Glucose intolerance due to heavy metals intoxication in the rat.

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Abstract

Data of the current study show that intraperitoneal (i.p.) injection of cadmium chloride (CdCl₂) (5 mg/kg) into male albino rats was found to induce a deterioration in glucose tolerance 24 hr post-treatment, which was accompanied by a reduced elevation in serum insulin levels in response to the glucose challenge. CdCl₂ produced a significant decrease in the liver content of both glutathione and protein contents 24 hours post CdCl₂ treatment while a significant elevation in liver thiobarbituric acid (TBA)-reactants was observed. A significant decrease in serum total proteins was noticed due to CdCl₂ treatment while the serum levels of the two aminotransferases enzymes AST & ALT were in significantly changed affected by cadmium intoxication. The present study suggest that the glucose intolerance observed due to CdCl₂ intoxication could be due to the elevation of lipid peroxidation (induced by cadmium) which may affect the rate of glucose transport into the cells. Impaired insulin synthesis and the inactivation of the glucose metabolizing enzymes which could be secondary effects to the glutathione depleting effects of cadmium, were also suggested to be contributing mechanisms to the deterioration of glucose tolerance in cadmium intoxicated rats.

The present study throw more light on one of the most serious phases of cadmium toxicity which emphasizes the importance of performing more studies to explore all the consequences of heavy metals pollution. This could be a gate way to determine means for protection against this pollution.

Key Words: Glucose tolerance; Cadmium; Rats

Introduction

Cd exists in the air and water pollutants. Its toxic effects on biological systems has been extensively reported (Mennear, 1979; Ahokas et al., 1980; Lewis, 1997 and Moustafa, 2000, 2002). Free radicals are evolved at the early stages of cadmium (Cd) intoxication (Ochi et al., 1987, Richelmi et al., 1989). Oxidative stress defined as a persistent imbalance between the production of highly reactive molecular species (chiefly oxygen and nitrogen) and antioxidant defense, is implicated in a broad variety of chronic and acute diseases, including such age-related diseases as diabetes (Oh-Ishi et al., 2003 and Varvarovska et al., 2003). Earlier studies have reported the involvement of oxidative stress in the development of impaired carbohydrate metabolism in systems involving different cytotoxins Moustafa (1998) investigating the effects of glutathione depletion due to allyl alcohol treatment on carbohydrates metabolism,. In spite of the existence of plenty of studies describing the deleterious effects of Cd on different biological functions, only
few reports have addressed the impact of cadmium toxicity on carbohydrate metabolism. The current study was undertaken to give more insight to the relationship between cadmium toxicity and glucose metabolism. The effect of cadmium chloride (CdCl₂) on glucose tolerance and other parameters related to glucose metabolism was also investigated.

**Materials and methods**

**Animals and Experimental protocol**

Male Albino rats were obtained from the National Research Center (Cairo, Egypt). Rats were housed three per cage and allowed free access to standard chow and water except in those experiments utilizing fasted rats, where food was withheld for 24 hr. Cadmium chloride (CdCl₂) dissolved in saline, was given intraperitoneally (i.p.) at the dose of 5 mg/kg (Moustafa, 2003). Control rats were given an equivalent volume of saline. Blood and tissue samples were obtained 24 hr. post treatment.

**Glucose tolerance and plasma insulin**

Intraperitoneal (i.p.) glucose tolerance test was performed 24 hr after CdCl₂ treatment. Glucose load (3 g/kg) was given to 24 hours-fasted rats and blood samples were obtained from lightly anesthetized rats with ether from the orbital sinus at 0, 30, 60 and 120 minutes post glucose loading.

**Determination of tissue and serum metabolites**

The rats were killed by decapitation and the livers were rapidly excised, immediately rinsed in saline, trimmed and quickly weighed. For the determination of liver GSH content, 4 parts of 0.15 M KCl₂ were added to each liver specimen for homogenization. The homogenates were used for the determination of GSH as described earlier by Tiez (1969). Liver homogenates 10 % w/v in cold water were used for the estimation of lipid peroxidation level by the thiobarbituric acid (TBA) test according to the method of Uchyama and Mihara (1978). Tissue homogenate (100 mg tissue/ml) was used for the determination of the liver content of total proteins.( Serum samples and liver homogenates were analyzed by the staff at the Clinical Pathology Laboratory in the Faculty of Medicine, Suez Canal University). A Hitachi 704 autoanalyzer was used for the determination of serum aminotransferases: aspartate amino-transferase (AST) and alanine amino-transferase (ALT), serum total proteins and the liver content of total proteins. Plasma insulin was measured using the insulin radioimmunoassay as described by Reeves (1983).

**Results**

**Effect of CdCl₂ on Glucose tolerance and plasma insulin**

CdCl₂ (5 mg/ kg)-treated rats showed significant increases in blood glucose levels 30, 60 and 120 minutes post glucose administration as compared to control rat (Fig 1). On the other hand, glucose intolerance in Cd-treated rats was accompanied by a significantly reduction elevation in insulin levels in response to glucose stimulation 30 and 60 minutes after glucose administration as compared to control rats (Fig. 2).

**Effects of CdCl₂ on hepatic Total proteins glutathione and lipid peroxidation (Thiobarbituric acid [TBA] reactants).**

The results in table (I) represent the toxic effect of CdCl₂ on hepatic cells, as
indicated by its marked depletion of hepatic total proteins & GSH and its induction to a significant elevation in TBA reactants in the liver of rat.

Effects of CdCl₂ on serum metabolites
Data in table (I) indicate a significant decrease in the serum total protein levels in the CdCl₂-treated group, while it didn’t induce any significant change in the serum levels of the liver-specific enzymes (AST & ALT) activities.

Statistical analysis
The data were analyzed using one way ANOVA.

Table 1. Effect of CdCl₂ intoxication on same liver and serum parameters in control and CdCl₂-treated rats.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>CdCl₂-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver GSH (µg/g)</td>
<td>10271.86 ± 2072</td>
<td>6325.75 ± 294.7*</td>
</tr>
<tr>
<td>Liver TBA</td>
<td>0.032 ± 0.008</td>
<td>0.119 ± 0.014*</td>
</tr>
<tr>
<td>Total protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver (mg/g)</td>
<td>120.52 ± 11.5</td>
<td>70.22 ± 9.2*</td>
</tr>
<tr>
<td>Serum (mg/dl)</td>
<td>5.09 ± 0.983</td>
<td>3.49 ± 0.06*</td>
</tr>
<tr>
<td>Serum AST (U/l)</td>
<td>121 ± 11.2</td>
<td>135 ± 12.1</td>
</tr>
<tr>
<td>Serum ALT (U/l)</td>
<td>47.8 ± 5.7</td>
<td>63.7 ± 7.9</td>
</tr>
</tbody>
</table>

Rats were intraperitoneally (i.p.) treated with 5 mg/kg. Data represent the means ± S.E of at least 5 animals. Serum and tissue samples were collected 24 hr-post treatment. *P<0.05.

Figure 1: Serum glucose responses to an intraperitoneal (i.p) glucose challenge (3 gm/kg) in control and i.p. injected rats with CdCl₂ (5 mg/kg). Experiments were performed 24 hr after CdCl₂ intoxication. Results represent the mean ± SE of at least five rats. Significantly different from control:* P< 0.05.
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Figure 2: Serum insulin responses to an intraperitoneal (i.p) glucose challenge (3gm/kg) in control and i.p. injected rats with CdCl₂ (5mg/kg). Experiments were performed 24 hr after CdCl₂ intoxication. Results represent the mean ± SE of at least five rats. Significantly different from control:* P<0.05.

Discussion

Glucose intolerance observed in cadmium chloride-intoxicated rats could be mediated through different mechanisms. Cd-mediated free radicals production could be involved in the increase in lipid peroxidation in the livers of CdCl₂-treated rats. Peroxidation of the lipid moiety of the cellular membrane may distort the structural integrity of these membranes, thereby modifying their functions. One of the most important functions of the cell membrane is the transport of various molecules into and out of the cells. Therefore, increased lipid peroxidation due to cadmium treatment may impair the rate of glucose transport into the cells. Glucose transport was reported to be the rate limiting step for overall glucose metabolism (Elbrink and Bihler, 1975). So impaired glucose transport could be the mechanism behind the development of glucose intolerance in Cd - treated rats (Moustafa, 1998), Alloxan-treated rats (Moustafa, 2003) and in aged rats (Moustafa et al., 1995).

On the other hand, accumulation of lipid peroxidation products in the process of organismal aging causes changes in the activity of erythrocyte membrane adenylate cyclase and protein kinase (Pfeffer & Swislocki, 1976), influences the functions and the stability of hemoglobin (Kikugawa et al., 1984) and disturbs aminophospholipid organization (Jain, 1984). These effects may impose serious consequences to glucose metabolism. They may affect cell hormonal interaction therefore, disturb the action of the hormones that regulate carbohydrate metabolism.

Another possible mechanism for the development of impaired glucose tolerance in CdCl₂-intoxicated rats is impaired insulin synthesis. The observed CdCl₂-induced hypoinsulinemia has also
been reported in the study of Merali and Singhal (1975). The same study has shown that Cd intoxication in the rat was associated with a decreased pancreatic secretory activity as evidenced by lowered insulinogenic indices and marked inhibition of phentolamine-stimulated insulin release. CdCl₂-induced hypoinsulinemia could be attributed to an adrenaline response since adrenaline is known to inhibit insulin secretion. Stress-induced increase in adrenaline secretion after drug intoxication has previously been reported (Kim and Na, 1991). Alternatively, the observed glutathione depletion in CdCl₂-treated rats may cause perturbations in the redox potential of cells which alters many important functions including biosynthetic reactions (Hazelton and Calvin, 1980). This could mean a direct effect of CdCl₂ on the biosynthetic capacity of the pancreatic islets, leading to impaired insulin synthesis. The decreased protein content in the serum and livers of CdCl₂-treated rats supports this hypothesis.

Glucose intolerance induced by cadmium chloride administration may be mediated through enhanced glycogenolysis and the stimulated glucose release from its stores. The stimulating effect of CdCl₂ on the glycogenolytic enzyme adenylyl cyclase as evidenced by its ability to increase the concentration of hepatic cyclic adenosine monophosphate markedly (Merali and Singhal 1975), and its effect of significantly increasing the activities of the hepatic enzymes fructose 1,6-diphosphatase and glucose 6-phosphatase (Merali and Singhal 1975) give a support support to this idea.

Additionally, Cd was reported to tie up the SH group of the protein layers resulting in S-Cd-S linkage (Kleinfeld, et al., 1955). Moreover, it has been reported that Heavy metals may react with enzymes, both soluble or membrane components, either by displacing the metal physiologically associated with the protein molecules or by binding to their functional groups (sulphydryl, carboxyl, etc.) (Eichorn, 1973). This effect of cadmium could mean the inactivation of the family of the SH-dependent enzymes that regulate carbohydrates metabolism. This could be one of the mechanisms accounting for the glucose intolerance resulting from CdCl₂ intoxication.

In conclusion the current study confirms the hazards of exposure to environmental pollution including exposure to heavy metals and reveals an important phase of cadmium toxicity, as its deleterious effects on carbohydrates metabolism. However further studies are needed to investigate the following points: a- The effect of CdCl₂ toxicity on the rates of insulin synthesis & secretion. b- Relationship between CdCl₂ toxicity and the number and the affinity of insulin receptors. c- Effect of lipid peroxidation on the rates of basal and insulin-stimulated glucose transport and metabolism (rat adipocytes are recommended as a cellular model for these studies).

References
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القصور في احتمال الجلوكوز نتيجة للتسوؤم بالمعادن الثقيلة في الجرذان

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تشير نتائج الدراسة الحالية إلى أن الحقن البيرتوني لذكور الجرذان بكميات كادميوم (5 مجم/كجم) قد نتج عنه التدهور في منحنى احتمال الجلوكوز والذي تم قياسه عقب 24 ساعة من الحقن بكلوريد الكادميوم. وقد صاحب هذا التدهور عدم زيادة هرمون الإنسولين بالقدر المعتاد عند التحميل بالجلوكوز. وقد أدى الحقن بكلوريد الكادميوم إلى النقص المعنوي في محتوى الكبد من كل من الجلوتاتيون والبروتين بعد 24 ساعة من الحقن وقد كان هذا النقص مصحوبًا بالارتفاع الملموس في محتوى الكبد في نواتج أكاسدة الدهون (TBA-reactants). وقد لوحظ ان تركيز البروتينات الكلوية بالمصل قد انخفض انخفاضًا ملحوظًا نتيجة للتسوؤم بكلوريد الكادميوم بينما لم يحدث تغيراً ملحوظاً في نشاط الإنزيمات الناقلة لمجموعة الأميل (AST & ALT) في المصل. وتقترح الدراسة الحالية أن القصور في احتمال الجلوكوز الذي حدث بسبب الحقن بكلوريد الكادميوم نتيجة تحفيز تكوين نواتج أكاسدة الدهون والتي ثبت أنها تعوق انتقال جزيء الجلوكوز إلى داخل الخلية. وقد اقترح أيضاً أن الخلط في تخلية الإنسولين وإبطال نشاط مجموعة من الإنزيمات التي تحفز عملية تمثيل المواد الكربوهيدراتية كنتنات مترتبة على إضاحي أمادة الجلوتاتيون يمكن أن يكون من الآليات المسببة القصور في احتمال الجلوكوز المصاحب للتسوؤم بعنصر الكادميوم.

وتلتقي الدراسة الحالية الضوء على أحد الجوانب الخطيئة للتسمؤم بعنصر الكادميوم وهو ما يؤكد على ضرورة إجراء المزيد من البحوث للكشف عن جميع جوانب التلوث بالمعادن الثقيلة والذي يفتح المجال إلى التوصل إلى أساليب الوقاية من هذا التسمؤم.