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Effect of *n*-propylthiouracil or thyroxine on arsenic trioxide toxicity in the liver of rat

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Abstract

Involvement of thyroid gland in the hepatotoxic manifestations of arsenic trioxide (As^{III}) has been studied in rat. The effects of *n*-propylthiouracil (PTU) (a thyrotoxic compound) and L-thyroxine (a thyroid hormone) have been studied with reference to T_3 and T_4 values in the serum, arsenic concentration in the liver, Ca^{2+} accumulation in the liver, aspartate transaminase, alanine transaminase and bilirubin values as the indicators of liver function, histopathological observations and finally the ultrastructural studies. It is concluded that hypothyroid condition protects against As^{III} toxicity. Scavenging of reactive oxygen species (ROS) that significantly contribute in As^{III} toxicity, by high intracellular concentration of reduced glutathione, as a consequence of PTU treatment is proposed as the plausible protective mechanism.

Keywords: Arsenic trioxide; Hepatotoxicity; *n*-propylthiouracil; L-thyroxine; Rat

Introduction

Arsenic is an ubiquitous metalloid. It is well known for its toxicity and carcinogenicity in humans [1,2]. Arsenic may exist in several forms, including inorganic forms – arsenic, arsenite and arsenate as well as methylated species (monomethylarsonous acid and dimethylarsinic acid). While both forms are toxic, they exhibit distinct biochemical properties. Trivalent arsenic (As^{III}) has a high affinity for sulfydryl groups [3], whereas pentavalent arsenic (As^{V}) mimics phosphate as uncoupler of oxidative phosphorylation [4]. Thus elucidation of the effects of arsenic is complicated owing to speciation of the metalloid. However, both *in vivo* and *in vitro* studies show that trivalent arsenical form is more toxic than pentavalent form [5,6] In biological systems, pentavalent arsenicals are reduced to trivalency before methylation. Earlier, methylation was considered a detoxification pathway, however, recent studies indicate that methylated arsenicals are more toxic than inorganic arsenic [7,8]. Nevertheless, it has been agreed that exposure to arsenic generates reactive oxygen species (ROS) *in vivo* such as dimethylarsenic radical ($(CH_3)_2As^{\bullet}$), dimethylarsenic peroxyl radicals ($(CH_3)_2AsOO^{\bullet}$), superoxide anion, singlet oxygen and hydroxyl radicals. Free radical theory of arsenic toxicity/carcinogenicity has been largely accepted [9].

Further, metabolism of arsenic has been found to be affected by several factors. Selenite is known to influence the disposition of arsenate and arsenite in rats [10]. Ascorbate is also known to modulate arsenic toxicity [11]. An earlier study from our laboratory has shown that thyroid hormones ameliorate oxidative stress induced by As^{III} in liver and kidney of rat [12]. Recent

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investigations have suggested that several environmental contaminants affect thyroid gland [13]. Thyroid status in turn might affect toxic manifestations of these chemicals [14]. Influence of thyroxine and *n*-propylthiouracil (PTU) on nephrotoxicity of inorganic arsenic has also been studied in our laboratory [15]. Therefore, a study on hepatotoxicity of arsenic trioxide in rats made hyper and hypothyroidic by administering L-thyroxine (T_4) and PTU, respectively, was considered important.

The present communication describes histopathological and ultrastructural effects of arsenic in the liver of hyper and hypothyroidic rats. Serum transminases, alkaline phosphatase and bilirubin have also been estimated to access the liver function. In addition, total calcium (Ca^{2+}) has been estimated in the liver to denominate hepatocellular injury.

Materials and methods

Chemicals

As^{III} was procured from Loba Chemie (Mumbai). T_4 was purchased from Glaxo Laboratories (Mumbai) and PTU was supplied by Sigma Chemical Company (USA). RIA kits for T_3 and T_4 were procured from Diagnostic Products Corporation (LA, USA). Glutaraldehyde, osmium tetraoxide, Epon 812, uranyl acetate and lead citrate were procured from Sigma Chemical Company (USA).

Animals and diet

Adult male Wistar rats weighing 150 ± 30 g were maintained under standard laboratory conditions (room temperature 20 ± 5 °C and relative humidity $= 50 \pm 10\%$). The rats were acclimated to laboratory housing conditions under 12 h light and dark cycle for 2 weeks. Each rat was housed separately in a polypropylene cage and offered pelleted food (Golden Feeds, New Delhi) and tap water *ad libitum*. All experiments were performed after the approval of the Institutional Ethical Committee according to the standards laid down by Ministry of Social Justice and Empowerment, Government of India.

Experimental procedure

After acclimatization, rats were divided at random into six groups of five rats each. Rats of groups B and E were injected T_4 (25 μg/100 g body weight) intramuscularly on every 4th day for 3 weeks. Similarly, rats of groups C and F were injected PTU (2.5 mg/100 g body weight) intramuscularly twice a week for 30 days. Rats of group D were administered As^{III} only (4 mg/100 g body weight) by gavage whereas rats of group A were offered saline to serve as controls. After these experiments, rats of groups E and F were further subjected to arsenic treatment. Each rat of these groups was administered a predetermined sublethal dose, i.e., 4 mg/100 g body weight of arsenic trioxide dissolved in saline through gavage on each alternate day for 30 days [12]. Gain or loss in the body weight of each rat was recorded daily. Suitable care was taken to maintain general health and hygiene of experimental rats. No mortality occurred during these investigations.

On the 31st day, all the rats were starved overnight and sacrificed next morning to make following observations.

Determination of T_3 and T_4

Blood was collected through cardiac puncture. Serum was separated by centrifugation and used for T_3 and T_4 estimations. RIA kits were used in this study. Coat-A-count procedure is a solid-phase radioimmunoassay wherein labeled T_3/T_4 competes with T_3/T_4 present in the sample for antibody sites. This reaction takes place in the presence of blocking agents that serve to liberate bound T_3/T_4 from carrier proteins. Hence the assay measures total T_3/T_4 since both free and protein bound T_3/T_4 from the sample are able to compete with respective radiolabelled antibody sites. After the tubes were decanted and counted, the concentration of hormones was read from a standard curve.

Estimation of arsenic

Liver was carefully removed from the rats. One gram of liver sample was digested in 10 mL of concentrated nitric acid at 80 °C for 16 h. The digests were stored at 4 °C before analysis. A 2 mL aliquot of the digest was analyzed for arsenic by hydride generation at pH 6 using sodium borohydride as the reducing agent. The hydride was collected on a cryogenic column before quantification by inductively coupled plasma-emission spectrophotometry (ICP-ES), using equipment supplied by GBC (Australia). These samples were analyzed at University Science Instrumentation Centre, Indian Institute of Technology, Roorkee (India).

Liver function tests

Serum concentrations of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using a commercial kits [16]. Serum alkaline phosphatase was determined by the method of Kind and King [17]. Phosphate concentration was determined as suggested by Fiske and Subbarao [18]. Serum bilirubin was analyzed using the method of White et al. [19].

Calcium estimation

A total of 10% (w/v) homogenates of liver samples were prepared in saline. Calcium in the liver sample was