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Prospective protective role of melatonin against arsenic-induced metabolic toxicity in Wistar rats

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Abstract

Subchronic exposure to arsenic is associated with alteration of glucose homeostasis. Arsenic treatment (as sodium arsenite) of male Wistar rats (weighing 130–150 g) at a dose of 5.55 mg kg^{-1} body weight (equivalent to 35% of LD_{50}) (i.p.) per day for a period of 30 days produced hypoglycemia, with associated increased urinary excretion of glucose and depletion of liver glycogen and pyruvic acid contents. Mobilization of free amino acids from kidney to liver was facilitated by arsenic treatment. Arsenic exposure significantly decreased the glutamate-pyruvate transaminase activity in kidney. Glucose 6-phosphatase activity in liver tissue was also significantly decreased after arsenic treatment. In addition to these, liver lactate dehydrogenase activity was elevated due to arsenic treatment.

Melatonin supplementation (i.p.) at a dose of 10 mg kg^{-1} day^{-1} for last five days prior to sacrifice reversed most of the above changes caused by arsenic. Melatonin, being a potent free radical scavenger may reduce arsenic-induced free radical production, and thereby, eliminating its toxic effects. So, arsenic-induced hypoglycemia, with associated glycogenolytic as well as glycolytic activities of liver can be partially counteracted by melatonin supplementation. Accordingly, it may be suggested that melatonin can serve as a prospective protective agent against arsenic-induced metabolic toxicity.

Keywords: Arsenic toxicity; Glucose metabolism; Melatonin supplementation

1. Introduction

Arsenic, being a potent environmental toxic agent, leads to development of various hazardous effects on human health. Arsenic is considered as a human carcinogen (Wang and Huang, 1994). Cellular mechanism of arsenic toxicity involves generation of reactive oxygen species (ROS) (Chen et al., 1998). Moreover, ROS is involved in genotoxicity caused by arsenic (Jha et

Abbreviations: LDH, lactate dehydrogenase; GOT, glutamate-oxaloacetate transaminase; GPT, glutamate-pyruvate transaminase; ROS, reactive oxygen species; i.p., intraperitoneal; γ-GCS, γ-glutamyl cysteine synthetase

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al., 1992), tissue degenerative changes (Prasad and Rossi, 1995) and unstability of cytoskeletal structure (Li and Chou, 1992). Enhanced generation of ROS after arsenic exposure alters cells' intrinsic antioxidant defenses and results in oxidative stress (Ercal et al., 2001). In addition to these findings, there are several reports of altered carbohydrate metabolism in arsenic intoxication (Boquist et al., 1988; Reichl et al., 1988; Szinicz and Forth, 1988). Liver glucose and glycogen depletion were found to be a major problem in arsenic toxicity (Kawaguchi, 1981; Reichl et al., 1990). This finding is further supported by the study of Reichl et al. (1991), who also indicated that liver glucose depletion is an important factor of arsenic toxicity. Recently, Pal and Chatterjee (2002) also demonstrated that hypoglycemia with associated glycogenolysis and other associated changes are supposed to be involved in arsenic-induced alteration of glucose homeostasis.

Melatonin (*N*-acetyl 5-methoxytryptamine), a pineal hormone, has been found to influence various physiological activities, such as neuroendocrine functions (Reiter, 1988; Falcon et al., 2003), seasonal reproduction (Malpaux et al., 1999; Bayarri et al., 2004) and immune functions (Srinivasan, 1989, Hotchkiss and Nelson, 2002). Studies by Bandyopadhyay et al. (2002) revealed that melatonin has protective effect against gastric ulceration. Several reviews have supported claims that melatonin possesses high antioxidant activity (Reiter, 1996, 1997). It detoxifies a variety of free radicals and reactive oxygen intermediates (Tan et al., 1993). The antioxidant effect of melatonin has been claimed as a protective factor towards carcinogenesis, neurodegeneration and aging (Hardeland et al., 1993; Poeggler et al., 1994; Melchiorri et al., 1995). In view of the above, the present investigation is carried out to reveal the effects of melatonin supplementation on altered glucose homeostasis due to arsenic treatment. Melatonin, being a potent antioxidant is expected to reduce the oxidative stress caused by arsenic. So, it is further intended to see whether melatonin can counteract the hypoglycemic effect of arsenic.

2. Materials and methods

2.1. Animal maintenance

Eighteen male rats of Wistar strain weighing 130–150 g were included for the present investigation. They were maintained in the laboratory conditions with 18% protein diet and sufficient drinking water for seven days. The 18% protein (casein) was used as it is considered as an adequate (normal) dietary protein level, which was used on earlier occasions (Chatterjee et al., 1984; Ghosh et al., 1992; Maiti and Chatterjee, 2000; Nayak and Chatterjee, 2001). The carbohydrate (amylum) content of the diet was kept at 71%. The other ingredients of the diet and supply of vitamins were same as reported earlier (Chatterjee et al., 1976, 1984). The animals were then divided into three groups of equal average body weight. They were continued with the 18% protein diet.

2.2. Arsenic treatment and melatonin supplementation

The animals of one of the groups were injected intraperitoneally (i.p.) with arsenic as sodium arsenite at a dose of 5.55 mg kg^{-1} body weight per day for a period of 30 days and marked as arsenic-treated group. The present dose of sodium arsenite is equivalent to 35% of LD_{50}, which was determined earlier (Maiti and Chatterjee, 2000). The animals of one of the groups were also treated with sodium arsenite at the same dose and duration along with melatonin supplementation (i.p.) 1 h before arsenic injection at a dose of 10 mg kg^{-1} body weight for last five days prior to sacrifice. The animals of the remaining group received the vehicle (0.9% NaCl) only and served as control. The animals of the control group and those of arsenic-treated group supplemented with melatonin were pair-fed to arsenic-treated group.

2.3. Collection of urine, blood and tissues

After the period of treatment was over, the animals of each group were kept fasted overnight in separate metabolic cages to collect 24 h urine. The animals were then sacrificed by cervical dislocation. Blood was collected from hepatic vein in a heparinized tube, containing sodium citrate as glycolytic inhibitor and kept in frozen condition. Liver and kidney tissues were removed, washed in ice-cold 0.9% NaCl, blotted dry and stored at −20 °C until analysis.