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Koncentracija malondialdehida u NOD miševa tretiranih akarbozom

Concentration of malondialdehyde in a NOD mice treated with acarbose

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Sažetak

Cilj: Malondialdehid (MDA) je jedan od toksičnih produkata lipidne peroksidacije, a koristi se u evaluaciji oksidativnog stresa tijekom šećerne bolesti. Cilj ovog rada bio je ispitati učinak akarboze (inhibitora α-glukozidaza) na koncentraciju glukoze u serumu i MDA u homogenatu jetre NOD (engl. *non-obese diabetic*) miševa.

Materijali i metode: U NOD miševa šećerna bolest inducirana je ***i.v.*** aplikacijom aloksan-monohidrata (75 mg/kg t. mase). NOD miševi podijeljeni su u 4 skupine (n=6): Kontrolni, zdravi NOD miševi (K), Kontrolni, zdravi NOD miševi tretirani 7 dana akarbozom (K/A), dijabetični NOD miševi (D) te dijabetični NOD miševi tretirani 7 dana akarbozom (D/A).

Koncentracija glukoze u krvi izmjerena je glukoza oksidaza-peroksidaza metodom, a koncentracija MDA u homogenatu jetre određena je upotrebom metode s tiobarbiturnom kiselinom.

Rezultati: Nakon sedmodnevnog tretmana akarbozom zabilježeno je statistički značajno smanjenje koncentracije glukoze u krvi u skupini D/A u odnosu na skupinu D ($p<0.05$), a isto tako je uočen i statistički značajan pad koncentracije MDA ($p<0.05$).

Zaključak: Ovi rezultati potvrđuju pozitivan antioksidacijski učinak akarboze što se može objasniti njezinim antihiperglikemijskim djelovanjem.

Ključne riječi: oksidativni stres, šećerna bolest, lipidna peroksidacija, akarboza

Abstract

Aim. Malondialdehyde (MDA) is one of the toxic product of the lipid peroxidation, and that plays an important role in the evaluation of oxidative stress in the diabetes mellitus. The aim of this study was to study the effect of acarbose (α-glucosidase inhibitor) on serum glucose concentration and MDA in liver homogenate of NOD (non-obese diabetic) mice.

Material and methods. Diabetes was induced in mice by i.v. administration of alloxan-monohydrate (75 mg/kg b.w.). The mice were divided into 4 groups (N=6): control, healthy mice (C), control healthy mice on 7-day treatment with acarbose (C/A), NOD mice (D), and NOD mice on 7-day acarbose treatment (D/A).

The levels of blood glucose and of MDA in liver homogenate were determined by glucose oxidase-peroxidase method and a method with thiobarbituric acid, respectively.

Results. Significant reduction in blood glucose levels was observed after 7-day administration of acarbose in D/A group compared to D group ($p<0.05$), together with a significant fall in MDA concentration ($p<0.05$).

Conclusion. This results confirmed the favorable antioxidative effect of acarbose which may be explained by its antihyperglycemic action.

Keywords: oxidative stress, diabetes, lipid peroxidation, acarbose

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Uvod

Proces lipidne peroksidacije je jedan oblik oksidativne promjene polinezasićenih masnih kiselina koji rezultira nastankom citotoksičnih produkata, a jedan od njih je malondialdehid (MDA) (1). MDA je prihvaćeni biljeg lipidne

Introduction

The proces of lipid peroxidation is one of oxidative conversion of polyunsaturated fatty acid to cytotoxic products, and one of them is the malondialdehyde (MDA) (1). Possible causes of oxidative stress in diabetes are as follows:

peroksidacije te se koristi u evaluaciji oksidativnog stresa (1). Mogući uzroci oksidativnog stresa u šećernoj bolesti su slijedeći: a) prekomjerno stvaranje reaktivnih kisikovih spojeva (ROS), posebno superoksid aniona (O^{2-}) b) smanjena ekspresija/aktivacija endotelne NO sintaze c) oštećena ekspresija/aktivacija superoksid dismutaze (SOD) d) smanjena aktivnost antioksidativnih enzima: katalaze (CAT) i glutation peroksidaze (GPx) e) smanjena razina antioksidanasa: glutationa, α-tokoferola i askorbata. f) povećana neenzimska glikacija proteina i stvaranje tzv. produkata kasne glikacije (AGE) g) povećana autooksidacija glukoze h) hiperaktivnost poliolnog puta i stvaranje sorbitola.
Reaktivni kisikovi spojevi (ROS) mogu promijeniti funkciju endotela krvnih žila indirektno preko povećane produkcije produkata kasne glikacije (AGE) ili povećanjem oksidacije lipoproteina niske gustoće (LDL). Najvažniji od reaktivnih kisikovih spojeva je superoksid anion (O^{2-}) koji uzrokuje kontrakciju glatkih mišića krvnih žila (2). Glavne posljedice oksidativnog stresa su: narušavanje strukture biomembrana, oštećenje nukleinskih kiselina, inhibicija enzima, razgradnja bjelančevina i lipidna peroksidacija. Kemijska modifikacija aminokiselina u proteinima tijekom lipidne peroksidacije rezultira formiranjem lipooksidacijskih produkata koji služe kao markeri oksidativnog stresa *in vivo*. Malondialdehid (MDA) i 4-hidroksinonenal (HNE) su dobro definirani oksidacijski produkti polinezasićenih masnih kiselina (3). MDA reagira s proteinima krvnih žila npr. s kolagenom i dovodi do promjena u njegovoj strukturi (4). Mnoge su studije pokazale da je njegova koncentracija znatno povišena u šećernoj bolesti (5). U radu Faure i sur. je opisano da je kontrola glikemije neophodna za smanjenje lipidne peroksidacije, odnosno koncentracije MDA (6). Akarboza je prvi lijek izbora u tretmanu šećerne bolesti tipa 2, a isto tako novija istraživanja pokazuju njen povoljan učinak na prevenciju šećerne bolesti u osoba sa oštećenom tolerancijom glukoze (7). Stoga je cilj ovog rada bio evaluirati oksidativni stres i lipidnu peroksidaciju u NOD miševa kojima je šećerna bolest izazvana aloksanom, određivanjem koncentracije MDA u homogenatu jetre jer je i jetra jedan od organa u kojem je dokazana povećana lipidna peroksidacija u tom patološkom stanju, te ispitati učinak akarboze na koncentraciju tog parametra lipidne peroksidacije.

Materijali i metode

Eksperimentalne životinje

U ovom radu korišteni su miševi soja NOD (engl. *non-obese diabetic*) u dobi od 3–4 mjeseca i tjelesne mase 23–30 g, uzgojeni u Laboratoriju za molekularnu endokrinologiju i transplataciju, Instituta "Ruđer Bošković". Životinje su držane u metaboličkim kavezima na temperaturi od 22–24 °C u 12-satnom svjetlo/tama ciklusu te su hranje-

a) excessive production of reactive oxygen species (ROS), particularly superoxide anions (O^{2-}); b) reduced expression/activation of endothelial NO synthase; c) impaired expression/ activation of superoxide dismutase (SOD); d) reduced activity of antioxidative enzymes catalase (CAT) and glutathione peroxidase (GPx); e) reduced level of antioxidants: glutathione, α-tocopherol and ascorbate; f) increased nonenzymatic protein glycosylation and formation of the so-called advanced glycosylation end products (AGE); g) enhanced glucose autooxidation; h) hyperactivity of polyol pathway and sorbitol production. Reactive oxygen species (ROS) may alter the function of vascular endothelium indirectly by increased production of AGE or by increased oxidation of low density lipoproteins (LDL). The most important ROS compound is superoxide anion (O^{2-}) as it causes vascular smooth muscle contraction (2). Major consequences of oxidative stress are destruction of biomembranes, nucleic acid damage, enzyme inhibition, protein degradation and lipid peroxidation. Chemical modification of amino acids in proteins during lipid peroxidation results in the formation of lipooxidative products that serve as markers of oxidative stress *in vivo*. Malondialdehyde (MDA) and 4-hydroxynonenal (HNE) are well characterized oxidation products of polyunsaturated fatty acids (3). MDA reacts with vascular proteins, e.g. collagen, and leads to changes in their structure (4). Many studies showed considerably elevated MDA concentration in diabetes mellitus (5). Faure *et al.* claim that glycemia control is necessary to reduce lipid peroxidation, i.e. MDA levels (6). Acarbose is the first drug of choice in the treatment of diabetes mellitus Type 2 (7). Therefore, the aim of this study was to evaluate oxidative stress in alloxan-induced non-obese diabetic (NOD) mice by measuring MDA levels in their liver homogenate, and investigate the effect of acarbose on the concentration of this parameter of lipid peroxidation.

Material and methods

Experimental animals

The mice selected for this study were of NOD (non-obese diabetic) strain, age 3-4 months and 23-30 g body weight, bred at the Laboratory for Molecular Endocrinology and Transplantation, Ruđer Bošković Institute, Zagreb, Croatia. The animals were kept in metabolic cages at the temperature of 22-24 °C and 12 h light/dark cycle, and were fed standard laboratory chow (Pliva Company, Zagreb, Croatia) with water available *ad libitum*. In our experiment, diabetes was induced in mice by i.v. administration of alloxan-monohydrate (Sigma, St. Louis, USA) in a dose of 75 mg/kg b.w. seven days before the onset of acarbose treatment. The animals were divided into four groups, with 6 mice in each: Control, healthy group of non-obese mi-

ne standardnom laboratorijskom hranom (Pliva, Zagreb) i davana im je voda *ad libidum*.
U našem eksperimentu šećerna bolest u NOD miševa izazvana je *i.v.* aplikacijom aloksan-monohidrata (Sigma, St. Louis, MO) u dozi od 75 mg/kg tjelesne mase sedam dana prije početka tretmana akarbozom. Životinje su podijeljene u 4 skupine, po 6 u svakoj. Kontrolna, zdrava skupina NOD miševa (K), kontrolna, zdrava skupina NOD miševa tretirana 7 dana akarbozom u dozi od 25 mg/100 g standardne laboratorijske hrane (K/A), skupina dijabetičnih NOD miševa (D), skupina dijabetičnih NOD miševa tretirana 7 dana akarbozom u dozi od 25 mg/100 g standardne laboratorijske hrane (D/A).
Neposredno prije žrtvovanja u eter narkozi NOD miševima je izvađena venska krv u kojoj je određena koncentracija glukoze.

Priprema homogenata jetre

Nakon žrtvovanja u eter narkozi, NOD miševima je izvađena jetra te višekratno isprana u fiziološkoj otopini te držana na hladnom do obrade. Jetra je izvagana, a zatim homogenizirana pomoću Ultra-Turax homogenizatora tip TP 1812 NR 3219 (tri puta pri 13 000 rpm/30 sek.). Homogenat u koncentraciji 100 g/L načinjen je uz pomoć 0,14 M otopine KCl-a te uz stalno hlađenje u vodi s ledom. Homogenati jetre su zatim centrifugirani na 12 000 g 30 minuta pri +4°C u Eppendorf centrifugi Hettich EBA 12 R. Nakon centrifugiranja homogenati jetre su čuvani na –20°C do analize. U tako dobivenom homogenatu jetre određena je koncentracija MDA.

Određivanje koncentracije MDA

Lipidna peroksidacija određena je mjerenjem malondialdehida (MDA) u homogenatima jetre NOD miševa uz pomoć tiobarbiturne kiseline (8). Metoda se temelji na slijedećem principu: zagrijavanjem tiobarbiturne kiseline i višestruko nezasićenih masnih kiselina u kiselom mediju kao sekundarni produkt nastaje ružičasti MDA, čija se apsorbancija mjeri spektofotometrijski kod valne dužine od 532 nm. Apsolutna količina MDA očitana je iz baždarnog dijagrama koji je pripremljen iz različitih razrijeđenja matičnog standarda 1,1,3,3-tetrametoksipropana (TMP).

Određivanje koncentracije glukoze

Glukoza u krvi NOD miševa određena je metodom glukoza oksidaza-peroksidaza (GOD-PAP) (9) reagensima tvrtke Trace. Apsorbancija uzorka očitana je prema slijepoj i standardu pri valnoj dužini od 500 nm.

Statistička metoda

Srednja vrijednost (sv) i standardna devijacija (sd) izračunate su pomoću računala korištenjem programa Excel, Microsoft®. Razina značajnosti (p) izračunata je korištenjem Student-t-testa, istog programa. Statistički značajnom promjenom smatrana je promjena za koju je $p<0,05$.

ce (C), control, healthy group of non-obese mice on 7-day acarbose treatment in a dose of 25 mg/100 g chow (C/A), group of non obese diabetic (NOD) mice (D), a group of NOD mice treated for 7 days with acarbose dose of 25 mg/100 g chow (D/A).
Immediately before sacrificing during ether narcosis, venous blood was collected from mice for determination of glucose concentration.

Preparation of liver homogenate

Following sacrificing under ether narcosis, liver was extracted from mice, multiply rinsed in physiologic solution, and kept cool until analysis. The liver was weighed, and then homogenized by Ultra-Turax homogenizer, type TP 1812 NR 3219 (three times at 13,000 rpm/30 sec). 100 g/l homogenate concentration was prepared using 0.14 M of KCl solution and continuous cooling in ice water. Liver homogenate was then centrifuged at 12,000 g for 30 minutes at +4 °C in Hettich EBA 12 R Eppendorf centrifuge. After centrifugation, it was stored at -20 °C until analysis. The liver homogenate obtained in this manner was used for determination of MDA concentration.

Determination of MDA concentration

Lipid peroxidation was established by measuring malondialdehyde (MDA) in mouse liver homogenates using thiobarbituric acid (8). The method is based on the following principle: the heating of thiobarbituric acid and polyunsaturated fatty acids in acid media results in the formation of pink-colored secondary product of MDA whose absorption is measured spectrophotometrically at 532 nm wavelength. The absolute amount of MDA was read from a calibration diagram prepared from different dilutions of the primary standard of 1,1,3,3-tetrametoxipropane (TMP).

Glucose level determination

Blood glucose was measured in mice by the glucose oxidase-peroxidase (GOD-PAP) (9) method using the Trace company reagents. Absorbance of the sample was read at 500 nm wavelength against the blank and the standard.

Statistical method

Mean value and standard deviation (SD) were calculated using a computer and Microsoft® Excel application. The level of significance (p) was calculated using Student t-test and the same application. A change was considered statistically significant if $p<0.05$.

Results

Serum glucose level in mice

Table 1 shows glucose concentration in mouse sera determined on day 7 since the beginning of acarbose treatme-

Rezultati

Koncentracija glukoze u serumu NOD miševa

Tablica 1. prikazuje koncentraciju glukoze u serumu NOD miševa izmjerenu sedmog dana od početka tretmana akarbozom. Koncentracija glukoze kontrolne, zdrave skupine NOD miševa (skupina K) je 4,63 ± 0,30 mmol/L, dok je u kontrolnoj skupini s dodatkom akarboze u hrani u dozi od 25 mg/100 g standardne laboratorijske hrane (skupina K/A) izmjerena koncentracija glukoze 2,85 ± 0,42 mmol/L tj. zabilježen je njen statistički značajan hipoglikemijski učinak (p<0,001). NOD miševi sa šećernom bolesti (skupina D) imaju statistički značajno višu koncentraciju glukoze u usporedbi s kontrolnom, zdravom skupinom NOD miševa (skupina K) (p<0,01). U skupini D izmjerena koncentracija glukoze u serumu bila je 31,28 ± 4,54 mmol/L, dok je u dijabetičnih NOD miševa tretiranih akarbozom u dozi od 25 mg/100 g standardne laboratorijske hrane (skupina D/A) izmjerena koncentracija glukoze bila 16,44 ± 9,5 mmol/L što je također statistički značajno niže u usporedbi s dijabetičnom skupinom D (p<0,05).

Koncentracija MDA u homogenatu jetre NOD miševa

Koncentracija MDA mjerena je u homogenatu jetre NOD miševa i rezultati su također prikazani u tablici 1. Koncentracija MDA mjerena u jetri kontrolnih, zdravih NOD miševa (skupina K) bila je 13,02 ± 1,84 μM/g. U skupini zdravih NOD miševa koja je tijekom sedam dana bila tretirana akarbozom u dozi od 25 mg/100 g standardne laboratorijske hrane (skupina K/A) izmjerena je statistički značajna niža koncentracija MDA u jetri 9,25 ± 1,04 μM/g (p<0,01). Dijabetična skupina NOD miševa (skupina D) imala je statistički značajno višu koncentraciju MDA u usporedbi s kontrolnom, zdravom skupinom (p<0,05). Promjena koncentracije MDA mjerena u dijabetičnoj skupini NOD miševa tretiranoj akarbozom u dozi od 25 mg/100 g standardnt. Glucose concentration in the control, healthy group of mice (group C) was 4.63 ± 0.30 mmol/l. In the control group with acarbose dose of 25 mg/100 g standard laboratory chow (group C/A), glucose concentration was 2.85 ± 0.42 mmol/l, i.e. glucose produced a significant hypoglycemic effect (p<0.001). NOD mice (group D) had significantly higher glucose level compared to the control, healthy group (group C) (p<0.01). The serum glucose level in group D was 31.28 ± 4.54 mmol/l, while in acarbose treated NOD mice given the dose of 25 mg/100 g standard laboratory chow (group D/A) glucose concentration was 16.44 ± 9.5 mmol/l, which was also significantly lower compared to the diabetic group D (p<0.05).

MDA concentration in liver homogenate of NOD mice

MDA concentration determined in liver homogenates of NOD mice and results are also presented in Table 1. The MDA concentration measured in the liver of control, healthy NO mice (group C) was 13.02 ± 1.84 μM/g. A significantly lower MDA concentration, i.e. 9.25 ± 1.04 μM/g (p<0.01) was measured in the group of healthy NO mice subjected to 7-day acarbose treatment in the dose of 25 mg/100 g standard laboratory chow (group C/A). The group of non-obese diabetic (NOD) mice (group D) had a significantly higher MDA concentration compared to the control, healthy group (p<0.05). The change in MDA concentration measured in the group of NOD mice subjected to acarbose treatment in the dose of 25 mg/100 g standard laboratory chow (group D/A) was significantly lower compared to group D (p<0.05) (12.65 ± 5.73 μM/g vs. 24.57 ± 3.75 μM/g).

Discussion

Oxidative stress is characterized by increased production of free radicals and/or reduced activity of antioxidative

Tablica 1. Koncentracija glukoze u serumu (mmol/L) i koncentracija MDA u jetri NOD miševa (μM/g jetre)

Table 1. Glucose concentration in the serum (mmol/L) and MDA concentration in the liver of NOD mice (μM/g liver tissue)

Groups	Serum glucose level mmol/L mean ± SD	Liver MDA level μM/g mean ± SD
Control group of NOD mice (C) n=6	4.63 ± 0.30	13.02 ± 1.84
Control group of NOD mice + acarbose (C/A) n=6	2.85 ± 0.42[a]	9.25 ± 1.04[d]
Group of non-obese diabetic (NOD) mice (D) n=6	31.28 ± 4.54[b]	24.57 ± 3.75[e]
Group of NOD mice + acarbose (D/A) n=6	16.44 ± 9.5[c]	12.65 ± 5.73[f]

[a] p<0.001 (C/A vs C) [d] p<0.01 (C/A, vs C)
[b] p<0.01 (D vs C) [e] p<0.05 (D vs C)
[c] p<0.05 (D/A vs D) [f] p<0.05 (D/A vs D)

dne laboratorijske hrane (skupina D/A) bila je statistički značajno niža u usporedbi sa skupinom D ($p<0,05$) (12,65 $\pm$ 5,73 μM/g vs. 24,57 $\pm$ 3,75 μM/g).

Rasprava

Oksidativni stres je karakteriziran povećanom produkcijom slobodnih radikala i/ili smanjenom aktivnosti antioksidativne obrane. U eksperimentalnom modelu šećerne bolesti, stanje oksidativnog stresa je izazvano auto-oksidacijom glukoze i glikacijom proteina (10). Oksidativnom stresu danas se posvećuje velika pažnja kao procesu koji je vjerojatno odgovoran za nastanak komplikacija u šećernoj bolesti (ateroskleroza, nefropatija, neuropatija i retinopatija) (11,12). Jedna od posljedica oksidativnog stresa je i lipidna peroksidacija. Od parametara lipidne peroksidacije mjere se: koncentracija lipidnih peroksida, koncentracija konjugiranih diena te koncentracija malondialdehida (MDA) koji je visokotoksičan konačni produkt lipidne peroksidacije vjerojatno nastao njihovim neenzimskim oksidativnim raspadom. Altomare i suradnici (13) uspoređivali su koncentracije MDA u skupinama: osoba sa šećernom bolesti sa slabo kotroliranom glikemijom, dobro kontroliranom glikemijom i usporedili rezultate sa zdravim, normoglikemičnim osobama. Njihovi rezultati pokazuju znatan porast koncentracije lipidnih peroksida u plazmi pacijenata sa slabo kontroliranom glikemijom u usporedbi s druge dvije skupine ispitanika. Pokus Armstronga i sur. (14) pokazao je da već i strogo nadziran način prehrane kod osoba sa šećernom bolesti dovodi do sniženja koncentracije MDA.

Hiperglikemija u šećernoj bolesti je primarni uzrok oksidativnom stresu i lipidnoj peroksidaciji. Ona naime uzrokuje promjene u staničnom redoks statusu preko nekoliko različitih mehanizama: mijenja regulaciju protein tirozin kinaza, mijenja regulaciju protein kinaze C, dovodi do nakupljanja sorbitola te povisuje omjer NADH/NAD^+ i smanjuje omjer NADPH/$NADP^+$ preko hiperaktivnosti sorbitol (poliolnog) puta. Posljedica svega toga je neravnoteža citosolnog redoks statusa ili to stanje se naziva i pseudohipoksija, zbog povišenog omjera NADH/NAD^+ koji prikriva hipoksiju tkiva (15).

Primjena lijekova koji snizuju hiperglikemiju (posebno postprandijanu) je važna u smanjivanju komplikacija u šećernoj bolesti uzrokovanih oksidativnim stresom.

Stoga smo u ovom radu ispitali hipoglikemijski učinak akarboze i njen utjecaj na oksidativni stres, odnosno lipidnu peroksidaciju u NOD miševa. Akarboza je pseudooligosaharid dobiven kao sekundarni metabolit iz kultura Actinomycetales. Molekula akarboze se sastoji iz nezasićenog cikloheksitolskog ostatka koji je vezan za aminošećer i dva glukozna ostatka putem α-1,4 glikozidnih veza. Njen antihiperglikemijski učinak temelji se na činjenici da je ona kompetitivni inhibitor intestinalnih α-glukozidaza,

defense. In an experimental model of diabetes, oxidative stress was induced by glucose autooxidation and protein glycation (10). Considerable attention is currently dedicated to oxidative stress as a process that is probably responsible for the occurrence of complications in diabetes (atherosclerosis, nephropathy, neuropathy and retinopathy) (11,12). One of the consequences of oxidative stress is also lipid peroxidation. Determination of lipid peroxidation parameters includes measurement of the concentration of lipid peroxides, conjugated diens and malondialdehyde (MDA); the last of these three parameters is a highly toxic final product of lipid peroxidation, probably the result of non-enzymic oxidative degradation of lipids. Altomare et al. (13) compared MDA concentrations in groups of diabetic patients with poorly and those with well controlled glycemia, and compared their results with healthy, normoglycemic individuals. Their results showed substantially elevated plasma lipid peroxide levels in patients with poorly controlled glycemia in comparison to the two other subject groups. An experiment conducted by Armstrong et al. (14) showed that strictly controlled diet in diabetic patients is sufficient to decrease MDA concentrations.

Hyperglycemia in diabetes is a primary cause of oxidative stress and lipid peroxidation. Actually, it brings about changes in cellular redox status via several various mechanisms: it changes the regulation of protein tyrosine kinase and of protein kinase C, it leads to sorbitol accumulation and increases NADH/NAD^+ ratio and decreases NADPH/$NADP^+$ ratio via hyperactivity of the sorbitol (polyol) pathway. The overall ensuing effect is cytosolic redox imbalance, the condition called pseudohypoxia, due to elevated NADH/NAD^+ ratio that conceals tissue hypoxia (15). Administration of hyperglycemia reducing medications (particularly post-prandial) is important to lessen diabetic complications caused by oxidative stress. Therefore we investigated in this study the hypoglycemic effect of acarbose, as well as its effect on oxidative stress, i.e. lipid peroxidation in NOD mice. Acarbose is a pseudooligosaccharide obtained as a secondary metabolite from Actinomycetales culture. An acarbose molecule consists of an unsaturated cyclohexitol residue bound to amino saccharide and two glucose residues via α-1,4 glycoside bonds. Its antihyperglycemic effect is based on the fact that acarbose is a competitive inhibitor of intestinal α-glucosidases that comprise glucoamylases, saccharases, maltases and α-dextrinases, the enzymes that are necessary for degradation of starch, saccharose and maltose (16). Acarbose binds to the above α-glucosidase with the affinity which is 10,000 to 100,000-fold higher than that of, e.g., saccharose. Inhibition of this enzymic activity by acarbose reduces the rate of monosaccharide production and their absorption in the intestine. A new investigation confirm beneficial effect of acarbose on postprandial

koje obuhvaćaju glukoamilaze, saharaze, maltaze i α- dekstrinaze, a ti su enzimi neophodni za razgradnju škroba, saharoze i maltoze (16). Akarboza se veže na navedene α-glukozidaze s afinitetom koji je 10 000 do 100 000 puta veći od istog npr. saharoze. Inhibicija ove enzimske aktivnosti akarbozom smanjuje brzinu nastanka monosaharida i njihovu apsorpciju u tankom crijevu. I novija istraživanja potvrđuju učinak akarboze na smanjenje postprandijalne hiperglikemije u osoba s oštećenom tolerancijom glukoze i osoba s tipom 2 šećerne bolesti (17). Akarboza također smanjuje makrovaskularne komplikacije, odnosno oštećenje endotela u postprandijalnoj hiperglikemiji (18). Znanstvena istraživanja su potvrdila da je oksidativni stres uključen u patogenezu kardiovaskularnih bolesti tijekom šećerne bolesti. Međutim, još je uvijek nejasno da li oksidativni stres nestaje smanjenjem postprandijalne hiperglikemije. U radu autora Assaloni i sur. dokazano je da se kontrolom postprandijalne hiperglikemije oralnom primjenom mitiglinida smanjuje oksidativni stres i lipidna peroksidacija i na taj način spriječava nastanak kasnih dijabetičnih komplikacija (19).

U ovom radu prikazana je statistički značajno povišena koncentracija MDA u jetri ($p<0,05$) i glukoze u serumu ($p<0,01$) u NOD miševa u kojih je šećerna bolest izazvana aloksanom (D) u usporedbi s kontrolnom, zdravom skupinom (K) što potvrđuje stanje oksidativnog stresa i lipidne peroksidacije u dijabetičnoj skupini NOD miševa. Pokusom je dokazan hipoglikemijski učinak akrboze u maloj dozi (25 mg /100 g standardne laboratorijske hrane) ($p<0,05$), a također je zabilježen i povoljan učinak akrboze na stanje oksidativnog stresa, naime u dijabetičnih NOD miševa nakon tretmana akrbozom u trajanju od sedam dana (skupina D/A), koncentracija MDA bila je statistički značajno niža ($p<0,05$) u usporedbi s dijabetičnom skupinom NOD miševa (skupina D). Povoljan učinak akarboze na koncentraciju MDA može se objasniti njenim antihiperglikemijskim učinkom.

hyperglicaemia in prediabetic and Type 2 diabetic patients (17). Acarbose, also, might reduce macrovascular complication by avoiding endothelial injury in postprandial hyperglycemic status (18). There is growing evidence that oxidative stress and inflammation are involved in the pathogenesis of cardiovascular disease in diabetes mellitus. However, it is still unclear whether this phenomenon can be controlled in clinical practice by modulating postprandial hyperglycemia. In study of Assaloni *et. al.* shows that controlling postprandial hyperglycaemia with mitiglinide significantly improves the cluster of oxidative stress and inflammation markers that are increased in the postprandial state in diabetic patients (19).

A significantly elevated serum glucose level ($p<0.01$) and MDA concentration in the liver ($p<0.05$) is reported in our study in NOD mice with alloxane induced diabetes (D), compared to the control, healthy group (C), the fact which confirmed oxidative stress in the group of non-obese diabetic mice. Hypoglycemic effect of a low acarbose dose (25 mg/100 g standard laboratory chow) ($p<0.05$) was experimentally proven; also, a favorable effect of acarbose on oxidative stress was recorded since MDA concentration in NOD mice after 7-day acarbose treatment (group D/A) was significantly lower ($p<0.05$) compared to the group of non-obese diabetic mice (group D). The favorable effect of acarbose on MDA concentration may be accounted for by its antihyperglycemic action.

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