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ALLOXAN DIABETES REGULATES ADENOSINE DEAMINASE ACTIVITY IN MICE : TISSUE- AND AGE- SPECIFIC CORRELATION

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Summary. Effect of alloxan induced diabetes on the activity of adenosine deaminase (ADA) was studied in the liver, spleen, stomach and small intestine of mice at two different postnatal ages (preweaned, 15-day and postweaned, 60-day). Alloxan significantly stimulates (133%) ADA activity in the liver of 15-day old mice, while it has no significant effect in the 60-day old animals. In contrast, ADA activity was moderately increased (25%) in spleen of both the ages. However, no significant influence of alloxan was observed on ADA activity of stomach at either age of mice. On the other hand, alloxan treatment increases (69%) intestinal ADA activity in 15-day old mice, with no significant change in 60-day old animals. Thus, alloxan diabetes increases ADA activity in an age- and tissue-specific manner. Stimulation of ADA activity in diabetic mice might play role in immune and other metabolic dysfunctions in diabetic conditions.

Keywords: Mice ADA activity; Alloxan; Diabetes

Introduction

Adenosine deaminase (ADA; EC 3.5.4.4), the purine salvage pathway enzyme, catalyzes the irreversible hydrolytic deamination of adenosine and 2′-deoxyadenosine, respectively (1,2). Its critical function is to regulate the steady state concentration of adenosine and deoxyadenosine with marked pleotropic effects, specially on immunological, neurological and vascular systems (3). Hereditary deficiency of ADA is associated with severe combined immunodeficiency (SCID) disorder (3-5), where both T- and B-lymphocytes are unable to proliferate and mount the antigenic challenges. Abnormalities of this enzyme have also been observed in other diseases of immune system, including acquired immune deficiency syndrome

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(AIDS), various lymphomas, leukemias, anemia and several other human disorders like, short-limbed dwarfism, hepatitis and hepato-cellular jaundice (6-10).

In mammals, ADA is phylogenetically ubiquitous and widely distributed in different tissues. However, the highest level of ADA is present in the alimentary canal, thymus and spleen (1,12,13). High level of ADA in these tissues possibly reflects its critical role in thymic as well as extrathymic T-cell maturation. Its activity changes markedly during development and differentiation in different species (12-14). Adenosine, a natural substrate of ADA, is believed to repress the insulin-stimulated glucose uptake and glycolysis, and that ADA might regulate these physiological activities by controlling adenosine concentration (15,16). Taking into account the role of adenosine and ADA in immune as well as other metabolic dysfunctions during diabetes, the present piece of work was conceived.

Materials and Methods

Animals and chemicals: Female Swiss albino mice (Balb/c strain) of two different postnatal age groups (15- and 60-day old) were used. The animals were maintained under normal laboratory conditions at 25±2 °C on a 12/12 h light/dark period and fed with a standard pellet diet (Amrut Laboratory, Pune) and water *ad libitum*.. All the chemicals used were of analytical grade and biochemicals were obtained from Sigma Chemical Co., USA.

Preparation and assay of ADA activity: Animals were killed by cervical dislocation at a fixed time of the day (14:00 h), their liver, spleen, stomach and small intestinal tubes were taken out, washed in chilled normal saline (0.9% NaCl) and blotted dry. A 20 % (w/v) homogenate of these tissues was prepared in ice-cold 100 mM sodium citrate buffer, pH 6.0 containing 0.25 M sucrose. Each homogenate was centrifuged at 27,500 x g for 60 min at 2 °C. The supernatant thus obtained was used for the assay of ADA. The activity of ADA was measured spectrophotometrically in a Hitachi Model U-2000 spectrophotometer by the method of Kalchar (17) and Yoshida and Aikawa (18) with certain modifications. Initial reaction rates were determined from the decrease in absorbance at 265 nm. The standard assay was carried out at 25 °C in 3 ml of 100 mM sodium citrate buffer, pH 6.0 with 100 µM adenosine and 50 µl of suitably diluted enzyme preparation that gave a linear decrease in absorbance at 265 nm.

Protein Estimation: Protein concentration of enzyme preparation was determined by the method of Bradford (19) using BSA as standard. The activity of ADA was expressed as units (µmol adenosine deaminated per min) per mg protein. The data were statistically analyzed and the level of significance (p-value) between two sets of data was calculated according to student's *t* - test.

Blood glucose estimation: Blood glucose levels were routinely determined using glucometer according to the user's guide with certain modifications. Blood was collected from the tail of the mice and a drop of blood was applied to the pad of the glucose stripes. After 20 sec, the pad surface was blotted dry and inserted into test

slot of the meter. The values of glucose level, indicated on the display screen, were recorded. The final blood glucose concentration was concurrently estimated with O-toluidine method (20).

Alloxan treatment: Two groups of overnight starved mice of 15- and 60-day old were injected with alloxan intraperitoneally at a dose of 15 mg/100 g body weight, in 0.3 ml of 0.15 M acetate buffer, pH 4.5 (16). A set of control animals received only 0.3 ml of 0.15 M acetate buffer. Thereafter, animals were kept under normal laboratory conditions and blood glucose level was routinely determined. Only the animals whose blood glucose level was increased to almost double compared to the control mice, were killed on the 7^{th} day of alloxan treatment. Tissues were removed and processed similarly as above.

Results and Discussion

Adenosine deaminase has gained considerable importance as its genetic deficiency leads to severe combined immunodeficiency (SCID) disorder where the patient is unable to upkeep a competent immune system (21). Many others, including our group, have earlier reported the tissue- and age-specific expression of ADA activity during pre- and postnatal development of mice (13, 14, 22). We have also shown that corticosterone inhibits ADA activity in liver, spleen, stomach and small intestine of mice, whereas dibutyryl-cAMP, an analog of cAMP, stimulates it (13,14). Alloxan has earlier been reported to induce diabetes in experimental animals (16, 23). Diabetogenic effect of alloxan may be regulated through Ca^{2+} which in turn might be responsible for β-cells damage in pancreas. The probable mechanism for Ca^{2+} mediated damage of β-cells has been suggested through activating endonuclease and causing DNA fragmentation in β-cells (24).

In our study, blood glucose levels (mg/dL) of treated animals were found elevated by 90-113% to that of the control and hence, ensured that animals had responded to alloxan and were diabetic. Both these ages responded to an almost equal extent as evident from Fig. 1. Alloxan treatment significantly stimulated (133%) ADA activity in the liver of preweanling (15-day old) mice, while it had no effect in the postweaned (60-day old) animals (Fig. 2A). In contrast, it stimulated (25%) ADA activity moderately in the spleen of both the ages (15-and 60-day) of mice (Fig. 2B). However, no significant influence of alloxan was observed on ADA activity of stomach at either age of mice (Fig. 2C). The activity of intestinal ADA was elevated (69%) by alloxan treatment as compared to that of the control in 15-day old mice, whereas in 60-day old animals, it was not significantly influenced by alloxan (Fig. 2D). Thus, alloxan-dependent increase in ADA activity is found to be tissue- and age-specific.

Diverse requirement of ADA has already been proposed in concurrence with its developmental expression in different tissues of animals (12,14). Several

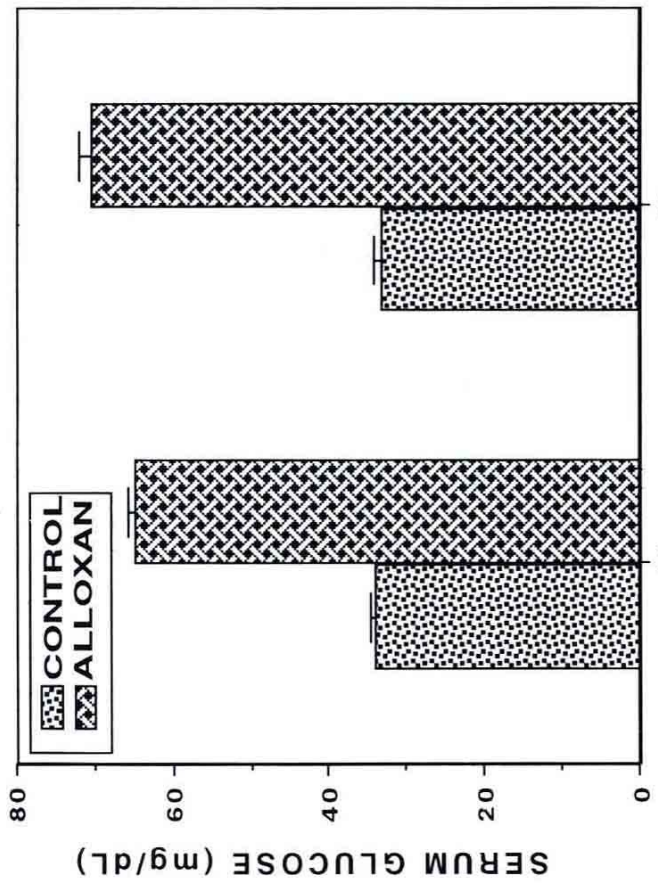

Fig. 1. Serum glucose level in alloxan treated and control mice of 15- and 60-day old. Values are mean of 4-5 mice in each group. Bars represent standard deviation. The observed differences are statistically significant ($P<0.01$) as compared to control.

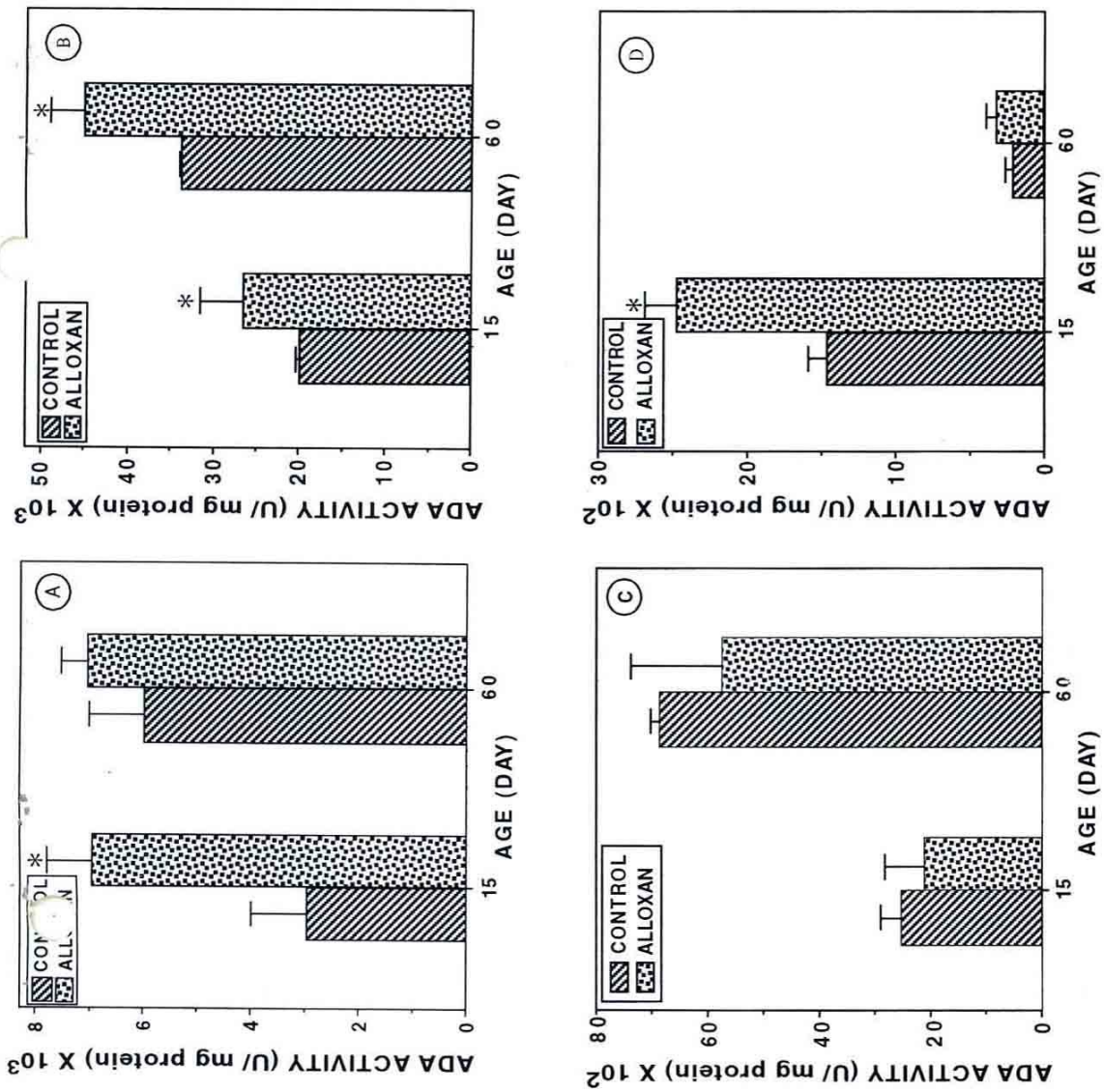

Fig. 2. Effect of alloxan diabetes on the activity of ADA in the liver (A), spleen (B), stomach (C) and small intestine (D) of 15- and 60-day old mice. Alloxan treatment and the other experimental conditions are described in Materials and Methods section. Values are mean of 4-5 mice in each group. Bars exhibit standard deviation. All the observed differences (asterisks) are statistically significant ($P<0.01$) as compared to control.

metabolic adjustments take place during diabetes to tailor high circulating glucose level. Adenosine and ADA might play critical role in such situations of diabetic condition. Adenosine has earlier been attributed in glucose transport and glycolysis (16). Our findings show that alloxan diabetes stimulates ADA activity in a tissue- and age- specific manner that might play a role in varied metabolic and immune dysfunctions during diabetes. Precise mechanism of diverse ADA regulation by alloxan is not clear. However, it will be of much significance, in future, to find out the correlation between alloxan induced diabetes and the regulation of adenosine deaminase.

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