Effects of Toluene on Rat Kidney

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Abstract

Introduction: Occupational exposure to Toluene is associated with development and progression of renal failure. However, the cellular mechanisms by which these agents cause renal dysfunction and injury remain elusive. The present study investigates the effect of Toluene on animal model (rat) kidney to present a broader understanding of the mechanism by which Toluene causes renal injury.

Methods and Materials: Adult male rats received Toluene at doses of 300, 600 and 900 mg/kg for seven consecutive days. Control group received vehicle only, and 24 hours later, animals were euthanized with overdose of sodium pentobarbital. Blood was collected for determination of BUN and creatinine (CR). Kidney tissues were removed, fixed and processed for light microscopy and determination of glutathione (GSH). Ten animals were used for each group.

Results: Biochemical and histopathological observations indicated that Toluene produced injury in the kidney. Statistical analysis indicated significantly increased BUN, CR and decreased GSH levels as compared to control values. Toluene induced dose-dependent injury in kidney.

Conclusions: The finding that Toluene induced injury in proximal convoluted tubular cells suggests that kidney may metabolize Toluene in situ to a nephrotoxic metabolite. The observation that Toluene depleted GSH level in kidney support the view that generation of oxidative stress is responsible for its toxicity.

Keywords: Toluene, kidney, glutathione (GSH).

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Introduction

Toluene (Tol) is widely used as an organic solvent. This chemical is used in the synthesis of various products. It is a major component in paints, varnishes, printing inks, degreasers, adhesives, fuel additives, glues, thinners, and plastics (1). Exposure to Tol may happen through various factors such as drinking water, food, consumer products and solvents in workplace or chemical abuse. In addition, automobile exhaust can emit Toluene into the air (1). Tol exposure has been associated with numerous health effects in human and experimental animals. The main effect of Toluene is on nervous system, but animals exposed to moderate or high levels of Toluene may also show harmful effects in their liver, kidneys, and lungs (1).

Occupational exposure to this agent can provoke fatigue, memory loss, headache, dizziness, auditory, and liver damage (2). Kidney damage was also noted in workers occupationally exposed to Tol(3). Renal function was measured in workers exposed to a mixture of Toluene and xylene. The levels of these parameters suggested mild tubular lesions (4). Metabolic acidosis and renal tubular injury due to pure Toluene inhalation in a 22-year old woman was reported (5). Charão et al. reported that reduced glutathione (GSH) levels decreased in painters who were occupationally exposed to organic solvents in comparison with non-exposed subjects (6).

Kronevi et al. showed that epicutaneous administration of Toluene in pigs caused histopathological damage in kidney, progressing nuclear pyknosis and junctional separation between the basement membrane and the basal cells (2). Acute renal failure induced by Toluene-containing adhesive was also reported. Histopathological alterations provoked by thinner inhalation are tubular damage and severe tubulointersticialnephrits in kidneys (7). Meydan et al. found that Toluene intraperitoneally injected into the rats with a dose of 500 mg/kgcaused alteration of glutathione peroxidase, catalase and malondealdehyde levels and produced kidney damage (8). Martinez-Alfaro et al. reported occupationally exposed to thinner contained 60-70% of Tol produced oxidative stress in exposed workers when compared to non-exposed subjects (9). The results of in vitro study indicated that Tol produced proximal cell damage (10). The effects of Toluene on animals are similar to those seen in humans (1). To our knowledge dose related effects of Tol on kidney has not been reported previously. The study of the effect of dose-related effects of Tol on kidney toxicity may be useful for a better understanding on the clinical pictures following its exposures in humans. The aim of the present study was to determine dose related effects of Tol on rat kidney.

Methods and Materials

Adult male Wistar rats (250-300 g) were housed in groups of three in clear polypropylene cages in a light cycle (12h light and 12h dark cycle) and 23± 2 Cº temperature. The animals were fed with concentrated food pellets and tap water ad libitum. Adult male rats received Toluene (Tol) at doses of 300, 600 or 900 mg/kg for seven consecutive days. Control group received vehicle only 24h later, animals were sacrificed with overdose of sodium pentobarbital. Blood was collected for determination of BUN and creatinine (CR). Kidney tissues were removed for determination of glutathione (GSH) or fixed and processed for light microscopy.

The levels of GSH in kidney homogenates were assayed by the method of Moren et al. (11).

For histopathology observation, the kidney tissues were removed, fixed and processed for light microscopy. The tissue was fixed in 10% buffered formalin for 24 hours, routinely processed and paraffin embedded. Five histological sections, each at least 15 μm apart, were taken from each

tissue block and stained with Hematoxylin and Eosin (H&E). The criteria for cell injury included: nuclear dilation, loss of staining capacity and obvious cellular swelling. Ten animals were used for each treated group. The protocol was approved by the Ethics committee of Ahvaz Jundishapur University of Medical Sciences.

Biochemical data were expressed as mean ± standard error of the mean. The results were analyzed by analysis of variance, completely randomized design, and treatment differences were identified by the method of Newman-Keuls and $p<0.05$ was used as the criterion of significance. Ten animals were used for each treatment group.

Results

A dose related increase in BUN concentration were observed after treating animals with Tol when compared to those in control group (Fig. 1). Similarly, the level creatinine was significantly increased in rats in dose-dependent manner (Fig. 2). However, the elevation of BUN and creatinine were more predominant in rats treated with higher dose of Tol. In contrast, the level of GSH decreased in dose-dependent manner in rats treated with Tol when compared to control group (Fig. 3).

Administration of vehicle alone did not produce detectable injury in rat kidney (Fig.4). However, dose-related injury in Tol-treated rats was noted. Light microscopy revealed that renal tubular cells were swollen, had loss of staining capacity, and nuclei appeared to be dilated, presence of blood clot was noted in Tol treated rats (Fig. 5).

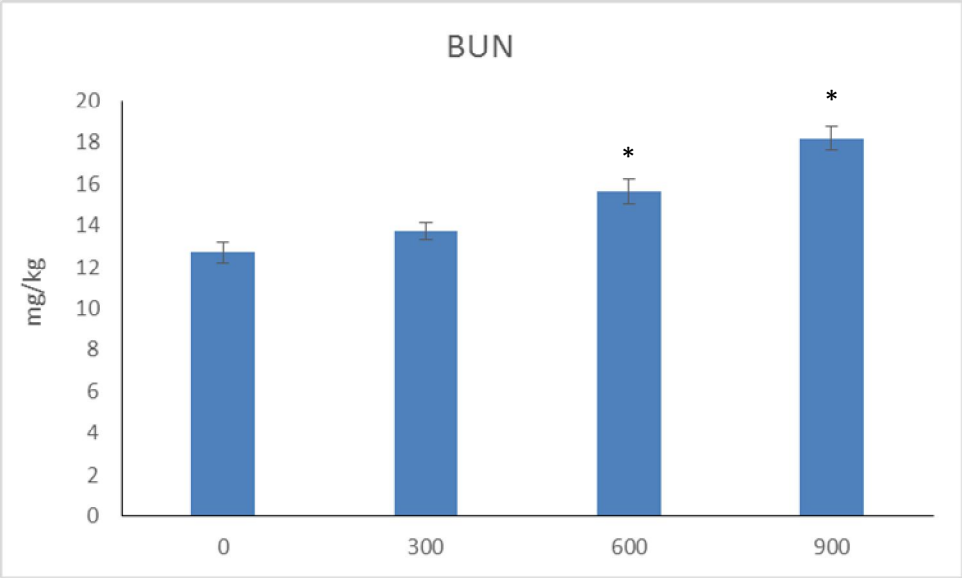

Fig 1: Effects of Toluene on rat BUN. * Significantly different from control group $p<0.05$

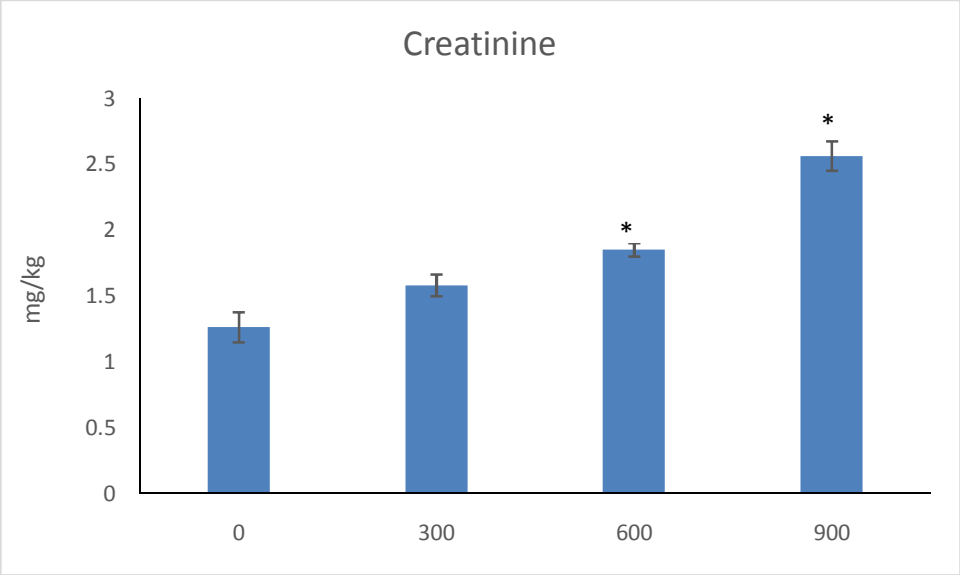

Fig 2: Effects of Toluene on rat creatinine. * Significantly different from control group p<0.05

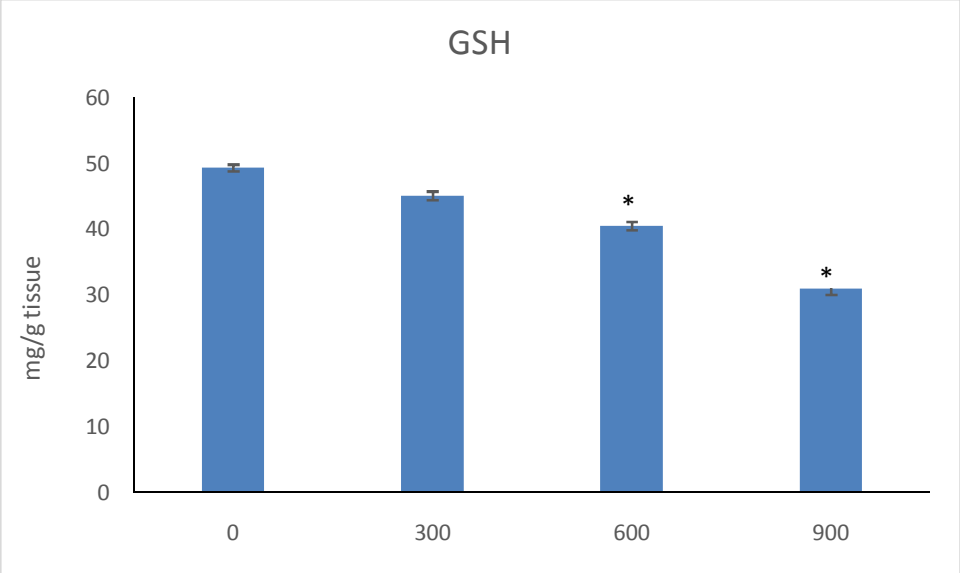

Fig 3: Effects of Toluene on rat glutathione (GSH). * Significantly different from control group p<0.05

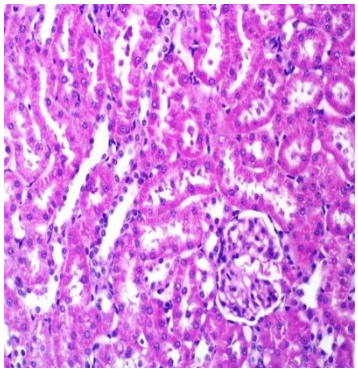

Fig 4: Light micrograph of rat kidney treated with vehicle (normal saline). There was no obvious injury in the kidney cells. *H&E* X200

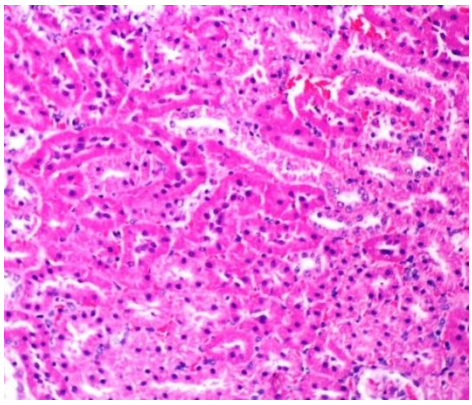

Fig 5: Light micrograph of rat kidney treated with 900 mg/kg Tol. Disorganization and congestion are apparent. *H&E* X200

Discussion

Toluene exposure results in structural and functional impairment of various organs (1). Kidney is the main organ for xenobiotic excretion. It is the target of numerous xenobiotic toxicants, including environmental chemicals. Anatomical, physiological, and biochemical features of kidney make it particularly sensitive to various environmental compounds. Occupational exposure to solvents is associated with development and progression of tubulointerstitial fibrosisand chronic renal failure (3,4). However, the cellular mechanisms by which these agents cause renal dysfunction and injury remain elusive.

Nephrotoxicity concern over occupational hazards associated with solvents has led to considerable interest to study of such agents on experimental animals. To our knowledge, the effects of dose-related Tol on kidney have not been reported previously. Blood urea nitrogen (BUN) and serum creatinine levels were measured for renal function. Dose-dependent elevation of BUN and creatinine in Tol-treated rats was observed when compared to control animals. Meydan et al. showed that intraperitoneal administration of 500 mg/kg Tol caused elevation of BUN and creatinine in rats when compared with the control group (8). Renal function impairment was noted in workers exposed to mixture of Toluene and xylene. Kidney function was measured in workers exposed to a mixture of Toluene and xylene. Renal function impairment indicators included total proteinuria, albuminuria, and urinary excretion of muramidase; the levels of these parameters suggested mild tubular damage (4)

Epicutaneous administration of Toluene in pigs produced renal injury (2). It was observed that Tol induced dose-related morphological alterations in rat kidney. Tol producing cell damage was mainly observed in proximal convoluted tubular cells (12). Franchini et al. (4) suggested that occupational exposure to Tol induced renal tubular lesion. In vitro study showed that Tol caused proximal cell damage in rat kidney (10). This observation supports the view that proximal cells of rat kidney have a potential for bioactivation of this chemical and may play an essential role in Tol toxicity. Occupational exposure to Tol was found to be associated with development and progression of renal failure. Death occurs in proximal tubular cells exposed to Tol. Long-term exposure to this chemical is associated with proximal apoptosis (10). Renal tubular injury due to pure Toluene inhalation in a 22-year old woman was reported (5).

The mechanism by which Tol causes nephrotoxicity is not completely understood. The in vivo dose-response

relationship between Toluene and reactive species (ROS) formation in rat was reported (13). We observed that Tol caused depletion of GSH in dose dependent manner in rat kidney.

It has been postulated by numerous investigators that modification, particularly depletion, of intracellular concentrations of GSH in the kidneys has a significant influence on the renal toxicity of xenobiotics (14, 15). El-NabiKamel andShehatashowed the effect of 650 mg/kg Toluene exposure (15, 30 and 45 days) on the oxidative stress and antioxidant status of rat kidney. They found that Tol induced time-related oxidative stress (14).

The results of the present study demonstrated that Toluene damages kidney tissue and that it is a nephrotoxic substance in a dose-related manner. Martínez-Alfaro found that glutathione depletion was induced by Toluene (9). Charão et al. reported that reduced glutathione levels were decreased in painters occupationally exposed to organic solvents including Toluene when compared to non-exposed subjects (6).Thus, it appears that Tol generation of reactive metabolites and depletion of GSH is responsible for Tol-induced nephrotoxicity. Kidney may be damaged by Tol through reactive metabolites generated by the cytochrome P450 monooxygenases(16).

As Tol is eliminated via kidney, another possibility for Tol-caused renal damage is that translocation of Tol metabolites from the liver to the kidney via general circulation produces kidney injury. However, the generation of metabolites in kidney may at least in part be responsible for kidney toxicity.

In conclusion, results of the present study indicated that Tol induced dose-dependent injury in rat kidney. Elevation of BUN and creatinine in dose-dependent manner indicated that impairment of renal function is related to Tol concentration. The finding that Tol diminished renal GSH levels in dose dependant manner supports the view that kidney has the ability to metabolize Tol and induces oxidative stress.

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