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Original Article

Protective Effects of Verapamil Against Hexachlorobutadiene Nephrotoxicity in Rat

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Abstract

Background: Hexachlorobutadiene (HCBD), a potent nephrotoxin can cause degeneration and necrosis in renal tubular epithelial cells in rodents. Its toxicity is due to conjugation with glutathione to form the related cysteine conjugate. This metabolite is then taken up by the kidney and cleared through renal tubular epithelial cells as a reactive thiol derivative by the enzyme β-lyase.

Objective: To evaluate the protective effect of verapamil against HCBC nephrotoxicity.

Method: Five groups of Winstar Albino rats were treated as follows: Group 1(corn oil), Group 2 HCBD (50mg/kg), Groups 3 and 4 verapamil (50 and 100µg/kg) one hour before HCBD (50mg/kg) and Group 5 verapamil (100µg/kg) one hour before HCBD (100mg/kg). All animals were killed after 24 hours.

Results: Histopathologic examinations showed substantial necrosis in straight portion of the proximal tubules. In verapamil-treated groups the kidney appeared normal. The concentration of urea and creatinine, as a marker of kidney damage, was significantly higher in HCBD-treated, as compared with the control group.

Conclusion: Verapamil, a calcium channel blocker, can protect the kidney against toxic effect of HCBD in rats.
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Keywords · hexachlorobutadiene · verapamil · nephrotoxicity · cysteine conjugate

Introduction

Verapamil, a calcium channel blocker, is used in patients with angina pectoris and cardiac arrhythmia. Calcium (Ca) is an important mediator of cell death in xenobiotic-induced cell injury. Alteration in intracellular calcium levels plays an important role in the cysteine conjugate-induced cell death. Thus, calcium channel blockers may affect the role of Ca in cellular toxicity. Some studies have shown that calcium channel blockers, protect kidney against nephrotoxins such as; mercuric chloride,[1] gentamicin,[2,3] cisplatin,[4] cephalosporins,[5] and cyclosporine.[6,7] As the toxicity mechanism of mercuric chloride is similar to that of HCBD and verapamil is shown to protect renal tissue against mercuric chloride,[1] it was decided to examine the protective effect of verapamil against HCBD-induced nephrotoxicity in rats.

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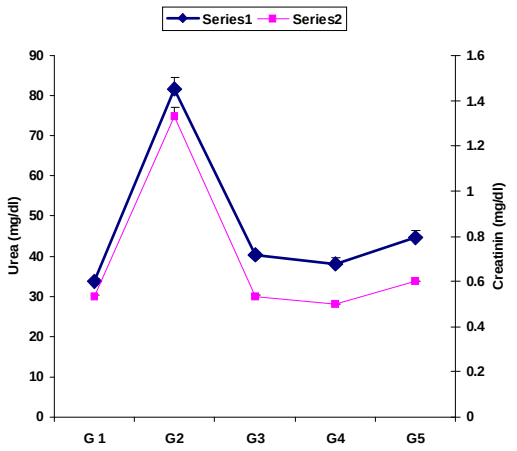

Fig 1: Concentration of urea and creatinine (mg/dl) in all treated groups. In HCBD-treated group concentration of both urea and creatinine are significantly higher that other groups.

HCBD is a potent nephrotoxin in rodents,[8] which can cause degeneration, necrosis, and regeneration in renal tubular epithelial cells.[9-11] Its toxicity is due to conjugation with glutathione (GSH) to form glutathione S-conjugate, and finally to the related cysteine-conjugate. This metabolite is then actively taken up by kidney and cleared through the renal tubular epithelial cells as a reactive thiol derivative by the enzyme β-lyase, which covalently binds to macromolecules.[12] The lyases are a group of enzymes, which catalyze the cleavage of C-S, C-N and C-C bonds. Some of these are capable of catalyzing the cleavage of cysteine S-conjugates by β-elimination. β-lyase has been distributed in many tissues of mammalians, helminthes and intestinal bacteria.[13-17] The S_3 region (pars recta) of the proximal tubule of rats' kidney is the most susceptible organ to the nephrotoxicity induced by cysteine conjugates.

Materials and Methods

HCBD was purchased from (Sigma-Aldrich Ltd, Poole, Dorset, UK), urea kit from (man Lab, Tehran, Iran) and verapamil, was kindly donated by (Alborz pharmaceutical company, Tehran, Iran).

In this study Wistar albino rats weighing 150-200g of either sex, provided by Animal Breeding Unit, Department of pharmacology, Ghaem hospital, Mashhad, Iran, were used. The animals were kept under light/dark cycle with 20° C and 50% humidity for 12 hours.

After acclimatization, they were divided randomly into 5 groups of 6 each that received

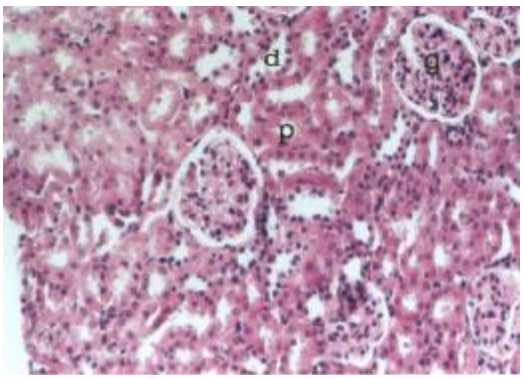

Fig 2: Representation of a normal rat kidney. All anatomical structures look normal (×20). g: glomerulus p: proximal tubule d: distal tubule

single doses of the following, via intraperitoneal route.

Group-1: Corn oil (1ml/kg).

Group-2: HCBD (50mg/kg).

Group-3: Verapamil (100µg/kg), one hour before HCBD (50mg/kg).

Group-4: Verapamil (50µg/kg), one hour before HCBD (50mg/kg).

Group-5: Verapamil (100µg/kg), one hour before HCBD (100mg/kg).

All animals were killed under light ether anesthesia 24 hrs after treatment. Blood samples were taken by cardiac puncture for measuring the levels of urea and creatinine that are indicators of kidney damage, using Technicon RA-1000 Autoanalyser. The right kidney was removed and fixed in 10% neutral buffered formalin, sectioned at 5µ and stained in Haematoxylin and Eosin (H&E) for histopatholoic studies.

Statistical analysis

The data were analyzed using one-way ANOVA and Tukey test.

Results

Concentrations of urea and creatinine are shown in Fig 1. As observed, concentration of urea (81.7±4.7 mg/100ml) in HCBD treated group was significantly higher ($p<0.01$) than control (33.8±1.3 mg/100ml,) and verapamil treated i.e., groups 3 (40.3±1.1 mg/100ml, $p<0.01$), 4 (38±5.4 mg/100ml, $p<0.01$) and 5 (44.8±6.2mg/100ml, $p<0.05$). Concentration of creatinine (1.08±0.3 mg/100ml in HCBD treated group was also higher than control (0.53± 0.05mg/ml $p<0.01$) and verapamil treated animals i.e. groups 3 (0.53±0.06mg/100, $p<0.05$), 4 (0.5±0.01, mg/100ml $p<0.01$) and 5 (0.6± 0.01 mg/100ml, $p<0.05$). There was no signifi-

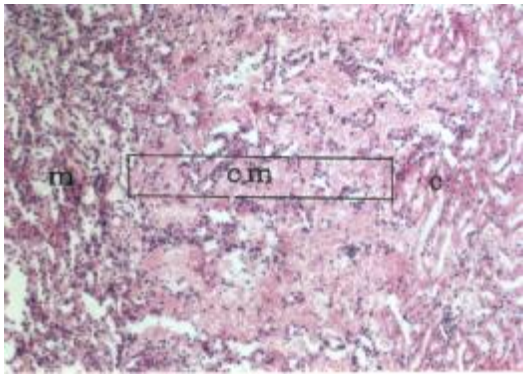

Fig 3: Response of rats in HCBD- treated group. Cortico-medullary junction (boxed area) has been extensively damaged (×10).
c: Cortex cm: Cortico-medullary junction
m: Medulla

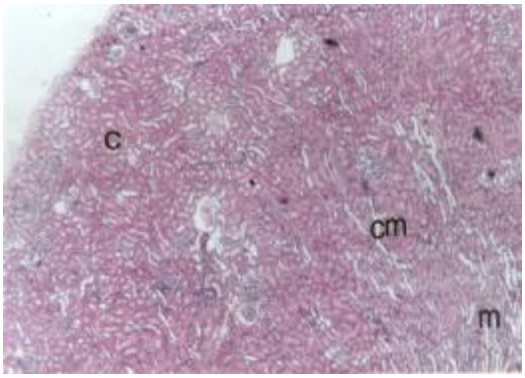

Fig 4: Response of rats in verapamil-treated group. Kidney shows a normal appearance (X 4).
c: Cortex cm: Cortico-medullary junction
m: Medulla

cant difference in urea and creatinine concentrations between corn oil and verapamil treated groups.

Light microscopic examination of sections of kidneys in control group stained with H&E showed that in control group glomerulus, bowman capsule, proximal, distal and collecting tubules appeared normal (Fig 2).

In HCBD treated group, an extensive damage in straight portion of proximal tubules, rich in β-lyase, was observed (Fig 3). Other parts of kidney, such as cortex and medulla, appeared normal. In verapamil-treated groups, all anatomical structures were normal as in control group (Fig 4).

Discussion

The present study has shown that verapamil had a protective effect against HCBD-induced nephrotoxicity in rat. In aspect of histopathology, the kidney of verapamil treated groups showed normal appearance similar to that of control group.

It is shown that calcium has an important role in cellular toxicity. As calcium homeostasis is precisely controlled, any alteration in intracellular calcium level will play an important role in the cysteine conjugate-induced cell death [18]. Thus, calcium channel blockers may affect the role of Ca in cellular toxicity. Toxicological consequences of increased intracellular Ca concentration are; i) activation of calpains leading to disruption of the protein components of cytoskeleton. ii) Activation of Ca-dependent endonuclease resulting in DNA single and double strand breaks and apoptotic or necrotic cell death. iii) Activation of Ca-dependent phospholipases (PLA_2), causing liberation of arachidonic acid and disruption of membrane integrity with ensuing cell death. iv) Activation of gene expression. The mechanism(s) for protective effect of verapamil is not clear, but may be mediated by: a) enhancing elimination of HCBD and/or its toxic metabolite, b) inhibiting the activation of Ca-dependent proteases (calpains), endonuclease and phospholipases, c) improving renal blood flow due to vasodilatory effect, which will reduce toxicity and d) preventing gene expression.

Several investigations have been carried out about the role of calcium channel blockers, against nephrotoxicity of mercuric chloride,[1] gentamicin[2,3] cephalosporines,[4] cycosporine,[6,7] cadmium[4] and cisplatin.[4] Verapamil is able to protect kidney against mercuric chloride toxicity. Animals treated with verapamil (75μg/kg, i.p) prior to mercuric chloride, showed normal kidney appearance.[1] The protective effect of other calcium blockers such as diltiazem and nifedipin against toxicity of HCBD and other nephrotoxins is yet to be investigated.

Acknowledgments

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