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The prevention of alloxan-induced diabetes in mice by the iron-chelator detapac: Suggestion of a role for iron in the cytotoxic process

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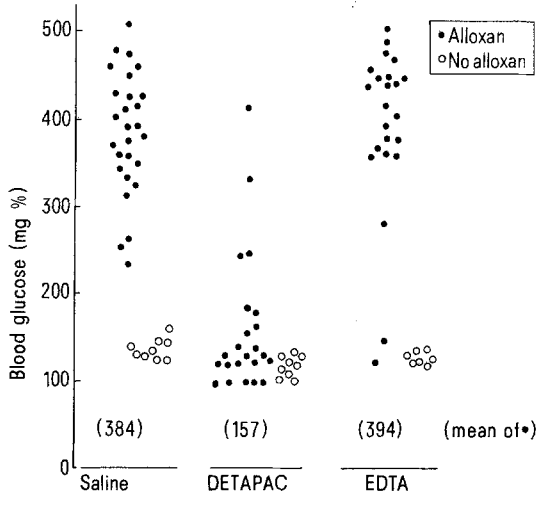
Summary. DETAPAC, an iron-chelating agent, given to male Swiss-Webster mice prior to alloxan, was able to protect the mice from the diabetogenic actions of alloxan. In contrast EDTA, another chelating agent, offered no protection. Possible mechanisms for these effects, including inhibition of hydroxyl radical formation, will be discussed.

The diabetogenic agent alloxan is a widely used tool in diabetes research. It has recently been reported that diethylenetriaminepentaacetic acid (DETAPAC) could prevent alloxan-induced damage in isolated islet cell preparations. It was suggested[2,3] that DETAPAC protection was due to its prevention of hydroxyl radical formation[4–7]. With the above knowledge, we speculated that DETAPAC administration might also prevent alloxan-induced diabetes in vivo. The data in the present report will show that this is indeed the case.

Materials and methods. Male Swiss-Webster mice (Perfection Breeders) were used in all experiments. Food was routinely withheld from the mice for 3–4 h. At this time the mice received an i.p. injection of DETAPAC (Sigma) or EDTA (Baker) (100–250 mg/kg, dissolved in distilled water) or a saline vehicle. 1 h later, the mice received an i.v. (tail) injection of 75 mg/kg alloxan monohydrate (Calbiochem). Food was returned to the mice approximately 1 h post-alloxan. Blood sugar was determined 48 h later by a glucose oxidase assay (Trinder method, Boehringer-Mannheim) after deproteinization of the blood with barium hydroxide and zinc sulfate.

Results. In saline-pretreated mice, alloxan at 75 mg/kg caused a large increase in blood glucose (fig. 1). Most of the mice had values greater than 300 mg%, which is generally considered diabetic. Mice that had been pretreated with 250 mg/kg of DETAPAC prior to alloxan were largely protected against the actions of alloxan. Only 2 of the 23 mice had blood glucose greater than 300 mg%. The non-alloxan treated mice (saline, DETAPAC or EDTA alone) had blood glucose values ranging from 105 to 158 mg%. Pretreatment with EDTA failed to protect against alloxan. Most animals (20 of 23) had glucose values above 300 mg%. The effects with DETAPAC showed a clear dose response relationship. In experiments not presented, mice pretreated with saline prior to alloxan had a mean blood glucose ± SD of 507 ± 178 mg%. In contrast, mice pretreated with 100 mg/kg of DETAPAC prior to alloxan had a blood glucose of 349 ± 108 mg% and mice pretreated with 200 mg/kg of DETAPAC prior to alloxan had a blood glucose of 229 ± 165 mg% (n = 8 to 10 mice in each group).

Discussion. Alloxan can be readily reduced to dialuric acid in vitro by standard reducing agents including ascorbic acid[8]. Dialuric acid is extremely unstable and rapidly reacts with oxygen (autoxidizes). Products of dialuric acid autoxidation include alloxan itself as well as several very reactive



The effects of DETAPAC or EDTA on alloxan-induced diabetes. Mice were pretreated with saline, or DETAPAC or EDTA at 250 mg/kg 1 h prior to 75 mg/kg of alloxan. Other mice received only the pretreatment (no alloxan). Blood glucose was measured 48 h later. Data represent individual values. Mean values for the alloxan-treated mice are in parentheses.

species derived from oxygen[9], namely the superoxide radical ($O_{2\cdot}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot$ OH). The latter species has been termed 'the most reactive oxidizing species known'.

It has been known for several years that $\cdot$ OH was a reaction product in several in vitro biochemical systems in which H_2O_2 and $O_{2\cdot}$ were present simultaneously[10]. In these studies, it was shown that both catalase, which decomposes H_2O_2, and superoxide dismutase, which decomposes $O_{2\cdot}$, could each by themselves prevent $\cdot$ OH formation. It was quite logically suggested[10] that the $\cdot$ OH was formed in a reaction between H_2O_2 and $O_{2\cdot}$. However, it has been demonstrated by several investigators using various techniques, that the direct reaction between H_2O_2 and $O_{2\cdot}$ is extremely slow[11-14]. Despite these apparent discrepancies a reaction sequence which can account for all of this seemingly inconsistent data can be written as follows[4-7]:

a) $Fe^3 + O_{2\cdot} \rightarrow Fe^{2+} + O_2$

b) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$

c) $O_{2\cdot} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$

Reaction c, the sum of reactions a and b, is commonly referred to as the Haber-Weiss reaction[15]. It has been suggested[4-7] that the iron-chelator DETAPAC could prevent $\cdot$ OH formation, probably by inhibiting reaction a or b above. It was also determined that EDTA, could promote the rate of $\cdot$ OH formation. In other studies, it has been shown that DETAPAC and other iron-chelating agents could prevent $\cdot$ OH formation only at certain iron-chelator ratios. And in fact, stimulation of $\cdot$ OH formation has been observed at certain ratios of iron to DETAPAC[16].

In recent in vitro experiments with isolated islet preparations, it has been shown that DETAPAC could counteract the toxic actions of alloxan[2,3]. In these same studies, catalase, superoxide dismutase and several potent $\cdot$ OH scavengers were also found to protect against alloxan. All of these data collectively seemed to suggest that $\cdot$ OH, generated from alloxan after its reduction to dialuric acid and further autoxidation, could damage beta cells in vitro.

The data of the present study, taken together with our previous data on protection against alloxan in vivo by several structurally diverse $\cdot$ OH scavengers[17-22], would appear to be consistent with the premise that DETAPAC effectively prevents $\cdot$ OH formation in vivo under the present experimental conditions and that $\cdot$ OH is responsible for alloxan-induced diabetes. Other mechanisms of protection, most likely involving iron, are also possible.

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β-Alanine and α-L-alanine inhibit the exploratory activity of spontaneously hypertensive rats[1]

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Summary. 1% β-alanine and α-L-alanine, when given for 7 days as the only drinking fluid, inhibited the exploratory activity of adult male spontaneously-hypertensive rats (SHR) but not that of the normotensive Wistar-Kyoto rats (WKR). β-Alanine decreased the taurine level in the liver of both strains and in the platelets of SHR. α-Alanine decreased the taurine level in the liver of WKR and in the platelets of SHR.

β-Alanine is a structural analog of taurine. It has been shown that β-alanine but not α-L-alanine inhibits the uptake of taurine into the platelets, cortical synaptosomes and retina[2,3]. β-Alanine also inhibits the transport of taurine into the isolated heart[4].

$H_2N-CH_2-CH_2-SO_3H$
Taurine

$H_2N-CH_2-CH_2-COOH$
β-Alanine

$H_2N-CH(CH_3)-COOH$
α-L-Alanine

We have previously shown that taurine enriched and taurine deficient diets stimulate the exploratory activity of SHR but not that of WKR. Both these treatments were assumed to increase the availability of taurine[5]. The present work was designed to study the actions of β-alanine and α-L-alanine in the open field situation and to evaluate the taurine levels in various tissues after alanine administration.

Materials and methods. Male rats of spontaneously hypertensive (SHR) (n = 24; weight mean ± SD 347 ± 17 g) and of normotensive strains of Wistar-Kyoto rats (WKR) (n = 24; weight 312 ± 8 g) were used in the study at the age of 3 months. The rats were housed in groups of 8 with food and fluid ad libidum and maintained on a 12-h light (7.00–19.00 h) -dark cycle.