 2012. do jula 2022. Literatura na engleskom jeziku selektovana je pretragom sledećih ključnih reči: metformin, karcinom pluća, kliničko ispitivanje. Ista baza podataka je korišćena i za prikaz rezultata koji se odnose na molekularne mehanizme antitumorskog dejstva metformina.

Metformin i hemioterapija NSCLC

U posmatranom periodu objavljeni su rezultati pet kliničkih studija nakon druge faze ispitivanja, a koje su uključivale pacijente u III i/ili IV stadijumu NSCLC koji nisu imali prethodno dijagnostikovan DM tip II kao i jedne studije koja je obuhvatala ovu grupu pacijenata (Tabela 1). Pet od šest spomenutih studija predstavlja rezultate kontrolisanih, randomizovanih, otvorenih kliničkih studija nakon IIa (20) i IIb (21-24) faze ispitivanja. Za razliku od njih studija *Parikh*-a i saradnika iz 2017. godine (25) za potrebe svog rada koristila je istorijsku kontrolu, što zbog poređenja različitih populacija ispitanika može uticati na ispravnost tumačenja rezultata.

Studije *Sayed*-a (20) i *Parikh*-a (25) analizirale su adjuvantnu ulogu metformina u citostatskoj terapiji (cisplatin/gemcitabin i karboplatin/pemetreksed) u uznapredovalim stadijuma NSCLC. Zajedničko za oba rada je da metformin ne dovodi do pogoršanja neželjenih efekata hemioterapije. Praćenjem parametara poput OS, PFS i ORR uočen je pozitivan trend rasta u grupi koja je u terapiji imala metformin. Izostanak statističke značajnosti u razlikama ovih prognostičkih parametara može se pripisati malom broju ispitanika koji su bili obuhvaćeni ovim istraživanjima (30, odnosno 14 pacijenata).

Dva velika istraživanja objavljena u julu 2021. godine bavila su se efektom metformina kod pacijenata sa lokalno uznapredovalim NSCLC, tokom i

Tabela 1. Rezultati prospektivnih kliničkih studija u kojima je ispitivan efekat metformina na prognozu pacijenata sa NSCLC na standardnoj hemioterapiji.

	Klinička studija	Dizajn studije	Broj ispitanika	Stadijum *NSCLC*	Ispitivana terapija	Rezultati
1	Sayed et al, 2015	randomizovana, otvorena, kontrolisana IIa faza kliničke studije	30	IV	gemcitabin, cisplatin	pozitivan trend rasta OS, PFS, ORR ali bez statistički značajne razlike
2	Parikh et al, 2017	otvorena, faza IIb kliničke studije sa istorijskom kontrolom	14	IIIb/IV	pemetreksed, karboplatin	
3	Marrone et al, 2018	otvorena, IIb faza kliničke studije sa istorijskom kontrolom	19	IIIb/IV	paklitaksel, karboplatin, bevacizumab	povećanje PFS i MOS u poređenju sa podacima iz literature
4	Skinner et al, 2021	randomizovana, otvorena, kontrolisana IIa faza kliničke studije	167	IIIa/IIIb	paklitaksel, karboplatin + radioterapija	pozitivan trend rasta OS i PFS ali bez statistički značajne razlike
5	OCOG-ALMERA (Tsakiridis et al, 2021)	randomizovana, multicentrična II faza kliničke studije	54	IIIa/IIIb	cisplatin + radioterapija	smanjenje OS i PFS kao i pogoršanje neželjenih efekata terapije
6	Lee et al, 2020	randomizovana, otvorena, kontrolisana IIb faza kliničke studije	164	IIIb/IV	gemcitabin, karboplatin	povećanje OS i PFS kod pacijenata sa većim

OS - ukupno preživljavanje; PFS - preživljavanje bez progresije bolesti; ORR - stopa odgovora na terapiju; MOS - medijana ukupnog preživljavanja; FDG – fluorodeoksiglukoza

Methods

In order to summarize current data about the role of metformin in the treatment of patients with NSCLC depending on the applied treatment modalities, literature was obtained through a search of PubMed database respecting the rules of MeSH (Medical Subject Headings) indexing. All clinical studies that were published between January 2012 and July 2022 were included in the analysis. The literature in the English language was selected by searching the following key words: metformin, lung cancer, clinical trial. The same database was used to present results related to molecular mechanisms of metformin's antitumor effects.

Metformin and chemotherapy in NSCLC

In the observed time period, the results of five clinical studies after phase II were published, and they included patients with NSCLC in the stage III and/or IV who did not have previously diagnosed DM type II, as well as the results of one study which included this group of patients (Table 1). Five out of six above mentioned studies present the results of controlled, randomized, open-label clinical trials after phase IIa (20) and IIb (21-24). On the other hand, the study of Parikh and associates from 2017 (25), used historical control, which may influence the accuracy of interpretation of the results due to the comparison of different populations of participants.

Studies of Sayed (20) and Parikh (25) analyzed the adjuvant role of metformin in cytostatic therapy (cisplatin/gemcitabine and carboplatine/pemetrexed) in advanced stages of NSCLC. In both studies, metformin did not cause worsening of side effects of chemotherapy. By observing the parameters such as OS, PFS and ORR, a positive trend of increase was noticed in the group that used metformin in the treatment. The absence of statistical significance regarding the difference between these prognostic factors may be attributed to the small number of participants that were included in these studies (30 and 14 patients, respectively).

Two large studies published in July 2021 investigated the effect of metformin in patients with locally advanced NSCLC, during and after concomitant chemoradiotherapy (CRT). OCOG-ALMERA study (23) is the first prospective clinical study that found significant worsening of side effects of chemoradiotherapy (cisplatin/etoposide), as well as distinctly worse results of treatment effects in the group of patients who used metformin (2000 mg/day). There are numerous limitations of this study, also stated by authors themselves, in particular the fact that only

Table 1. Results of the prospective clinical studies that investigated the effect of metformin on survival of NSCLC patients treated with standard chemotherapy

	Clinical study	Study design	Sample size	NSCLC stage	Lung cancer treatment	Results
1	Sayed et al, 2015	randomized, open-label, controlled phase IIa	30	IV	gemcitabin, cisplatin	increase in OS, PFS, ORR but statistically non-significant
2	Parikh et al, 2017	open-label, phase IIb with historical control	14	IIIb/IV	pemetrexed, carboplatin	
3	Marrone et al, 2018	open-label, phase IIb with historical control	19	IIIb/IV	paclitaxel, carboplatin, bevacizumab	increase in PFS and MOS in comparition with historical control
4	Skinner et al, 2021	randomized, open-label, controlled phase IIa	167	IIIa/IIIb	paklitaxel, carboplatin + radiotherapy	increase in OS and PFS but statistically non-significant
5	OCOG-ALMERA (Tsakiridis et al, 2021)	randomized, multicentric phase II	54	IIIa/IIIb	cisplatin + radiotherapy	decrease in OS, PFS and increased toxic events
6	Lee et al, 2020	randomized, open-label, controlled phase IIb	164	IIIb/IV	gemcitabin, carboplatin	increase in OS and PFS in patients with higher FDG uptake

OS - overall survival; PFS - progression- free survival; ORR - overall response rate; MOS - median overall survival; FDG - fluoro-deoxy-glucose

nakon konkomitantne hemioradioterapije (HRT). OCOG-ALMERA studija (23) predstavlja prvu prospektivnu kliničku studiju koja je uočila značajna pogoršanja neželjenih dejstava HRT (cisplatina/etopozid) kao i upadljivo lošije vrednosti efekata lečenja u grupi pacijenata koji su bili na terapiji metforminom (2000 mg/dnevno). Brojna su ograničenja ove studije koja navode i sami istraživači, od kojih se posebno ističe da je svega 56% pacijenata u ispitivanoj grupi primilo HRT po protokolu lečenja (u poređenju sa 77,8% u kontrolnoj grupi). Takođe, kod 20% pacijenata ispitivane grupe u potpunosti je prekinuta zračna terapija zbog pojave teških neželjenih efekata, dok su svi pacijenti kontrolne grupe primili predviđenu dozu zračenja (60-65 Gy). Drugo značajno ograničenje ispitivanja je činjenica da se radi o otvorenoj studiji u kojoj je ispitivana grupa bila na terapiji metforminom, dok kontrolna grupa nije uzimala placebo, što može uticati na pojavu neželjenih reakcija na lek kod ispitivane grupe.

Da su rezultati OCOG-ALMERA studije (23) najverovatnije posledica malog broja ispitanika i dodatno smanjene komplijantnosti prema terapiji, potvrđuje studija *Skinner*-a i saradnika iz 2021. godine (24) koja je obuhvatila 167 pacijenta na konkomitantnoj HRT (cisplatin/paklitaksel; 60-65Gy). Rezultati ove do sada najbronije prospektivne studije, pokazali su da prilikom adjuvantne primene metformina ne dolazi do povećanja učestalosti neželjenih efekata HRT, ali takođe da nema ni poboljšanja u parametrima preživljavanja i progresije bolesti. Potencijalno ograničenje ove studije je podatak da je svega 39% pacijenata ispitivane grupe uzimalo predviđenu dozu metformina (2000 mg/dnevno), dok je kod ostatka ove grupe tolerancija na lek i komplijantnost prema terapiji bila smanjena.

U radu *Lee* i saradnika iz 2020. godine (22) uočeno je da pacijenti sa skvamocelularnim tipom NSCLC čije tumorske promene preuzimaju više fluorodeoksiglukoze (FDG) prilikom PET snimanja, imaju više vrednosti OS i PFS nakon primene kombinovane terapije metforminom i hemioterapeuticima (karboplatin/gemcitabin). S obzirom da su ovim istraživanjem bili obuhvaćeni pacijenti sa i bez dijagnostikovanog DM, zaključeno je i da nema razlika u vrednostima prognostičkih parametara između ove dve populacije.

Istraživanje *Marrone*-a iz 2018. (21) koje je pored primene hemioterapeutika obuhvatalo i imunoterapiju anti-VEGF antitelom-bevacizumabom prekinuto je zbog izmene preporučenih terapijskih protokola. Rezultati ove studije pokazali su povećanje PFS kod pacijenata na terapiji metforminom, ali u poređenju sa podacima iz literature što samo po sebi ima upitan značaj.

Metformin i radioterapija *NSCLC*

Klinička studija *Chun*-a i saradnika (26) bavila se efektom metformina tokom hipofrakcionisane stereotaktičke radioterapije pacijenata u I i II stadijumu *NSCLC*. Efekti lečenja opisivani kroz PERCIST kriterijume u zavisnosti od stepena preuzimanja FDG tokom PET snimanja. Kod 70% pacijenata koji su bili na terapiji metforminom (2000 mg/dan) uočen je kompletan metabolički odgovor 6 meseci nakon radioterapije. Nažalost, placebo grupu je činio samo jedan pacijent tako da nije bilo moguće izvršiti adekvatnu komparativnu analizu.

Metformin i biološka terapija *NSCLC*

Istraživanje Arriete i saradnika iz 2019. godine (27) obuhvatalo je 139 ispitanika u odmaklim stadijumima adenokarcinoma pluća koji su bili pozitivni na EGFR mutaciju. U ovom radu pokazano je da kombinovana terapija metforminom i EGFR TKI (erlotinib, afatinib, gefitinib) dovodi do produžavanja PFS i OS. Međutim, u radu *Li* i saradnika objavljenom iste godine (28), uočeno je da nema razlike u prognostičkim faktorima kod pacijenata na kombinovanoj terapiji metforminom i gefitinibom. Diskrepance u ovim rezultatima potencijalno proističu iz razlike u dizajnu navedenih studija. Naime, studija *Li* i saradnika (28) predstavlja dvostruko-slepu randomizovanu kliničku studiju sa upotrebom placeba i dvostruko većim brojem ispitanika, što neminovno daje prednost pri tumačenju rezultata u odnosu na studiju koja je bila otvorenog tipa i nije bila kontrolisana placebom.

Kliničko ispitivanje koje se bavi efektima terapijske primene anti-PD1 antitela (nivolumaba) i metformina kod neoperabilnih NSCLC trenutno je u toku (registarski broj NCT03048500) (29).

Metformin i mogući mehanizmi citotoksičnog dejstva pokazani *in vitro*

Veliki je broj originalnih naučnih članaka koji su pokušali da objasne moguće mehanizme ćelijske smrti do koje dovodi metformin u ćelijama NSCLC, kao i aktivaciju signalnih puteva koji dovode do citotoksičnog efekta. Različiti rezultati koji se sreću u literaturi su verovatno posledica razlika u diza-

56% of patients in the examined group received chemoradiotherapy according to the protocol (in comparison to 77.8% in the control group). Also, in 20% of patients from the examined group, radiotherapy was stopped because of serious side effects, while all of the patients from the control group received the planned dose of radiotherapy (60-65 Gy). The second significant limitation of this study is the fact that it is an open-label study in which the examined group used metformin in the treatment, while the control group did not take placebo, which could influence the occurrence of side effects in the examined group.

The study of Skinner and associates from 2021 (24) that included 167 patients using concomitant CRT (cisplatin/paclitaxel; 60-65 Gy) confirmed that the results of OCOG-ALMERA study (23) were most probably the consequence of the small number of participants and reduced compliance with the therapy. The results of this prospective study, which has included most participants so far, have shown that the adjuvant treatment with metformin, does not increase the frequency of side effects of CRT, but at the same time the parameters of survival and disease progression do not improve. A potential limitation of this study is the fact that only 39% of patients from the examined group used metformin in the planned dose (2000 mg/day), while the tolerance to this medication and compliance with the therapy were reduced in the rest of the participants from this group.

In the study of Lee and associates from 2020 (22), it was found that patients with squamocellular NSCLC with high fluorodeoxyglucose (FDG) tumor uptake during PET imaging, had higher values of OS and PFS after the administration of combined therapy with metformin and chemotherapeutics (carboplatine/gemcitabine). Considering that this study included patients with and without DM, it was concluded that there were no differences regarding the values of prognostic factors between these two populations.

The study of Marrone from 2018 (21), in which immunotherapy with anti-VEGF antibody – bevacizumab was used in addition to chemotherapeutics, was ended due to the change in the recommended treatment protocols. The results of this study showed the increase in PFS in patients who used metformin, but in comparison with the literature data which in itself has questionable significance.

Metformin and radiotherapy in NSCLC

The clinical study of Chun and associates (26) assessed the effect of metformin during hypofractionated stereotactic radiotherapy of patients in stages I and II of NSCLC. The effects of the treatment were described through PERCIST criteria depending on the degree of FDG uptake during PET scanning. A complete metabolic response was noticed 6 months after radiotherapy in 70% of patients who used metformin in the treatment (2000 mg/day). Unfortunately, placebo group included only one patient and therefore, it was not possible to do an adequate comparative analysis.

Metformin and biological therapy of NSCLC

The study of Arriete and associates from 2019 (27) included 139 participants with advanced stages of adenocarcinoma who were EGFR-positive. It was shown that the combined therapy with metformin and EGFR-TKI (erlotinib, afatinib, gefitinib) led to the extension of PFS and OS. However, in the study of Li and associates that was published in the same year (28), it was found that there was no difference regarding prognostic factors between patients who used the combined therapy of metformin and gefitinib. The discrepancy between these results was potentially caused by difference regarding the studies' design. Namely, the study of Li and associates (29) was a double-blind randomized placebo-controlled trial with twice as many participants, which unavoidably gives advantage during the analysis of results in comparison with the study that was open-label and was not controlled by placebo.

The clinical trial that investigates the effects of therapeutic application of anti-PD1 antibody (nivolumab) and metformin in inoperable NSCLS is currently in progress (registration number NCT03048500) (28).

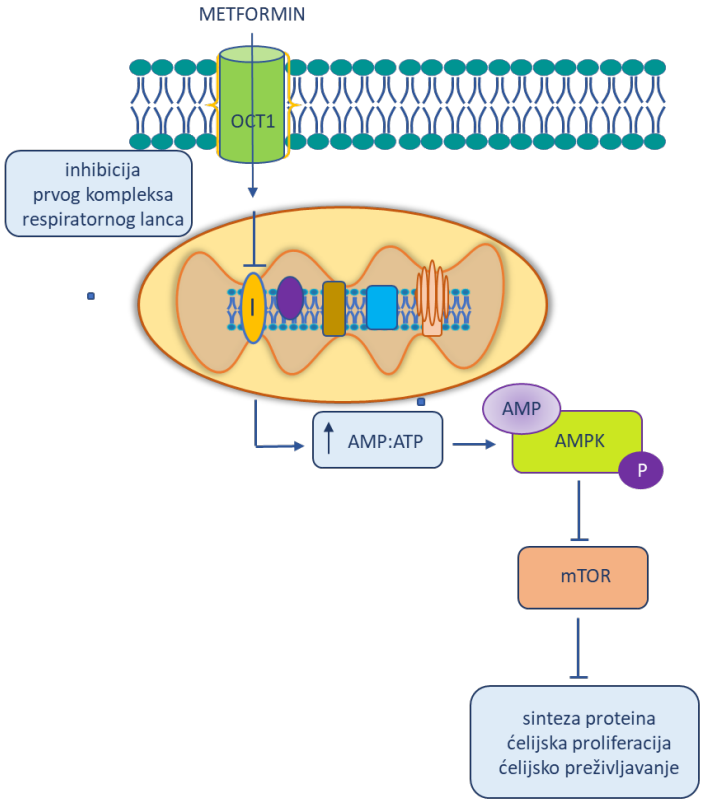
Metformin and the possible mechanisms of cytotoxic effects shown *in vitro*

Many original scientific articles have tried to explain the possible mechanisms of cell death caused by metformin in NSCLC cells, and the activation of signaling pathways which lead to the cytotoxic effect. Different results presented in literature are probably the result of differences in preclinical studies' design, primarily regarding the

jnu pretkliničkih studija, i to pre svega u pogledu odabira ćelijskih linija NSCLC (više od 10 različitih ćelijskih linija sa prisutnim ili odsutnim „*driver*" i drugim mutacijama), dužine trajanja tretmana kao i koncentracije metformina koja je korišćena.

Pokazano je u više istraživanja da sam metformin pokazuje citotoksični efekat prema ćelijama *NSCLC in vitro*, pri čemu se kao mehanizam ćelijske smrti opisuje apoptoza i to pokretanje spoljašnjeg puta praćeno aktivacijom kaspaze 8 (30). Takođe, opisuje se i pokretanje apoptoze koje je posledica smanjenja antiapoptotskog Bcl-2 i povećanja proapoptoskog Bax proteina, što aktivira mitohondrijalni, unutrašnji put pokretanja apoptoze (31). Iako ovi rezultati deluju međusobno isključujuće, aktivacija i spoljašnjeg i unutrašnjeg puta apoptoze, iako se ređe sreće, poznati je fenomen iz literature (32,33). Sa druge strane, neki od autora navode da metformin nema direktan citotoksičan efekat, već da dovodi do zaustavljanja progresije ćelijskog ciklusa u G1 fazi i time smanjuje proliferaciju ćelija karcinoma pluća (34,35).

Iako mehanizam ćelijske smrti do koje metformin dovodi nije u potpunosti jasan, veliki je broj istraživanja koja su ispitivala signalne puteve koji se pokreću kao posledica dejstva metformina, a bez ispitivanja modaliteta ćelijske smrti. Tako je pokazana aktivacija adenozin-monofosfatom aktivirane kinaze i posledična aktivacija GSK3β (36,37). Od ranije predloženi i u literaturi najčešće prikazivan molekularni mehanizam kojim metformin ostvaruje cititoksično dejstvo prema maligno izmenjenim ćelijama jeste efekat na energetski metabolizam koji podrazumeva inhibiciju I kompleksa respiratornog lanca mitohondrija. Smanjenje oskidativne fosforilacije tako dovodi do smanjene produkcije ATP-a i povećanja odnosa AMP:ATP, što dalje aktivira adenozin monofosfatom-aktiviranu kinazu (AMPK). Aktivacija nishodnog signalnog puta AMPK za posledicu ima inhibiciju PI3K/Akt/mTOR signalnog puta odgovornog za sintezu proteina, progresiju ćelijskog ciklusa i ćelijsku proliferaciju (38, 39) (Slika 1), što je i pokazano i na ćelijama NSCLC (34, 40). U suprotnosti sa ovim rezultatima je da metformin aktivira Akt i tako dovodi do citotoksičnog efekta nezavisnog od aktivacije AMPK (41). Zanimljivo je da aktivacija ćelijske smrti po tipu autofagije kao posledica dejstva metformina nije detaljnije ispitivana iako je poznato da je jedan od osnovnih mehanizama pokretanja autofagije



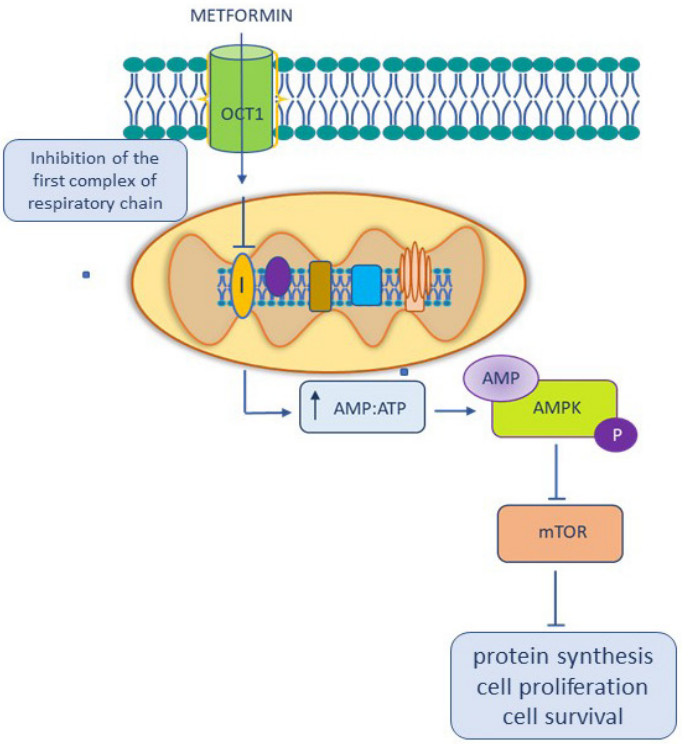
OCT1 – organski katjonski transporter 1; AMP – adenozin-monofosfat; ATP – adenozin-trifosfat; AMPK – adenozin-monofosfatom aktivirana kinaza; P – fosforilisan protein; mTOR – mehanistička meta rapamicina

Slika 1. Pretpostavljeni mehanizam citotoksičnog dejstva metformina aktivacijom AMPK

choice of cell lines of NSCLC (more than 10 different cell lines with the presence or absence of "driver" and other mutations), duration of treatment, as well as the concentration of metformin that was used.

It has been shown in several studies that metformin has a cytotoxic effect on NSCLC cells *in vitro*, while apoptosis has been described as the mechanism of cell death, namely the triggering of extrinsic pathway accompanied by the activation of caspase 8 (30). The activation of apoptosis as the consequence of decrease in antiapoptotic Bcl-2 and increase in proapoptotic Bax protein which activates the mitochondrial, intrinsic pathway of apoptosis activation (31), has also been described. Although these results seem as mutually excluding, the activation of both the extrinsic and intrinsic pathways of apoptosis is, although rare, a phenomenon well-known from the literature (32,33). On the other hand, some of the authors state that metformin does not have a direct cytotoxic effect, but rather induces arrest in the G1 phase of the cell cycle, thus reducing the proliferation of lung cancer cells (34,35).

Although the mechanism of cell death caused by metformin is not completely clear, many studies have examined signaling pathways triggered by metformin, without investigating modalities of cell death. The activation of adenosine monophosphate-activated kinase was shown, as well as the resulting activation of GSK3ß (36, 37). The molecular mechanism most frequently presented in literature which has a cytotoxic effect on malignant cells, is the effect of metformin on energy metabolism, primarily the inhibition of mitochondrial respiratory-chain complex 1. Thus, reduced oxidative phosphorylation leads to the reduced production of ATP and increase in the AMP:ATP ratio, which further activates adenosine monophosphate-activated kinase (AMPK). The activation of AMPK downstream signaling results in the inhibition of PI3K/Akt/mTOR signaling pathway which is responsible for the protein synthesis, progression of cell cycle and cell proliferation (38, 39) (Picture 1), which has been confirmed in NSCLC cells (34,40). Contrary to these results, metformin can also activate Akt, leading to the AMPK-independent cytotoxic effect (41). It is interesting



OCT1 – organic cation transporter 1; AMP – adenosine-monophosphate; ATP – adenosine-triphosphate; AMPK – adenosine monophosphate activated protein kinase; P – phosphorilated protein; mTOR – mechanistic target of rapamycin

Figure 1. Suggested cytotoxic mechanism of metformin mediated by AMPK activation

aktivacija AMPK i posledična inhibicija PI3K/Akt/mTOR signalnog puta (42). Samo u jednom radu je pokazano da metformin pokreće apoptozu i istovremeno autofagiju koja je citotoksičnog karaktera (43). Važna činjenica koju treba napomenuti u vezi sa prethodno pomenutim studijama je da je u najvećem broju njih korišćena koncentracija metformina koja značajno prevazilazi koncentracije koje se mogu postići u krvotoku osoba koje su na terapiji metforminom. Naime, koncentracije ovog leka u laboratorijskim uslovima bile su stotinu i više puta veće (5-80 mM) u poređenju sa onim koje su registrovane u plazmi pacijenata na terapiji metforminom (20-50 µM). U *in vivo* uslovima ovako visoke koncentracije leka dovele bi do letalnih ishoda kao posledice teškog oblika laktatne acidoze. U svega jednom istraživanju koje je pokazalo citotoksični efekat metformina su korišćene klinički relevantne koncentracije (100 µM), a mehanizam je objašnjen aktivacijom AMPK i inhibicijom signalnog puta Akt/mTOR (44). Druga grupa autora je pak pokazala da ovaj lek u terapijskim koncentracijama (50 µM) nema citotoksični efekat na ćelije karcinoma pluća kao i da ne utiče na promenu potencijala mitohondrijalne membrane i produkciju ROS (45, 46). Dodatno, pokazano je da metformin ni na netumorskim ćelijama u niskim koncentracijama (µM) ne pokazuje očekivane efekte - inhibiciju respiratornog lanca i aktivaciju AMPK (47), čime se možda može objasniti nekonzistentnost u rezultatima između pretkliničkih tj. *in vitro*, i kliničkih studija.

U istraživanjima koja su ispitivala potencijalni sinergistički efekat metformina sa hemioterapeuticima koji se koriste u lečenju NSCLC, pokazano je da metformin povećava osetljivost ćelija rezistentnih na cisplatin, i to inhibicijom MAPK signalnog puta (48), aktivacijom unutrašnjeg puta apoptoze i oksidativnog stresa (49), inhibicijom mTOR nezavisno od AMPK (50), ili preko p53 (51). Takođe, opisano je i da u ovim ćelijama smanjuje nivo aspartata i NAD, čime se objašnjava povećanje citotoksičnosti (52). Sa druge strane, jedna studija je pokazala da zajednički tretman cisplatinom i metforminom modifikuje apoptozu aktivacijom Akt-a i pokretanjem autofagije koja onda dovodi do odloženog i smanjenog citotoksičnog dejstva cisplatina (53). Upravo ovakav, za tumorske ćelije protektivni mehanizam kojim se štite od dejstva toksičnih jedinjenja i na taj način stiču rezistenciju, i ranije je opisivan u literaturi (54). Svim studijama je zajedničko da su korišćene koncentracije metformina u opsegu između 2 i 20 mM.

Rezultati studije koja je ispitivala sinergističko dejstvo metformina i paklitaksela su pokazali da koncentracije metformina od 100 µM i veće dovode do inhibicije p38 komponente MAPK signalnog puta te se na taj način ostvaruje potencijalno klinički značajan efekat (55). Slično, tokom in vitro istraživanja pokazano je da metformin u terapijskim koncentracijama (50 µM) dovodi do potencijacije efekta paklitaksela stimulišući proces apoptoze u tumorskim ćelijama NSCLC (56). Kombinovana terapija metforminom i pemetreksedom takođe potencira apoptozu i zaustavlja ciklus ćelija karcinoma pluća u S fazi (57).

Uticaj metformina na zračnu terapiju ćelija karcinoma pluća je ređe ispitivan, ali je pokazano da metformin u visokim koncentracijama povećava radiosenzitivnost *NSCLC* ćelija i to preko smanjene transkripcije antioksidantnih proteina i zaustavljanja deobe u G2/M fazi ćelijskog ciklusa (58), dok je u drugom istraživanju pokazano da i koncentracije niže od 100 µM povećavaju osetljivost na radijaciju putem aktivacije AMPK i inhibicije Akt/mTOR signalnog puta (44). Takođe, postoje i podaci da metformin može povećati radiosenzitivišući efekat cisplatina, zavisno od tipa korišćene ćelijske linije NSCLC (59).

Jedini rad koji je pronađen pretragom literature, a koji se odnosi na primenu anti-PD1 pembrolizumaba i metformina (500 µM) na ćelije nesitnoćelijskog karcinoma pluća je pokazao povećanje citotoksičnog dejstva kao i povećanje aktivnosti citotoksičnih T limfocita prema tumorskim ćelijama u odnosu na primenu samog pembrolizumaba (60).

U *in vitro* studijama je ispitivan veći broj specifičnih i nespecifičnih tirozin kinaznih inhibitora, tj. uticaj metformina (koncentracije 1 mM i veće) na njihov antitumorski potencijal. U slučaju EGFR mutiranih NSCLC ćelija koje su rezistentne na TKI gefitinib i osimertinib, metformin dovodi do njihove resenzitizacije povećanjem apoptoze (61), ili aktivacijom AMPK i posledičnom inhibicijom ERK signalnog puta (62). Dokazana je pojačana apoptoza uz inhibiciju preuzimanja glukoze i smanjenje glikolize kod tretmana kombinacijom afatiniba i metformina (63). U slučaju stečene rezistencije na erlotinib, metformin doprinosi resenzitizaciji povećanjem ekspresije EGFR koji je hiperfosforilisan (64) te stoga dolazi do prekida prenosa proliferativnog signala. Dodatno, za gefitinib je pokazano da može

that autophagic cell death caused by metformin has not been examined in detail, although it is known that one of the main mechanisms of autophagy induction is the activation of AMPK and the resulting inhibition of PI3K/Akt/mTOR signaling pathway (42). In only one paper it has been shown that metformin activates apoptosis and simultaneously cytotoxic autophagy (43). An important fact related to previously mentioned studies is that the concentration of metformin used significantly exceeded the concentration that could be achieved in the bloodstream of patients who are treated with metformin. Namely, the concentrations used in laboratory setting were a hundred or more times higher (5-80 mM) in comparison to those measured in the plasma of patients who used metformin (20-50 μM). In *in vivo* conditions, such high concentrations of metformin would lead to lethal outcome as the consequence of severe forms of lactic acidosis. Only in one study the cytotoxic effect of clinically relevant concentrations (100 μM) of metformin was shown, while the mechanism was explained by the activation of AMPK and inhibition of Akt/mTOR signaling pathway (44). However, another group of authors have shown that therapeutic concentrations of metformin (50 μM) do not have the cytotoxic effect on the lung cancer cells, nor do they influence the mitochondrial membrane potential and production of ROS (45, 46). In addition, it has been shown that metformin in low concentrations (50 μM) does not have the expected effects on non-tumor cells – inhibition of respiratory chain and activation of AMPK (47), which can explain the inconsistency of results between preclinical (*in vitro*), and clinical studies.

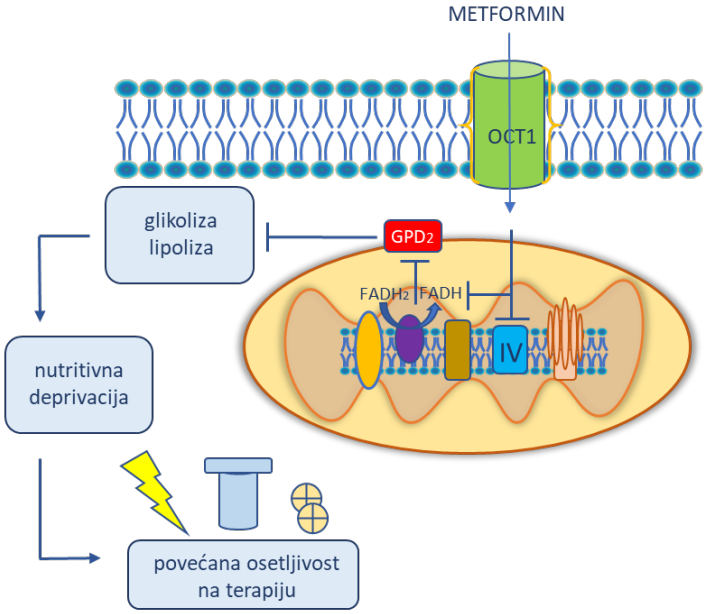
In studies that investigated the potential synergistic effect of metformin with chemotherapeutics used in the treatment of NSCLC, it has been shown that metformin increases the sensitivity of cells resistant to cisplatin, by inhibiting MAPK signaling pathway (48), activating the intrinsic pathway of apoptosis and oxidative stress (49), inhibiting mTOR independently of AMPK (50), or through p53 (51). In addition, metformin reduces the levels of aspartate and NAD in lung cancer cells, which can also be the explanation of increased cytotoxicity (52). On the other hand, one study has shown that the combined treatment with cisplatin and metformin modifies apoptosis by activating Akt and triggering autophagy, which then leads to the postponed and reduced effect of cisplatin (53). The same protective mechanism in which tumor cells protect themselves from the effect of toxic compounds and develop resistance, has been described in the literature before (54). Common for all these studies is that the concentrations of metformin used were between 2 and 20 mM.

The results of the study assessing the synergistic effect of metformin and paclitaxel have shown that concentrations of metformin of 100 μM and higher lead to the inhibition of p38 component of MAPK signaling pathway, and thus, the effect which is potentially clinically significant is achieved (55). Similarly, one *in vitro* study showed that metformin in therapeutic concentrations (50 μM) increases the potency of paclitaxel by stimulating the apoptotic process in NSCLC tumor cells (56). The combined therapy with metformin and pemetrexed also potentiates apoptosis and induces S phase cell cycle arrest (57).

The effect of metformin on radiotherapy of lung cancer cells has been rarely examined, but it has been shown that metformin in high concentrations increases radiosensitivity of NSCLC cells through the reduced transcription of antioxidant proteins and division arrest in G2/M phase of cell cycle (58), while in another study it has been shown that concentrations lower than 100 μM increase the sensitivity to radiation through AMPK activation and inhibition of Akt/mTOR signaling pathway (44). Also, there are data which show that metformin may increase the radiosensitizing effect of cisplatin, depending on the type of cell line of NSCLC (59).

The only paper which was found during the literature search which assessed the use of anti-PD1 agent pembrolizumab and metformin (500 μM) in the treatment of non-small cell lung cancer, showed the increase of cytotoxic effect, as well as the increase of activity of cytotoxic T-lymphocytes towards tumor cells in comparison to the use of pembrolizumab only (60).

In *in vitro* studies, a number of specific and non-specific tyrosine kinase inhibitors was investigated, examining the influence of metformin (concentrations 1 mM and higher) on their antitumor potential. In EGFR-mutated NSCLC cells that are resistant to TKIs gefitinib and osimertinib, metformin leads to their resensitization by increasing apoptosis (61), or activating AMPK and consequently inhibitiing ERK signaling pathway



OCT1 – organski katjonski transporter 1; GPD2 – glicerol-3-fosfat dehidrogenaza 2;
FAD – flavin-adenin dinukleotid; $FADH_2$ – dihidroflavin-adenin dinukleotid

Slika 2. Pretpostavljeni mehanizam citotoksičnog dejstva metformina inhibicijom GPD2

pokrenuti autofagiju u ćelijama karcinoma pluća koja je mogući mehanizam nastanka rezistencije, pri čemu dodatak metformina inhibicijom pokrenute autofagije povećava apoptozu (65).

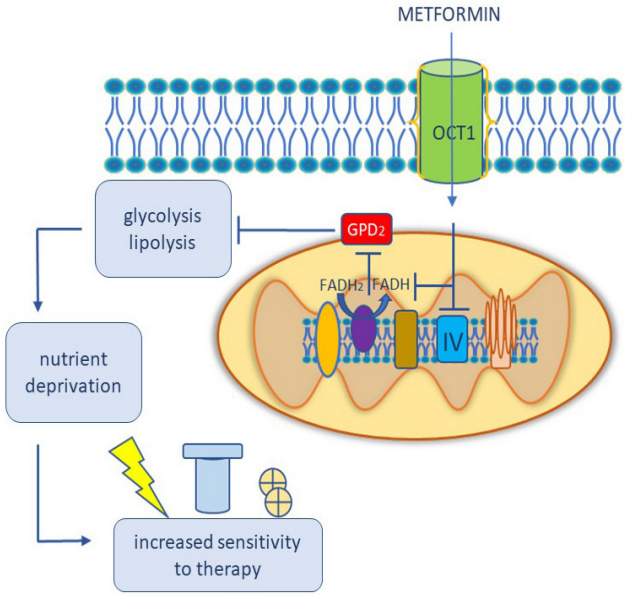
Rezultati studija koje su ispitivale dejstvo metformina na efekte krizotiniba kod ALK+ ćelija NSCLC su nekonzistentni - sa jedne strane je pokazano da metformin omogućava prevazilaženje rezistencije (66), dok druga studija ukazuje na to da ne postoji sinergistički efekat (67). Ćelije karcinoma pluća koje poseduju NRAS i BRAF mutacije su u slučaju tretmana metforminom i trametinibom pokazivale veći procenat smrtnosti i to inhibicijom Akt/mTOR od strane metformina i inhibicijom MAPK signalnog puta od strane trametiniba (40,68).

Novija istraživanja ukazuju da terapijske koncentracije metformina dovode do remećenja metaboličkog statusa tumorskih ćelija inhibicijom mitohondrijalne glicero-3-fosfat dehidrogenaze (GPD2) (69). Ovaj enzim posredstvom energetskih supstrata stimuliše procese poput glikolize i lipolize i kao takav se u većoj meri aktivira u tumorskim ćelijama zadovoljavajući povišene energetske zahteve neophodne za brzu deobu ovih ćelija (70). Samim tim inhibicija GPD2 od strane metformina potencijalno uvodi tumorsku ćeliju u nutritivnu deprivaciju, čineći je osetljivijom na efekat konvencionalnih terapijskih protokola (Slika 2). Uticaj metformina na GPD2 i eventualna povezanost sa citotoksičnim efektima na ćelijama NSCLC još uvek nije ispitivana.

Zaključak

Uprkos uvođenju novih terapijskih pristupa u lečenju uznapredovalih stadijuma NSCLC, veliki problem i dalje predstavljaju inicijalno neadekvatan odogovor kao i pojava rezistencije na terapiju što uslovljava rekurenciju i progresiju tumorskih promena. U cilju uvođenja lekova koji potencijalno mogu poboljšati odgovor na konvencionalne terapijske modalitete, antidijabetik metformin koji je tokom pretkliničkih i retrospektivnih opservacionih studija pokazao obećavajuće rezultate se ispituje u brojnim kliničkim studijama kao mogući dodatak terapiji. Ono što karakteriše većinu do sada sprovedenih prospektivnih studija je mali broj ispitanika i neadekvatna komplijantnost prema terapiji metforminom, verovatno uslovljena pojavom neželjenih efekata poput mučnine i dijareje, kao i činjenicom da se radi o grupi pacijenata koji nisu oboleli od *Diabetes mellitus*-a.

Kada su u pitanju molekularni mehanizmi kojima metformin potencijalno ostvaruje povoljne efekte na NSCLC, u literaturi se sreću različiti i povremeno suprotni rezultati. Sinergizam sa hemio- i radioterapijom je pokazan, dok su uključeni signalni putevi najčešće AMPK i Akt/mTOR. Veliki nedostatak većine pretkliničkih studija je genetička raznorodnost ćelijskih linija koje se koriste u eksperimentima, kao i nepostojanje konsenzusa oko odabira koncentracija metformina za *in vitro* istraživanja čije bi vrednosti bile primenljive i u *in vivo* studijama.



OCT1 – organic cation transporter 1; GPD2 – glycerol-3-dehydrogenase 2;
FAD – flavine-adenine dinucleotide; $FADH_2$ – dihydroflavine-adenine dinucleotide

Figure 2. Suggested cytotoxic mechanism of metformin mediated by GPD2 inhibition

(62). Increased apoptosis was shown with the inhibition of glucose uptake and reduction of glicolysis in the combined treatment with afatinib and metformin (63). In case of acquired resistance to erlotinib, metformin contributes to the resensitization by increasing the expression of EGFR that is hyperphosphorylated (64), and therefore, to the disruption of transduction of proliferative signal. In addition, it has been shown that gefitinib triggers autophagy in lung cancer cells, this possibly being the resistance mechanism, while the addition of metformin increases apoptosis by inhibiting triggered autophagy (65).

The results of studies which investigated the influence of metformin on the effects of crizotinib in ALK+ NSCLC cells are not consistent – on the one hand it has been shown that metformin makes it possible to overcome resistance (66), whereas another study points to the fact that there is no synergistic effect (67). Lung cancer cells with NRAS and BRAF mutations that were treated with metformin and trametinib showed decreased viability explained by Akt/mTOR inhibition by metformin and MAPK signaling pathway inhibition by trametinib (40, 68).

Latest studies have shown that therapeutic concentrations of metformin lead to the disruption of metabolic status of tumor cells via the inhibition of mitochondrial glycerol-3-phosphate dehydrogenase (GDP2) (69). This enzyme stimulates the processes such as glycolysis and lipolysis, and is significantly activated in tumor cells, satisfying the increased energy demands necessary for the fast division of these cells (70). Therefore, the inhibition of GPD2 caused by metformin potentially causes nutrient deprivation of tumor cells, making them more sensitive to the effect of conventional therapeutic protocols (Picture 2). The influence of metformin on GPD2 and potential link with the cytotoxic effects on NSCLC cells have not been examined yet.

Conclusion

Although new therapeutic approaches have been introduced in the treatment of advanced stages of NSCLC, the initially inadequate response to therapy, as well as the occurrence of resistance still presents a great problem, which causes the recurrence and disease progression. In order to use drugs that may potentially improve the response to conventional treatment modalities, the antidiabetic metformin, which has showed promising results in preclinical and retrospective observational studies, is being examined in numerous clinical studies as the possible addition to therapy. Prospective clinical studies, which have been conducted so far, are characterized by a small number of participants and inadequate compliance with metformin therapy, which is probably due to side effects such as nausea and

Na osnovu do sada objavljenih rezultata prospektivnih kliničkih i pretkliničkih studija, efekti primene metformina u nesitnoćelijskom karcinomu pluća i dalje nisu razjašnjeni te se ne može sa sigurnošću doneti zaključak da li metformin dovodi do promene efekata standardnog lečenja i koji su ćelijski mehanizmi ovih promena. Stoga je neophodno da buduće kliničke studije obuhvate veći broj ispitanika koji su jasno selektovani, kao i da iste budu osmišljene kao dvostruko slepe sa upotrebom placeba, jer bi se na taj način isključili različiti vidovi pristrasnosti. Dizajn pretkliničkih studija je potrebno unaprediti uz jasniji odabir ćelijskih linija koje se koriste, kao i odabir klinički relevantnih koncentracija metformina, a kako bi dobijeni rezultati imali veću šansu za ponovljivost u kliničkim okolnostima.

Konflikt interesa

Autori su izjavili da nema konflikta interesa.

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diarrhea, and to the fact that this group includes patients who do not have Diabetes Mellitus.

With regard to the molecular mechanisms of metformin that potentially have favorable effects on NSCLC, different and occasionally opposing results may be seen in literature. Synergistic effects with chemo- and radiotherapy have been shown, while most frequently involved signaling pathways are AMPK and Akt/mTOR. The significant limitation of most preclinical studies is genetic diversity of cell lines that are used in experiments, as well as the absence of consensus regarding the concentration of metformin used for *in vitro* studies, with levels that would be applicable in *in vivo* studies, as well.

According to the results of prospective clinical and preclinical studies that have been published so far, the effects of the use of metformin in non-small cell lung cancer have not been fully elucidated, and therefore it cannot be concluded with certainty whether metformin leads to the change of effects of standard treatment and what are the cell mechanisms of these changes. Therefore, future clinical studies should necessarily include more participants, with clear enrollment criteria, and they should be designed as double-blind with placebo controls, thus excluding different types of bias. Design of preclinical studies should be necessarily improved with the clearer selection of cell lines to be used, and the selection of clinically relevant concentrations of metformin, so that obtained results would have a greater chance to be repeated in clinical conditions.

Competing interests

Authors declare no competing interests.

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