Utjecaj akarboze na katalitičke aktivnosti alanin aminotransferaze i aspartat aminotransferaze u jetri kontrolnih i dijabetičnih CBA miševa

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Source / Izvornik: **Acta Pharmaceutica, 2006, 56, 87 - 93**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:163:655999

Acta Pharm. 56 (2006) 87–93 Short communication

Effect of acarbose on alanine aminotransferase and aspartate aminotransferase activities in the liver of control and diabetic CBA mice

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The purpose of this study was to examine the short-term effects of diet containing 0.1% (*m/m*) of acarbose in standard laboratory chow on specific liver enzyme activities: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in control and diabetic CBA mice. Diabetes was induced by intravenous injection of alloxan monohydrate in a dose of 75 mg kg^{-1} mouse body mass seven days before the treatment with acarbose. There were four groups of CBA mice in the experiment: control (C) mice (n = 6) and diabetic (D) mice (n = 8) fed standard chow; control (C/A-100) mice (n = 8) and diabetic (D/A-100) mice (n = 8) fed standard chow containing 0.1% acarbose. Diabetes induced a decrease of the ALT catalytic activities to 69.6% of the control value. A similar level of decreased ALT catalytic activity was detected in the liver of control and diabetic mice fed chow containing 0.1% acarbose. No changes in the specific and total activities of AST in the liver of experimental groups were observed.

Keywords: acarbose, alanine aminotransferase, aspartate aminotransferase, diabetes

Accepted October 10, 2005

Acarbose, a complex oligosaccharide that acts by competitive and reversible inhibition of small intestine brush-border α-D-glucosidases thereby delaying absorption of carbohydrates in the gut, is increasingly used for the treatment of diabetes type II (1). Additional findings indicate that α-D-glucosidase inhibitors also act specifically on the entry of free glucose into the enterocytes (2). Due to these effects, treatment with acarbose results in reduced postprandial blood glucose levels, and reduced postprandial hyperinsulinemia (3).

Delayed carbohydrate digestion increases the amount of fermentable carbohydrate in the bowel, which does not appear to cause calorie loss because of the metabolism to other absorbable nutrients by colonic microflora (4).

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Ahr *et al.* (5) studied absorption, disposition, metabolism and excretion of acarbose following a single administration of ^{14}C-labelled compound to rats and dogs via different routes (intravenous, oral, intraduodenal) in the dose range of 2–200 mg kg^{-1} as well to man in a single oral dose of 200 mg. After oral administration ^{14}C-acarbose was very poorly adsorbed (1–2% of the dose in rats and man and 4% in dogs). Additionally, up to 35% of the radioactivity of ^{14}C-acarbose was absorbed after degradation by digestive enzymes and/or intestinal microorganisms.

Since acarbose is minimally absorbed in unchanged form after oral administration, the drug is widely believed to be safe, with only flatulence as a commonly reported complaint, and rarely a severe gastrointestinal disturbance such as ileus (6, 7).

Contrary to this opinion, acarbose has been incriminated in several reports of dose--related hepatotoxicity and higher serum alanine aminotransferase (ALT) activity with normal aspartate aminotransferase (AST) activities in humans (8, 9). This effect could be induced by reabsorbed acarbose or its metabolites on the structure and metabolic activity of the liver (10). Therefore acarbose has been put on the list of drugs which may induce acute hepatitis (11).

In our previous study, we studied short-term effects of 0.075, 0.1 and 0.15% (*m/m*) acarbose mixed in standard chow on specific intestinal disaccharidase activities and on hyperglycaemia in diabetic CBA mice (12). We found that feeding with 0.1 or 0.15% acarbose in standard laboratory chow for seven days caused a statistically significant decrease in specific maltase and sucrase activities in duodenum, jejunum and ileum while antihyperglycaemic effect was observed only in the group of diabetic mice fed 0.12 acarbose.

For this reason, we selected this antihyperglycaemically effective dose to test the possible short-term effect of acarbose mixed in standard laboratory chow on the ALT and AST activities in liver of control and diabetic CBA mice.

EXPERIMENTAL

Animals

Three-month IRB bred, male CBA mice, body mass 25–30 g, were used in the study. The mice were housed in metabolic cages on a 12-h light/dark cycle at a temperature of 22–24 °C. All mice were fed *ad libitum* with standard laboratory chow (Pliva, Croatia) and had free access to water. Diabetes was induced by intravenous injection of alloxan monohydrate (Sigma, USA) in Hank's solution (pH 7.0) in a dose of 75 mg kg^{-1} body mass seven days before the treatment with acarbose. Seven days after alloxan injection, blood was collected from the tail vein of control and diabetic mice for measuring glucose. Diabetic mice with blood glucose beyond 20 mmol L^{-1} were selected for acarbose diet.

There were four groups of CBA mice in the experiment: control (C) mice (n = 6) and diabetic (D) mice (n = 8) fed on standard chow; control (C/A-100) mice (n = 8) and diabetic (D/A-100) mice (n = 8) fed a mixture containing 100 mg acarbose per 100 g of standard chow. They were fed this diet *ad libitum* for seven days. Chows with mixed acarbose

were prepared daily. Body mass was measured before and after seven days of feeding, while food and water intake were measured daily. Blood was collected again from the tail vein before mice were sacrificed under ether anaesthesia, between 9:00 and 10:00 h, without fasting.

This study was approved by the Research Ethics Committee of the Ruđer Bošković Institute, Zagreb.

Liver homogenates

Liver was immediately excised, washed in ice-cold saline and then blotted on tissue paper. Liver tissues were homogenized (100 g L^{-1}) in cold 0.14 mol L^{-1} KCl using a Teflon homogenizator (Measuring & Scientific Equipment, UK). Homogenates were centrifuged at 12000 *g* for 30 minutes in a Mistral 2 L-refrigerated centrifuge (Measuring & Scientific Equipment). Supernatants were stored at –20 °C until analysis.

Methods

ALT and AST activities were determined using IFCC recommended UV methods (HD dijagnostika, Croatia) on a Technicon RA-1000 biochemical analyser (Bayer, Italy). Protein concentration was determined by the method of Lowry *et al.* (13) using bovine serum albumin as standard. Blood glucose concentrations was determined by the glucose oxidase method (HD dijagnostika).

Statistics

Data are shown as means ± standard deviation. They were compared using the ANOVA one-way test of variance. The value of $p < 0.05$ was considered the significance level. SigmaStat program for Windows, version 2.0 (Jandel Corporation, USA), was used for statistical analysis.

RESULTS AND DISCUSSION

In order to test the effect of feeding, body masses of experimental mice were measured before and after seven days of feeding with standard chow or with 0.1% acarbose in standard chow. No significant differences in body mass between the groups were found, either before or after the treatment. Mean blood glucose concentration decreased significantly to 71.6% ($p < 0.05$) in the group of diabetic mice (D/A-100) fed 0.1% acarbose for seven days compared to untreated diabetic mice (D) (Table I).

No differences between the groups were found in the content of total proteins in the liver (Table II). Significantly lowered liver masses were found in control mice (C/A-100) (88%, $p < 0.05$) and diabetic mice (D/A-100) (89%, $p < 0.05$) fed acarbose compared to control mice (C) fed standard chow. The same result was recorded for diabetic mice (D/A-100) (89%, $p < 0.05$) fed acarbose in comparison with diabetic mice (D) on standard diet. Due to the fact that the diabetic mice had lower body mass at the beginning of the treatment with acarbose, the ratio of the liver mass to body mass is better indication

Table I. Body mass and blood glucose of CBA mice[a]

Group	*n*	Body mass (g)		Blood glucose (mmol L^{-1})
		Before treatment	After treatment	
Control mice (C)	6	29.3 ± 2.9	30.0 ± 3.2	8.76 ± 0.86
Control mice on acarbose (C/A-100)	8	28.7 ± 2.8	28.1 ± 2.6	7.50 ± 1.21
Diabetic mice (D)	8	24.0 ± 2.3	22.7 ± 0.4	24.45 ± 3.46[b]
Diabetic mice on acarbose (D/A-100)	8	25.1 ± 3.6	25.5 ± 2.7	17.50 ± 5.75[b, c]

[a] Data are presented as mean ± standard deviation.
[b] Significantly different from control group (C) ($p < 0.05$).
[c] Significantly different from diabetic group (D) ($p < 0.05$).

of the changes in the liver structure and possible metabolic activity. In our study, ratio of liver mass to body mass was significantly higher in diabetic mice on standard diet (D) (134%, $p < 0.05$) compared to control mice on standard diet (C). Ratio of liver mass to body mass decreased significantly in the group of diabetic mice (D/A-100) fed acarbose for seven days.

In our study, this ratio increased in diabetic mice (D), probably due to the storage of many lipid granules in the hepatocytes, as observed by Degirmenci *et al*. (14), who investigated the effect of acarbose on the liver ultra-structure in streptozotocin-induced diabetes in neonatal rats. They also found lysosomal bodies in the hepatocytes of diabetic rats treated with acarbose. This is in agreement with the results of Lembcke *et al*. (15), who additionally found that the effect of lysosomal storage of glycogen depends on the type of alpha-glucosidase inhibitors (emiglitate > miglitol > acarbose).

Acarbose is minimally absorbed in an unchanged form after oral administration and the mechanism of acarbose-induced liver injury is still unknown.

Table III presents specific and total activities of ALT and AST in the liver of experimental groups. Fourteen days after induction of diabetes, both specific and total ALT activities were significantly lowered in the diabetic mice on standard diet (D) (68.6 and 50%, respectively, $p < 0.05$) compared to the control mice on standard diet (C). Similar

Table II. Liver mass and content of proteins in the liver of CBA mice[a]

Group	*n*	Liver (g)	Liver/body mass ratio	Total proteins in liver (g)
Control mice (C)	6	1.52 ± 0.18	0.050 ± 0.002	0.307 ± 0.044
Control mice on acarbose (C/A-100)	8	1.34 ± 0.09[b]	0.048 ± 0.005	0.283 ± 0.031
Diabetic mice (D)	8	1.53 ± 0.11	0.067 ± 0.005[b]	0.304 ± 0.091
Diabetic mice on acarbose (D/A-100)	8	1.36 ± 0.17[b,c]	0.054 ± 0.008[c]	0.296 ± 0.066

[a] Data are presented as mean ± standard deviation.
[b] Significantly different from control group (C) ($p < 0.05$).
[c] Significantly different from diabetic group (D) ($p < 0.05$).

levels of decreased specific and total ALT activities were observed in the liver of control (C/A-100) and diabetic (D/A-100) mice fed acarbose for seven days. No changes in specific and total activities of AST in the liver of experimental groups were observed. The difference between the ALT and AST activity profiles could be explained by the slightly increased permeability of plasma membrane, probably caused by acarbose alone, and the loss of ALT as a cytoplasmatic enzyme from the cells. Those effects are probably consequence of markadly higher amounts of acarbose applied in the current experiment (111.9 mg kg^{-1} b. m.) compared to the usual amount of acarbose administered to human patients (4 mg kg^{-1} b. m.). Another explanation of the metabolic and structural differences of the liver lobules and the distribution of acarbose or its metabolites in the liver could be also taken into account. ALT is a cytosolic, periportally prevailing enzyme while AST is a cytosolic as well as mitochondrial perivenously located enzyme (16). This explanation is supported by our results on the same experimental model, where we proved decreased glucose-6-phosphatase activity, another enzyme predominantly located in the periportal part of the liver (17, 18). The use of acarbose in the usual doses might be presummed safe in the treatment of diabetes mellitus.

Table III. Specific and total enzyme activities in the liver of CBA mice[a]

Group	*n*	ALT		AST	
		Specific activity (U g^{-1} proteins)	Total activity (U per total liver)	Specific activity (U g^{-1} proteins)	Total activity (U per total liver)
Control mice (C)	6	372 ± 26	114 ± 14	889 ± 71	272 ± 36
Control mice on acarbose (C/A-100)	8	241 ± 83[b]	68 ± 21[b]	740 ± 127	210 ± 41
Diabetic mice (D)	8	259 ± 108[b]	57 ± 23[b]	1146 ± 520	266 ± 153
Diabetic mice on acarbose (D/A-100)	8	234 ± 76[b]	67 ± 21[b]	944 ± 320	271 ± 85

[a] Data are presented as mean ± standard deviation.
[b] Significantly different from control group (C) ($p < 0.05$).
[c] Significantly different from diabetic group (D) ($p < 0.05$).

CONCLUSIONS

Decreased ALT catalytic activity was detected in the liver of control and diabetic mice fed chow containing 0.1% acarbose. No changes in specific and total activities of AST in the liver of experimental groups were observed, indicating possibly slightly increased permeability of liver cells plasma membrane. A better insight into the effect of on ALT and AST activity profiles could be gained by higher amounts of acarbose and by monitoring its metabolites in the liver.

Acknowledgement. – This work was supported in part by the Ministry of Education, Science and Sports of the Republic of Croatia (CEEPUS HR-044).

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S A Ž E T A K

Utjecaj akarboze na katalitičke aktivnosti alanin aminotransferaze i aspartat aminotransferaze u jetri kontrolnih i dijabetičnih CBA miševa

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Svrha ovog rada bila je ispitati kratkotrajni učinak 0.1% (*m/m*) akarboze u suhoj hrani na katalitičku koncentraciju specifičnih jetrenih enzima: alanin aminotransferaze (ALT) i aspartat aminotransferaze (AST) u jetri kontrolnih i dijabetičnih CBA miševa. Dijabetes je bio izazvan *i.v.* injekcijom aloksan-monohidrata u dozi od 75 mg kg^{-1} tjelesne mase miša sedam dana prije početka ishrane s akarbozom. U pokusu su ispitane četiri skupine CBA miševa: kontrolna (C) (n = 6) i dijabetična (D) (n = 8) skupina bile su sedam dana na standardnoj ishrani, te kontrolna (C/A-100) (n = 8) i dijabetična (D/A-100) (n = 8) skupina koje su hranjene 0.1% akarbozom umiješanom u standardnu hranu. U skupini D katalitička koncentracija ALT-a bila je značajno snižena u usporedbi s kontrolnom skupinom C. Sličan pad katalitičke koncentracije ALT-a zabilježen je i u jetri kontrolnih i dijabetičnih miševa hranjenih suhom hranom u koju je bila umiješena akarboza (0.1%). U ispitanim skupinama nisu zabilježene promjene u specifičnoj i ukupnoj aktivnosti AST-a.

Ključne riječi: akarboza, alanin aminotransferaze, aspartat aminotransferaze, dijabetes

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