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Alloxan in vivo Does Not Only Exert Deleterious Effects on Pancreatic B Cells

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Summary

The aim of the experiment was to investigate the mechanism of harmful alloxan action *in vivo*. 75 mg/kg b.w. of this diabetogenic agent were administered to fasting rats. Two minutes later the animals were decapitated. It was observed that alloxan caused a distinct rise in blood insulin and glucose levels with a concomitant drop of free fatty acids. The amount of sulfhydryl groups in the liver of alloxan-treated rats was decreased and glutathione peroxidase activity was substantially higher. These results indicate that some changes observed in alloxan-induced diabetes can not only be the consequence of B cells damage by alloxan but may also be the result of its direct influence on other tissues. It was also observed that glucose given 20 min before alloxan injection only partially protected against the deleterious effects of alloxan.

Key words

Alloxan – Glucose – Insulin – Sulfhydryl groups – Glutathione peroxidase

Introduction

At present there is not a fully acceptable way of inducing experimental diabetes in animals. One of the methods used for the first time in 1943 by Dunn, Sheehan and McLethie was alloxan injection (Dunn *et al.* 1943). During the following years alloxan was often used as a diabetogenic agent. It was observed that a few days after single intravenous, intraperitoneal or subcutaneous administration of this compound, experimental animals exhibited a number of symptoms characteristic for diabetes. The elucidation of alloxan action on pancreatic B cells *in vitro* was the aim of several investigations and is at present quite well characterised (Takasu *et al.* 1991, Zhang *et al.* 1992). But there is lack of information about the direct effect of alloxan on the whole organism shortly after its administration. It was previously presumed that after giving a diabetogenic dose, its cytotoxic action concerns the pancreatic B cells only. This assumption is now often questioned. In many experiments, alloxan-treated rats manifested a number of side effects and animals succumbed. In addition, changes observed in animals shortly after alloxan administration seem to be caused not by damage of pancreatic B cells only. Thus, the information about the direct *in vivo* action of alloxan on other tissues will certainly be helpful in elucidating the cause of its toxic effects.

The purpose of the present experiments was to ascertain if a standard diabetogenic dose of alloxan injected into rats had a direct effect only on pancreatic B cells. The protective action of glucose against the cytotoxicity of alloxan *in vivo* was also tested.

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Material and Methods

In our experiments, 24 male Wistar rats weighing 150±5 g and kept under standard conditions were used. Prior to the experiment, rats were fasted for about 16 h and were divided into 4 groups (n=6). Six animals received water intragastrically and physiological saline intravenously and these served as controls (Group I). The second group received intragastrically glucose and intravenously saline. The third group of animals was given water intragastrically and intravenously alloxan. The fourth group received glucose intragastrically and alloxan intravenously. Glucose was always given in a dose of 400 mg/100 g body weight. The dose of alloxan dissolved in saline was 75 mg/kg body weight. The amount of water or glucose solution given into the stomach as well as saline with or without alloxan given intravenously was 1 ml/100 g body weight. Intravenous injections were made under light ether anaesthesia. Intragastric administrations were made 20 min before the intravenous injections. Rats were decapitated 2 min after intravenous injections of saline or alloxan. The serum and liver were collected for analysis. The serum was used for the estimation of blood glucose, insulin, free fatty acids and triglyceride contents, and the liver for the determination of the sulfhydryl content and for glutathione peroxidase activity. Glucose was photocolorimetrically assayed by means of the enzymatic method with glucose oxidase and *o*-dianisidine. Insulin was determined using a radioimmunoassay kit (Swierk, Poland). Free fatty acids were determined according to Duncombe (1964) and triglycerides were assayed by the method of Foster and Dunn (1973). The concentration of sulfhydryl groups in the liver was determined according to Sedlak and Lindsay (1968) and glutathione peroxidase activity was assayed by the method described by Rice-Evans *et al.* (1991) with hydrogen peroxide as substrate. The protein content in liver homogenates was determined according to Lowry *et al.* (1951). All the reagents used in this experiment were obtained from Sigma. The results were statistically evaluated using one-way analysis of variance and Duncan's multiple range test.

Table 1. Blood glucose, insulin, free fatty acids, triglycerides, sulfhydryl content of liver and liver glucose peroxidase activity in rats after alloxan administration and the protective effect of glucose.

Parameter	Group I i.g. water i.v. saline	Group II i.g. glucose i.v. saline	Group III i.g. water i.v. alloxan	Group IV i.g. glucose i.v. alloxan
Glucose (mmol/l)	3.30 ± 0.41^{A}	5.22 ± 0.32^{B}	4.58 ± 0.40^{B}	7.47 ± 0.25^{C}
Insulin (μU/ml)	6.62 ± 0.96^{A}	19.89 ± 2.40^{B}	75.24 ± 7.20^{C}	80.59 ± 9.60^{C}
Free fatty acids (mmol/l)	0.79 ± 0.09^{A}	0.31 ± 0.02^{B}	0.55 ± 0.07^{C}	0.22 ± 0.03^{D}
Triglycerides (mmol/l)	2.16 ± 0.12	2.73 ± 0.20	2.08 ± 0.09	2.57 ± 0.14
-SH groups (mmol/100 g w.t.)				
- total	2.57 ± 0.11^{A}	2.63 ± 0.10^{A}	2.14 ± 0.04^{B}	2.26 ± 0.05^{B}
- nonprotein	0.22 ± 0.01^{A}	0.27 ± 0.01^{B}	0.19 ± 0.01^{C}	0.21 ± 0.01^{A}
- protein-bound	2.36 ± 0.01^{A}	2.36 ± 0.01^{A}	1.95 ± 0.04^{B}	2.05 ± 0.04^{B}
GPx activity in the liver (nmol/min/mg protein)	635 ± 86^{A}	736 ± 92^{A}	1257 ± 105^{B}	1047 ± 80^{C}

Values are means ± S.E.M. for six animals. Means with different letter superscripts are significantly different ($p<0.05$). Intravenous injections (i.v.) were made 20 min after intragastric (i.g.) administrations. The amount of glucose was 400 mg/100 g b.w. and alloxan was given in the dose of 75 mg/kg b.w. GPx – glutathione peroxidase, w.t. – wet tissue.

Results

The results obtained in the experiment are presented in Table 1.

Alloxan administration significantly increased blood glucose and insulin concentrations. Treatment with glucose prior to alloxan injection did not prevent these changes. In alloxan-treated animals, a decrease in serum concentration of free fatty acids with a simultaneous slight drop in triglycerides were also observed. The administration of alloxan also caused a distinct decrease in liver sulfhydryl groups. The

protective effect of glucose only concerned nonprotein sulfhydryl groups. The activity of glutathione peroxidase in the liver of alloxan-treated animals increased significantly. Glucose given to rats prior to the alloxan injection partially prevented the increase in activity of this enzyme.

Discussion

In the experiment performed two minutes after intravenous injection of alloxan in the dose of 75 mg/kg body weight, a considerable increase of insulin concentration in the blood serum of rats was observed. The animals were sacrificed 2 min after alloxan administration because this compound is very unstable under physiological conditions (half-life is about one minute), so that short time intervals should be used to ascertain its direct effects. The rise of insulin observed at this time was particularly high in comparison with the increase of glycaemia and significantly exceeded insulin concentrations usually found in peripheral blood of fasted rats under physiological conditions. This serves as evidence that the observed effect was caused by the direct influence of alloxan on pancreatic B cells. In another *in vitro* experiment, alloxan caused a sudden but short-lasting release of insulin and then completely inhibited the hormone secretion (Kliber *et al.* 1996). Dean and Matthews (1972) demonstrated depolarization of the cell membrane of pancreatic B cells in the presence of alloxan. This, in turn, opened the voltage-dependent calcium channels (Komatsu *et al.* 1989) and caused insulin release. Using the perfused rat pancreas it was found that alloxan-induced insulin secretion was independent of the glucose presence (6.6 mmol/l) in the perfusion medium (Kliber *et al.* 1996). In this experiment performed *in vivo*, the administered glucose caused a rise of glycaemia from 3.3 to 5.2 mmol/l but did not prevent a rapid increase of insulinaemia in alloxan-treated rats. It seems that the increase of insulinaemia was caused by a lack of the inhibitory action of glucose on the alloxan uptake by pancreatic B cells (Gorus *et al.* 1982). In another experiment performed under similar conditions, Bansal *et al.* (1980) showed that glucose completely protected the islets against alloxan, but only when a smaller dose of alloxan had been used.

The increase in insulin concentration 2 min after alloxan injection was accompanied by a rise in blood glucose concentration and a simultaneous decrease in the content of free fatty acids. These changes suggest that the use of lipids as a source of energy is enhanced. This assumption is additionally supported by a slight decrease of blood triglycerides in alloxan-treated rats. The observed changes were certainly not only the consequence of pancreatic B cell damage by alloxan. They may also be interpreted as symptoms of impaired insulin sensitivity as the result of direct alloxan action on the cells.

Extremely high blood insulin levels noted in alloxan-treated animals may additionally be caused by reduced degradation of this hormone. The main organ internalizing insulin in normal conditions is the liver (Field *et al.* 1980). Alloxan acting on the liver may affect this process.

To ascertain if the direct action of alloxan also concerns the liver, the content of sulfhydryl groups as well as glutathione peroxidase activity in this tissue were determined. It was observed that as early as 2 min after alloxan administration, the content of sulfhydryl groups in the liver dropped considerably. It is well known that alloxan undergoes reduction to dialuric acid in the presence of reducing agents. The main source of protons in this reaction *in vitro* is often reduced glutathione (Sakurai and Ogiso 1991). In this experiment, a considerable decrease in nonprotein sulfhydryl groups was accompanied by a simultaneous drop in protein-bound sulfhydryl groups in livers of alloxan-treated rats. This demonstrates a substantial effect of alloxan on protein-bound sulfhydryl groups *in vivo*. The oxidation of these essential groups may lead to the inactivation of some enzymes (Lenzen *et al.* 1988) and to other deleterious changes in the liver (Thor *et al.* 1985) beginning shortly after alloxan administration.

Glucose given to fasted rats increased the content of nonprotein sulfhydryl groups without any effect on protein-bound ones. Similar results were obtained in other experiments (Keck *et. al.* 1990). The increase of nonprotein sulfhydryl groups can be attributed to NADPH generation as a result of glucose metabolism. NADPH is then used to reduce oxidized glutathione in the reaction catalyzed by glutathione reductase. In this experiment, glucose provided only partial protection against the alloxan-induced oxidation of sulfhydryl groups. It seems that in our experiment the main reason for the lack of the expected glucose effect was that the applied dose of sugar was too small in comparison with the injected amount of alloxan. Harman and Fischer (1982) using the suspension of isolated rat hepatocytes demonstrated that only a very high glucose level protected against the alloxan-induced decrease of the glutathione content.

Further evidence confirming the direct influence of alloxan on the liver concerned the considerable increase of glutathione peroxidase (GPx, E.C. 1.11.1.9) activity in this tissue two minutes after injection of this diabetogenic agent. Alloxan decomposition is accompanied by the formation of superoxide radicals which undergo dismutation to form hydrogen peroxide (Takasu *et al.* 1991). Increased GPx activity is one of the protective responses against oxidative stress. Thus, the augmentation of GPx confirmed the presence of reactive oxygen species in the liver of alloxan-treated rats. The decrease of GPx in alloxan-injected animals pretreated with glucose suggested that this sugar partially protected

hepatocytes *in vivo* against the toxicity of alloxan. It is quite possible that glucose reduces alloxan-induced generation of hydrogen peroxide or makes H_2O_2 decomposition possible by a different mechanism.

The results obtained in this experiment clearly indicate that alloxan administered to rats in a commonly applied diabetogenic dose is not selectively toxic for pancreatic B cells. Thus, the deleterious changes observed in alloxan-diabetic animals may by partially caused by direct action of alloxan on other tissues. It was also shown that higher blood glucose levels only partially counteract the alloxan-induced cytotoxicity to hepatocytes *in vivo*.

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