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Protective effect of telmisartan treatment against arsenic-induced testicular toxicity in rats

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Abstract: Oxidative/nitrosative stress, inflammation, and apoptosis play a crucial role in the pathogenesis of arsenic-induced testicular injury. Telmisartan, the angiotensin II-receptor antagonist, possesses antioxidant and anti-inflammatory activities. The protective effect of telmisartan against arsenic-induced testicular damage was investigated in rats. Testicular damage was induced by sodium arsenite (10 mg kg–1/day, p.o., for 2 consecutive days). Telmisartan (10 mg kg–1/day, i.p.) was given for 3 consecutive days, starting 1 day before sodium arsenite administration. Telmisartan significantly attenuated the arsenic-induced decrease in the levels of serum testosterone and testicular reduced glutathione, and significantly decreased the elevation of the levels of testicular malondialdehyde, nitric oxide, and arsenic levels, as well as myeloperoxidase activity resulting from sodium arsenite administration. Histopathological and immunohistochemical examination revealed that telmisartan markedly attenuated testicular tissue changes, and decreased the arsenic-induced expression of vascular endothelial growth factor, inducible nitric oxide synthase, tumor necrosis factor-α, cyclooxygenase-2, nuclear factor-κB, and caspase-3. Telmisartan, via its antioxidant and/or anti-inflammatory effects, may represent a potential candidate to protect against the deleterious effects of arsenic on testicular tissue.

Keywords: arsenic; rat testes; telmisartan.

1 Introduction

Inorganic arsenic, particularly arsenite (As³⁺), is a ubiquitous environmental pollutant with multiple toxic effects in animals and humans. Ground water containing high arsenic levels is the major source of exposure. The permissible arsenic limit in drinking water recommended by the WHO is 10 ppb. However, this limit is much exceeded in many countries all over the world [1, 2]. Also, industrial production of agricultural pesticides and wood preservatives, as well as glass production are another source of arsenic contamination [3]. The testes are highly susceptible to arsenic exposure, whether acute or chronic. It was reported that arsenic intoxication can lead to reduction of testicular weight, impairment of spermatogenesis and androgenesis, and male reproductive dysfunction [4, 5].

The mechanism(s) by which arsenic causes male reproductive toxicity are not fully elucidated. Growing evidence supports the role of oxidative/nitrosative stress, inflammation, and apoptosis in the pathophysiology of tissue injury mediated by arsenic [6, 7]. Oxidative and nitrosative stress up-regulates inflammatory cascades, and necrotic and apoptotic pathways lead eventually to cell death [8]. It has been reported that arsenic-induced testicular toxicity can be ameliorated by antioxidants and anti-inflammatory agents [6, 9, 10].

In addition, activation of the angiotensin II type 1 (AT1) receptor by angiotensin II, the main active component of the renin-angiotensin system, induces oxidative stress, inflammation and apoptosis [11]. Telmisartan, the selective AT1-receptor blocker [12], exhibited significant antioxidant and anti-inflammatory properties in previous studies [13–15]. Our recent work demonstrated that telmisartan significantly ameliorated cadmium-induced testicular toxicity in rats [16], and it has been reported to attenuate germ cell toxicity in diabetic rats [17]. Considering these facts, the possible protective effect of telmisartan against arsenic-induced testicular toxicity appeared likely, but to the best of our knowledge, a possible protective effect of AT1-receptor blockers against arsenic-induced testicular injury and dysfunction has not been investigated. The present study was designed to test this hypothesis.
2 Materials and methods

2.1 Drugs and chemicals

Telmisartan (Sigma-Aldrich, St. Louis, MO, USA) was prepared in 1% (v/v) aqueous solution of Tween 80, and sodium arsenite (Loba Chemie, Mumbai, India) was dissolved in 0.9% (w/v) NaCl. The doses of telmisartan and sodium arsenite used in the present study were selected based on our preliminary experiments and in accordance with previous reports [6, 16].

2.2 Animals and treatments

Male Sprague-Dawley rats, weighing 250 ± 10 g were obtained from the Animal House, College of Medicine, King Faisal University. The animals were housed at 24 ± 1°C, 45 ± 5% humidity, and 12 h light-12 h dark cycle, supplied with standard laboratory chow and water ad libitum, and left to acclimatize for 1 week before the experiments. The experimental protocol had been approved by the Scientific Research Ethical Committee at King Faisal University (approval number: 160104). The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The rats were randomly divided into four groups (n = 8, each) as follows:

- The first (control) group received 0.9% NaCl (vehicle of sodium arsenite), p.o., for 2 consecutive days.
- The second group received sodium arsenite (10 mg kg⁻¹/day, p.o., for 2 consecutive days) and a daily i.p. injection of 1% aqueous solution of Tween 80 (vehicle of telmisartan) for 3 consecutive days starting 1 day before arsenite administration.
- The third group received sodium arsenite and telmisartan (10 mg kg⁻¹/day, i.p.) for 3 consecutive days starting 1 day before arsenite administration.
- The fourth group of animals received telmisartan only for 3 consecutive days.

2.3 Sample preparation and biochemical measurements

The rats were euthanized by thiopental (100 mg kg⁻¹, i.p.) 24 h after the last administration of sodium arsenite. Blood samples were collected through a puncture in the left ventricle, left for 60 min to clot, and centrifuged for 10 min at 2,430 g. The obtained clear sera were stored at −80°C, and subsequently the serum testosterone level was measured using the Johnsen score [18]. A scale from 1 to 10 was given to 100 tubules per slide as follows: 1 = atrophic tubules without seminiferous epithelial cells; 2 = no germ cells, but only Sertoli cells; 3 = spermatogonia only; 4 = few spermatocytes; 5 = no spermatids, but many spermatocytes; 6 = few spermatids; 7 = no spermatozoa but many spermatids; 8 = few spermatozoa; 9 = slightly impaired spermatogenesis; 10 = full spermatogenesis.

2.4 Histopathological examination

The left testis obtained from each animal was fixed in Bouin’s solution, dehydrated, and embedded in paraffin. Sections of 4-μm thickness were prepared, stained with hematoxylin and eosin (H&E) and examined under the light microscope at 200× magnification by a pathologist unaware of the treatment protocol. In addition, the level of spermatogenesis in the testicular tissue was assessed using the Johnsen score [18]. A scale from 1 to 10 was given to 100 tubules per slide as follows: 1 = atrophic tubules without seminiferous epithelial cells; 2 = no germ cells, but only Sertoli cells; 3 = spermatogonia only; 4 = few spermatocytes; 5 = no spermatids, but many spermatocytes; 6 = few spermatids; 7 = no spermatozoa but many spermatids; 8 = few spermatozoa; 9 = slightly impaired spermatogenesis; 10 = full spermatogenesis.

2.5 Immunohistochemical examinations

Four 4-μm thick sections prepared from the different animal groups were deparaffinized, rehydrated, and endogenous peroxidase activity was blocked with 3% H₂O₂ in methanol. Sections were pre-treated in 10 mM citrate buffer (pH 6.0) in a microwave and then incubated with rabbit polyclonal antibodies directed against rat vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX2), nuclear factor-κB (NF-κB), and caspase-3 (Thermo Scientific, Fremont, CA, USA; dilution 1:1000), and tumor necrosis factor-α (TNF-α) (US Biological, Swampscott, MA, USA; dilution 1:500). The sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with diaminobenzidine (DAB). Slides were counterstained with hematoxylin. The slides were examined under a light microscope, and the extent of cell immunopositivity was assessed. The area (μm²) occupied by immunopositive cells was measured in five separate microscopic fields in each slide using a digital imaging software program (cellSens, Olympus Corporation, Center Valley, PA, USA), and the mean area for each slide was obtained, then the mean ± S.E.M. was calculated for each group. The same procedures were repeated using normal rabbit serum instead of the primary antibodies to obtain a negative control and indicate the specificity of the used antibodies [19].

2.6 Statistical analysis

Data are expressed as mean ± S.E.M., and the results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for post hoc comparisons using SPSS for Windows (version 18). p < 0.05 was selected as the criterion for statistical significance.
3 Results

Significant decreases of serum testosterone and testicular GSH, and significant increases in testicular MDA, NO, angiotensin II, and arsenic levels, as well as MPO activity were observed in rats that had received sodium arsenite, as compared to the control group. Testes of rats treated with arsenite and telmisartan had significantly higher levels of serum testosterone and testicular GSH, and significantly lower levels of MDA, NO, and arsenic, and of MPO activity than rats treated with arsenite only (Figure 1A, B). The results obtained from the group that had received telmisartan alone were comparable to those of the control group, without any significant difference.

Figure 2 reveals that sodium arsenite caused marked testicular damage in the form of necrosis and vacuolization of the seminiferous tubular cells, impaired spermatogenesis, interstitial tissue edema, congestion and hemorrhages. However, telmisartan markedly ameliorated the arsenic-induced damage of testicular tissue and preserved spermatogenesis in most of the seminiferous tubules. The Johnsen score was significantly decreased in the vehicle plus arsenic group as compared to the control group, but telmisartan treatment resulted in a significant increase in the score.

Telmisartan treatment significantly decreased the expression of VEGF, iNOS, TNF-α, COX-2, NF-κB, and caspase-3 induced by arsenic in the cells of the seminiferous tubules as compared to the vehicle plus arsenic group (Figure 3A–F). Samples of the vehicle plus arsenic group incubated with normal rabbit serum instead of the primary antibodies were not stained, indicating the specificity of the used antibodies (figures not shown). Telmisartan alone did not have a detectable effect on all these parameters.

4 Discussion

Telmisartan treatment significantly suppressed oxidative, nitrosative, inflammatory, and apoptotic biomarkers in rats exposed to a toxic level of arsenic, as evidenced by the biochemical, histopathological, and immunohistochemical examinations.

Testicular tissue is known to contain all components of the renin-angiotensin system [20], and there is growing evidence that the pathophysiology of arsenic testicular toxicity includes activation of this system [21, 22]. The main active component, angiotensin II, increases the generation of reactive oxygen and nitrogen species thereby inducing oxidative/nitrosative stress, activates inflammatory reactions, and enhances the production of cytokines and chemokines, including TNF-α [23]. In addition, oxidative stress and inflammation are involved in blood pressure regulation by the renin-angiotensin system [24]. Arsenic also induces iNOS thereby leading to increased production of NO which interacts with superoxide to generate the potent cytotoxic agent, peroxynitrite [25]. The major effect of peroxynitrite is the nitration of cellular proteins leading to nitrosative stress, and depletion of the endogenous antioxidant defense systems [26]. Also, exposure to oxidative stress increases VEGF expression [27]. VEGF enhances angiogenesis, endothelial proliferation, cell migration, and vascular permeability. It causes NO overproduction, increased peroxynitrite formation.

Figure 1: (A) Serum testosterone level; (B) testicular tissue levels of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), angiotensin II (Ag II), arsenic, and myeloperoxidase (MPO) activity in rats of the untreated (control), arsenite only, telmisartan + arsenite, and telmisartan only group, respectively. Data are mean ± S.E.M. of 8 rats, *p < 0.05 vs. control group, †p < 0.05 vs. vehicle plus arsenic group. As = arsenic, TEL = telmisartan.
and nitrosative stress [28]. In addition, arsenic exposure has been reported to increase the production of inflammatory prostaglandins via activation of COX-2, the inducible form of cyclooxygenases [29]. Moreover, arsenic exposure resulted in increased testicular MPO activity, which is a good indicator of neutrophil infiltration and tissue inflammation [30].

Telmisartan is a selective AT1-receptor blocker, and has prominent antioxidant and anti-inflammatory properties. Telmisartan exerts a direct antioxidant effect, and also activates the endogenous antioxidant defenses [31, 32]. Telmisartan attenuates lipid peroxidation, prevents depletion of the antioxidant defense systems [13, 14], and suppresses nitrosative stress by decreasing iNOS activity and NO production [33]. The anti-inflammatory activity of telmisartan is attributed to reduction of the levels of inflammatory cytokines and chemokines [15], and suppression of COX-2 and MPO activities [34, 35].

Arsenic exposure also induces the NF-κB signalling pathway with subsequent enhancement of the transcription of the genes encoding TNF-α, iNOS, and COX-2 [6, 8, 36]. It has been reported that agents inhibiting TNF-α production and NF-κB activation significantly ameliorated arsenic-induced tissue injury [6, 7]. Likewise, telmisartan was found to provide a significant anti-inflammatory effect by suppressing NF-κB activation [37].

Induction of caspase proteases and apoptotic cell death is also involved in arsenic-induced tissue injury [38], and we have shown here that telmisartan significantly decreased the arsenic-induced expression of caspase-3 in the testicular tissue. Caspase-3 is an important marker of a cell’s entry into the apoptotic death

**Figure 2:** Photomicrographs of rat testes (H&E, 200×) from: (A) control group, showing normal testicular histology; (B) arsenite only group, showing widespread necrosis, vacuolization of seminiferous tubular cells (black arrows), marked reduction in spermatogenesis, interstitial tissue edema (arrow heads), congestion and hemorrhages (white arrows); (C) telmisartan + arsenite group, showing a histological picture comparable to that of the control group with preservation of normal spermatogenesis in most seminiferous tubules; (D) Johnsen score for the level of spermatogenesis in the different groups. Data are mean ± S.E.M. of eight rats, *p < 0.05 vs. control group, †p < 0.05 vs. the arsenite only group. As = arsenite, TEL = telmisartan. See materials and methods for the definition of the scale of the Johnsen score.
cascade [39]. Telmisartan provides a significant anti-apoptotic effect in germ cells of diabetic rats by reducing caspase-3 activity [17].

Chelation is an important natural detoxification pathway of heavy metals via formation of complexes with thiol-containing endogenous molecules [40]. GSH is a potent chelator of heavy metal ions, including arsenic, during their sequestration, transportation, and excretion, and is considered a biomarker for heavy metal overload [41, 42]. In the present study, telmisartan significantly attenuated arsenic overload in the rat testes most probably due to preservation of testicular GSH content which in turn reduced the arsenic burden in the testes.

5 Conclusions

Testicular injury and dysfunction caused by arsenic in rats were significantly ameliorated by telmisartan treatment. Telmisartan reduces arsenic-induced testicular oxidative/nitrosative stress as well as inflammatory and apoptotic responses.

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Conflict of interest statement: The authors declare that there are no conflicts to disclose.

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Figure 3: Areas of immunopositivity (µm²) in the histograms of immunohistochemical examinations of rat testes stained for: (A) vascular endothelial growth factor (VEGF); (B) inducible nitric oxide synthase (iNOS); (C) tumor necrosis factor-α (TNF-α); (D) cyclooxygenase-2 (COX-2); (E) nuclear factor-κB (NF-κB); (F) caspase-3, each in the control, arsenite only, telmisartan + arsenic, and telmisartan only group, respectively. Data are mean ± S.E.M. of 8 rats, *p < 0.05 vs. control group, †p < 0.05 vs. arsenite only group. ND = non-detectable, TEL = telmisartan, As = arsenite. The respective immunohistograms are shown in Supplementary Materials Figure S1.
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