ARTICLE IN PRESS

International Immunopharmacology xxx (2013) xxx-xxx



Contents lists available at SciVerse ScienceDirect

International Immunopharmacology



journal homepage: www.elsevier.com/locate/intimp

Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats $\stackrel{\sim}{\sim}$

Q13 Bo Xu¹, Yu-bo Zhang¹, Zhao-zhu Li, Mo-wen Yang, Shuai Wang, Da-peng Jiang*

Department of Pediatric Surgery, Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, 150086, Harbin, China

ARTICLE INFO

Article hi	story:
Received	7 May 2013
Received	in revised form 31 May 2013
Accepted	1 11 June 2013
Available	e online xxxx
Keyword	s:
Hydroge	n-rich saline
Interstiti	al fibrosis

Unilateral ureteral obstruction
 Inflammation

ABSTRACT

Hydrogen has been demonstrated to have effective protection against tissue injuries caused by oxidative stress, 20 inflammation, and apoptosis. This study investigated the efficacy of hydrogen-rich saline (HS) on the prevention 21 of renal injury induced by unilateral ureteric obstruction (UUO) in rats. Male Sprague–Dawley rats were divided 22 randomly into 4 groups: sham group, UUO group, UUO + saline group, and UUO + HS group. UUO was induced 23by ligation of the left ureter. 5 ml/kg HRSS or saline was administered beginning 1 day after UUO and for 10 days 24 thereafter. Rats were killed at 10 days after UUO. Left kidneys were excised immediately for the tissue histologic 25 examinations and biochemical assays. Renal injury scores in the UUO group and the UUO + saline group were 26 significantly higher compared with those in the sham group. However, administration of HS significantly 27 reduced the injury score. Apoptosis index was significantly increased in UUO group and the UUO + saline 28 group. HS treatment also reduced the apoptosis index. Interstitial fibrosis and macrophage infiltration were ob- 29 vious in UUO kidneys. However, HS treatment significantly reduced the fibrosis and infiltration of macrophage in 30 UUO kidneys. Significant increase in the MDA level and decrease in the SOD activity were observed in UUO group 31 and the UUO + saline group. MDA level of UUO + HS group was significantly reduced. In addition, SOD activity 32 of was significantly improved after treatment of HS. The data provide a biochemical and histologic basis for HS 33 acting as a novel therapeutic strategy for preventing the renal injury induced by UUO. 34

© 2013 Published by Elsevier B.V. 35

36

39 38

40 1. Introduction

Renal interstitial fibrosis, a common morphological feature of ob-41 structive nephropathy, is the major cause of chronic kidney disease 42 in children and young adults. Unilateral ureteral obstruction (UUO), 43 which is a representative model of tubulointerstitial renal fibrosis that 44 45 have many readily quantifiable cellular and molecular events during the ignition and progression of renal fibrosis, results in renal functional 46 loss and histological changes [1]. The pathogenesis of renal fibrosis is 47 characterized by relentless production and deposition of extracellular 48 49 matrix (ECM) proteins, such as fibronectin and collagens [2]. Although much progress has been made in investigating the biological effects of 50interstitial fibrosis and the prevention of its inherent deleterious effects, 5152there remains a gap in our knowledge of cellular and molecular mechanisms that facilitate fibrogenesis [3]. Knowledge regarding 53 protect patients from the damage of renal interstitial fibrosis, there-5455fore, has become more important.

56 In the UUO kidney many cellular and molecular events occur during 57 the initiation and progression of renal injury, including inflammation,

1567-5769/\$ – see front matter © 2013 Published by Elsevier B.V. http://dx.doi.org/10.1016/j.intimp.2013.06.033

oxidative stress, fibrosis, and apoptosis [4-6]. Report has indicated 58 that the interstitium in the setting of UUO is under the continuous oxi- 59 dant stress produced from tension, stress, or hypoxia [7]. Reactive oxy- 60 gen species (ROS) has been proposed as one major cause responsible for 61 the initiation and progression of chronic allograft nephropathy follow- 62 ing transplantation [8]. It play a role in inducing synthesis of collagen, 63 change of cell phenotypes, increase of interstitial cells, and cell prolifer- 64 ation. Excessive increase in ROS contributes to progression of kidney fi- 65 brosis following UUO [9]. ROS also leads to lipid peroxidation (LPO), 66 leukocyte activation, DNA damage, protein oxidation, and apoptosis, 67 all of which contribute to tissue damage [10]. In addition, inflammation 68 is known to contribute to the development of renal injury in UUO cases. 69 A large number of macrophages accumulate in the tubulointerstitial 70 space, which leads to renal inflammation and fibrosis in kidneys with 71 UUO [11]. Increased production of proinflammatory cytokines has also 72 been implicated following UUO. Inflammatory response could results 73 in overproduction and deposition of collagen, which could induce tissue 74 fibrosis [3]. 75

For the above reasons, antioxidant had been used to protect the 76 renal from injury induced by UUO [12]. Hydrogen (H₂), which pos-77 sesses anti-oxidative effects through selective reduction of the levels 78 of hydroxyl radical, has been demonstrated to have effective protec-79 tion against tissue injuries in testis caused by oxidative stress [13]. 80 H₂ also attenuates the activation of inflammatory signal pathway in 81 the liver tissue [14]. H₂ saturated in saline (HS) can be easily and 82

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033

^{*} Corresponding author. Tel./fax: +86 451 86605247.

E-mail address: jdp509@163.com (D. Jiang).

¹ These two authors contributed equally to this work.

2

ARTICLE IN PRESS

B. Xu et al. / International Immunopharmacology xxx (2013) xxx-xxx

safely applied. Recent study demonstrated that HS had a protective
 effect on interstitial congestion, edema, inflammation, and hemor rhage in renal tissue following ischemia-reperfusion [15]. These pro tective effects were due to the antioxidant properties of HS.

Considering the complexity of renal interstitial injury that includes aberrant expression of various cytokines and interstitial matrix molecules, infiltration of inflammatory cells, and tubular and vascular cell loss, we hypothesized that HS is one of the reasonable ways to protect kidney injury induced by UUO. In this current study, we investigate the potential protective and ameliorating effect of HS in the treatment of renal injury in rats using biochemical and histologic parameters.

94 **2. Materials and methods**

95 2.1. Animals

Sixty male Sprague–Dawley rats, weighing 180 to 200 g, were used
in this study. Rats were maintained with free access to food and water
under a constant 12-h light/12-h dark photoperiods, and were cared
for according to a protocol approved by the Animal Care and Usage
Committee at Harbin Medical University.

101 2.2. Experimental protocol

HS was prepared by gassing with hydrogen as described previous ly [16]. Hydrogen was dissolved in physiological saline for 4 h under
 high pressure (0.4 MPa). HS was stored under atmospheric pressure
 at 4 °C in an aluminum bag with no dead volume and was freshly pre pared every week.

The rats were randomly divided into 4 groups, each consisting of 107 108 15 animals. The groups consisted of a sham group, UUO group, UUO + saline group, and UUO + HS group. One week after acclimati-109 zation, UUO was induced. On the day of surgery, the rats were anesthe-110 tized with xylazine and ketamine. All operations were performed under 111 112 sterile conditions. The abdominal cavity was exposed via midline incision and the left ureter was ligated next to the uretero-pelvic junction 113114 with 5-0 silk. A two-layer running suture was used for abdominal clo-115sure with 4-0 dexon and 2-0 nylon. In sham-operated group, the left ureters underwent a physical manipulation but not ligated. In the 116 UUO group, rats were subjected to unilateral ureteral ligation and re-117 118 ceived no treatment. In UUO + saline group, the same surgical procedure was done as in the UUO group, but saline (5 ml/Kg) was injected 119 intraperitoneally daily. In UUO + HS group, the same surgical proce-120dure was done as in the UUO group, in addition, 5 ml/kg HRSS was in-121 traperitoneally injected into rats. Either HS or the same volume of 122saline was administered beginning 1 day after UUO and for 10 days 123 thereafter. Animals were killed with an overdose (200 mg/kg) of sodi-124 125um pentobarbital at 10 days after UUO. Left kidneys were excised immediately after sacrifice for the tissue histologic examinations and 126127biochemical assays.

128 2.3. Light microscopy

The kidney tissues were fixed in 10% formaldehyde, embedded in 129130paraffin, sectioned, and stained with H&E, and Masson's trichrome to determine general histology and interstitial collagen deposition. The 131 tubulointerstitial injury score was evaluated as described previously 132[17]. Briefly, tubule dilatation, distortion of tubular basement mem-133 branes and atrophy were analyzed by using a score of 0, no morpho-134logical deformities; 1, less than 10%; 2, less than 25%; 3, less than 50%; 1354, less than 75%; and 5, 75% or greater deformities. Ten fields per slide 136 were examined for counting. To minimize variation during the scor-137 ing process, the kidney sections were evaluated and scored by two 138 139 pathologists in blinded fashion.

The degree of interstitial fibrosis was assessed using Masson's 140 trichrome staining in 16 randomly selected tubulointerstitial fields 141 in each section. Tissue sections were analyzed at $400 \times \text{magnification}$. 142

2.4. Analysis of apoptosis 143

Tubular epithelial cell apoptosis was examined using TUNEL 144 (terminal deoxynucleotidyl transferase-mediated deoxynridine triphos- 145 phate nick-end labeling) assay. TUNEL-positive cells were quantified in 146 20 randomly selected cortex areas in a section. Apoptotic cells were 147 recorded using light microscopy at the magnification of $400 \times$. 148

2.5. Immunohistochemistry

The inflammatory cells were identified by immunohistochemistry 150 using standard techniques. Paraffin embedded tissues were cut into 151 4 μ m sections, mounted on glass slides and stained using indirect 152 immunoperoxidase. Slides were incubated overnight at 4 °C with 153 1:200 monoclonal anti-ED1 antibody (Serotec, Sydney, NSW, Australia) 154 for macrophage detection. The quantification of ED1 positive cells in the 155 interstitium was carried out with 400 \times magnification. The results rep- 156 resent the percentages of ED1 positive area in the total measured area. 157

|--|

To investigate LPO, levels of MDA as the final product of LPO were 159 measured in the rat kidney. For the MDA assay, kidney sample was 160 weighed and homogenized in cold sodium phosphate buffer. After the 161 homogenate had been centrifuged, the MDA concentrations in supernatant were determined spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances. The final concentration 164 of MDA was then expressed in nmol/mg protein. 165

Renal cortical tissue was weighed, minced, and homogenized. 166 Total SOD activity was measured by reduction of nitroblue tetrazoli-167 um by xanthine-xanthine oxidase system. SOD activity was expressed as U/mg protein. 169

2.7. Statistical analysis

Data are presented as means \pm standard deviation (SD). Analysis 171 utilized the Kruskal–Wallis test and Mann–Whitney U-test. Statistical 172 analyses were carried out with SPSS 11.0 statistical package. All values 173 of P < 0.05 were accepted as statistically significant. 174

3. Results

3.1. HS protects kidney from tubulointerstitial injury 176

The findings of the histological examination by H&E staining for 177 each group are shown in Fig. 1A. Renal tissues in the Sham group did 178 not show any morphological changes (Fig. 1A). However, desquamation 179 of epithelial cells in the renal tubular epithelium, tubular dilatation, and 180 atrophy were observed in the UUO group and the UUO + saline group. 181 Renal tissues in the UUO + HS group showed an improved histological 182 appearance (Fig. 1A). Renal injury score findings paralleled histological 183 findings. Scores of tubular damage in each group are shown in Fig. 1B. 184 The tubulointerstitial score was progressively increased after UUO. 185 However, HS treatment substantially reduced the tubulointerstitial 186 score in the renal tissue sections. 187

3.2. HS ameliorates the renal fibrosis

188

We determined collagen deposition, a typical index of fibrosis, by 189 Masson trichrome staining. Extensive peribiliary and interstitial colla- 190 gen deposition was evident in the UUO group, as shown by positive 191 Masson's trichrome staining (Fig. 2A). However, HS administration 192

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033

158

170

175

149

ARTICLE IN PRESS

B. Xu et al. / International Immunopharmacology xxx (2013) xxx-xxx



Fig. 1. HS protects kidney from tubulointerstitial injury. (A) Representative photomicrographs of H&E-stained sections of rat kidney tissues on day 10 after UUO. (original magnification × 400). (B) The statistical analyses of tubulointerstitial injury after UUO. Renal injury score was expressed as means \pm SD. **P* < 0.05 versus the sham group, #*P* < 0.05 versus the UUO and UUO + saline groups.

significantly ameliorated renal interstitial fibrosis. In the measurement of
the fibrous area in obstructed kidneys, fibrous area of kidney was greater
in untreated-UUO rats than in sham-operated and saline-treated rats,
and HS treatment significantly reduced the fibrous area (Fig. 2B).

197 3.3. HS decreases infiltration of macrophages in UUO kidney

Key feature of inflammation associated with UUO is intense mac-198199rophage infiltration. We evaluated the influence of HS on the macro-200 phages migration by immunohistochemical detection of ED1 positive cells. Kidneys in sham-operated group displayed rare cells positive for 201 macrophages antigen (Fig. 3A). Surface density of ED1 positive cells in 202 the interstitium was obviously increased in the UUO rats compared 203 with the sham-operated rats (Fig. 3A). Treatment with HS caused a 204 significant reduction in the density of the inflammatory macrophage 205marker (ED1 antigen) compared to non-treated and saline-treated 206 animals (Fig. 3B). 207

208 3.4. HS decreases UUO-induced tubular apoptosis

209To determine renal tubular apoptosis after UUO, the TUNEL assay was210performed. The findings of TUNEL-positive cells in all groups are shown211in Fig. 4A. Apoptotic tubular cells were rarely detected in sections of212sham control kidney (Fig. 4A). UUO group and the UUO + saline group



Fig. 2. HS ameliorates the renal fibrosis. (A) Representative kidney tissue sections stained with Masson's trichrome. (original magnification \times 400). (B) Graph showing relative percentages of tubulointerstitial fibrosis in the cortex of the kidneys after Masson's trichrome staining. Each bar represents the mean \pm SD. **P* < 0.05 versus the sham group, #*P* < 0.05 versus the UUO and UUO + saline groups.

had a significantly increased mean number of TUNEL positive cells 213 (Fig. 4B). Treatment of rats with HS markedly reduced tubular apoptosis 214 compared with the UUO group and the UUO + saline group (Fig. 4B). 215

3.5. HS decreased level of MDA and increased activity of SOD after UUO 216

The results of renal MDA levels and SOD activities in all groups are 217 shown in Fig. 4. Tissue MDA levels significantly increased in the UUO 218 group and the UUO + saline group as compared with the Sham group 219 (Fig. 4C). Rats with HS administration showed reduced levels of MDA. 220 Moreover, UUO significantly decreased renal SOD activity at day 10 221 (Fig. 4D). As shown in Fig 4C, HS treatment was significantly capable 222 of preventing this effect. There were no significant differences in tissue MDA levels and SOD activities between the UUO group and the UUO + saline group. 225

4. Discussion

UUO is an experimental model of renal injury that mimics the complex pathophysiology of chronic obstructive nephropathy. It has been 228 well established that UUO causes tubulointerstitial damage through 229 many molecular mechanisms, including interstitial inflammatory infiltration, progressive fibrosis, apoptosis, oxidative stress, myofibroblast activation, and ECM deposition [11,18]. A network of inflammatory and apoptotic processes results in the tubular atrophy and tubulointerstitial fibrosis [19]. Furthermore, oxidative stress contributes to the pathogenesis of UUO [20]. Increased renal concentrations of ROS have been observed in obstructed kidneys, together with decreased activities of the 236

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033

226

4

ARTICLE IN PRESS

B. Xu et al. / International Immunopharmacology xxx (2013) xxx-xxx



Fig. 3. HS decreases infiltration of macrophages in UUO kidney. (A) The distribution of macrophages (arrows) was evaluated using immunostaining with an anti-ED1 antibody. (original magnification \times 400). (B) Density of interstitial macrophage in obstructed kidneys at day 10. **P* < 0.05 versus the sham group, #*P* < 0.05 versus the UUO and UUO + saline groups.

major protective antioxidant enzymes [21]. Increased production of ROS
overwhelms the capacity of endogenous free radical scavengers [22].
ROS can cause LPO in the cellular and mitochondrial membranes. Therefore, medical therapies directed towards the mediators responsible for
inflammation, apoptosis, and oxidative stress should be used for decreasing the renal injury after UUO.

H₂ has a protective effect against acute pancreatitis through inhibiting 243oxidative stress and apoptosis [23]. It can also attenuate hepatic fibrosis 244via its antioxidant effect [24]. Inflammation, apoptosis, oxidative stress, 245246and progressive fibrosis have been implicated in the pathogenesis of 247renal injury after UUO. Although H₂ has been investigated for multiple 248purposes, its effect on injury of the kidney after UUO has not yet been evaluated. In the present study, we investigated whether HS treatment 249exerted a protective effect on the kidney. We measured a variety of pa-250251rameters related to kidney damage induced by UUO.

Hydrogen can diffuse rapidly into tissues and can be incorporated
into the body by drinking [25]. Moreover, it was reported that dissolving hydrogen in saline is easy to deliver intraperitoneally and safe
[13]. So, HS was intraperitoneally injected into rats in our study. Similar
to previous study, no adverse effect of HS on the rats' overall well-being
was observed in this study.

In the present study, we found that UUO induced tubule morphological changes, such as dilatation, distortion of tubular basement membranes and atrophy. Renal injury score was increased in the UUO group and the UUO + saline group when compared with that of the 261 sham group. HS ameliorated the UUO-induced histological damage in 262 the kidney in our study. All of those findings were statistically signifi-263 cant. We histologically confirmed that HS is capable of protecting the 264 kidney from the development of tubulointerstitial lesions after UUO. 265

It has been shown that renal tubular apoptosis after UUO was 266 thought to contribute to the severity of tubular dilatation, progressive 267 loss of renal function and accumulation of ECM [5]. In our TUNEL assay, 268 we found that apoptosis was increased in the obstructed kidney com-269 pared with that of the sham group. Similar to the findings of tubule mor-270 phological changes, HS treatment significantly reduced tubular apoptosis. 271

Kidney injury after UUO has a complex pathophysiology with a 272 number of contributing factors, such as local neutrophil accumula- 273 tion, lymphocyte/macrophage activation, and release of inflammatory 274 cytokines, which lead to cell injury [20,26]. An invasion of macro- 275 phage into the renal interstitium in the obstructed kidney coincides 276 with a decline in blood flow and glomerular filtration rate [27]. A recent 277 paper reported that HS could reduce airway inflammation and remod- 278 eling by inhibiting the activation of NF-KB [28]. Thus, we investigated 279 the effect of HS on macrophages/lymphocytes infiltration in obstructed 280 kidneys using immunohistochemistry techniques. We found that, HS 281 clearly decreased inflammation. Renal fibrosis involves infiltration of 282 the kidney by inflammatory cells, including macrophages/lymphocytes, 283 activation and possible transformation of intrinsic renal cells [3]. We con-284 sider that the decrease in inflammatory cells infiltration by HS may lead 285 to suppression of renal fibrosis. 286

Fibrogenic process after UUO plays a critical role in ultimately287leading to permanent loss of the normal structural and functional in-288tegrity of the kidney. Study showed that macrophage/monocyte infil-289tration has a critical function in the development of renal interstitial290fibrosis in a UUO model [12]. Oxidative stress has been regarded as291a major contributor to the development of various renal disorders in-292cluding renal fibrosis [29]. The aim of this study was to test whether293HS has therapeutic effects to ameliorate renal fibrosis. The accumula-294tion of ECM proteins is the key feature of renal tubulointerstitial fibro-295sis. We found that HS markedly decreased matrix deposition induced296by UUO, as represented by Masson;s trichrome staining. Our results297suggest that an important anti-fibrotic effect of HS may be a promis-298ing therapeutic target for fibrotic renal diseases.299

The role of oxidative stress has been implicated in renal injury in the UUO model [30]. ROS could disrupt the cellular cytoskeleton and cause 100 in the cell. MDA is the product of peroxidative decomposition of polyenic fatty acids in the LPO process and is widely used as a reliable marker of tissue damage. Our study showed that obstructed kidneys had significantly higher tissue MDA. Moreover, intraperitoneal administration of HS significantly decreased MDA concentration in rats exposed to the UUO injury. 307

SOD is a key component in cell growth and protection. Decreased 308 antioxidant level in cortical tubules of obstructed kidney is the major 309 factor contributing to renal injury and fibrogenesis. SOD from tubular 310 cells in the obstructed kidney show downregulation in our study, 311 which increases the vulnerability of the kidney to oxidative damage. 312 The antioxidant effect of H₂ is supported by the findings that hydro-313 gen can protect testis from ischemia–reperfusion-induced injury via increasing endogenous SOD in vivo [13]. In our study, SOD activities 315 were preserved by the treatment of rats with HS. Anti-oxidant actions 316 represent one plausible mechanism by which HS inhibits renal injury 317 in UUO. 318

The study presented here provided novel insights into the role of 319 HS in the pathogenesis of renal injury in obstructive nephropathy. 320 We demonstrated that HS effectively protects kidney from damage 321 through the suppression of renal fibrogenesis, inflammation, and ox- 322 idative stress in the UUO kidney of rats. Due to its efficacy and conve- 323 nience, HS should be considered as a new strategy for the treatment 324 of fibrotic kidney diseases. For this purpose, further clinical studies 325 will be needed. 326

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033

B. Xu et al. / International Immunopharmacology xxx (2013) xxx-xxx



Fig. 4. HS ameliorates tubular apoptosis, decreases level of MDA, and increases activity of SOD after UUO. (A) Apoptotic tubular cells (arrows) were determined by TUNEL-positive staining, (original magnification \times 400). (B) The number of apoptotic tubular cells in renal tissues in all groups. Apoptotic tubular cells was counted and expressed as means \pm SD. (C) Effects of HS treatment on MDA in rat kidney tissues on day 10 after UUO. (D) Effects of HS treatment on SOD levels in rat kidney tissues on day 10 after UUO. *P < 0.05 versus the sham group, #P < 0.05 versus the UUO and UUO + saline groups.

327 Acknowledgements

This work was supported by grants from the National Natural Sciences 328 Foundation of China (Nos. 30901516, 81272049, 81150024), Reserve Tal-329 ents of Universities Overseas Research Program of Heilongjiang, and Nat-330 ural Science Foundation of Heilongjiang (QC2011C049). 331

References 332

336

337

338

339

340

344

345

- [1] Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney 333 334 Int 2006:69:213-7 335
 - Li L, He D, Yang J, Wang X. Cordycepin inhibits renal interstitial myofibroblast activation probably by inducing hepatocyte growth factor expression. J Pharmacol Sci 2011;117:286-94.
 - [3] Kim J, Padanilam BJ. Loss of poly(ADP-ribose) polymerase 1 attenuates renal fibrosis and inflammation during unilateral ureteral obstruction. Am J Physiol Renal Physiol 2011;301:F450-9.
- 341[4] Ning XH, Ge XF, Cui Y, An HX. Ulinastatin inhibits unilateral ureteral obstruction-342induced renal interstitial fibrosis in rats via transforming growth factor β 343 (TGF-β)/Smad signalling pathways. Int Immunopharmacol 2013;15:406–13.
- [5] Manucha W, Valles PG. Apoptosis modulated by oxidative stress and inflammation during obstructive nephropathy. Inflamm Allergy Drug Targets 2012;11: 346303-12
- [6] Zhou TB, Qin YH, Lei FY, Zhao YJ, Huang WF. Association of PAX2 with cell apoptosis 347 in unilateral ureteral obstruction rats. Ren Fail 2012;34:194-202. 348
- [7] Kamijo-Ikemori A, Sugaya T, Matsui K, Yokoyama T, Kimura K. Roles of human liver 349 type fatty acid binding protein in kidney disease clarified using hL-FABP chromo-350 351somal transgenic mice. Nephrology (Carlton) 2011;16:539-44.

- [8] Djamali A, Reese S, Yracheta J, Oberley T, Hullett D, Becker B. Epithelial-to-mesenchymal 352 transition and oxidative stress in chronic allograft nephropathy. Am J Transplant 353 2005;5:500-9. 354
- [9] Manucha W, Carrizo L, Ruete C, Molina H, Valles P. Angiotensin II type I antagonist 355 on oxidative stress and heat shock protein 70 (HSP 70) expression in obstructive 356 nephropathy. Cell Mol Biol (Noisy-le-Grand) 2005;51:547-55.
- [10] Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A. Spermatic cord 358 torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. 359 Mol Aspects Med 2004;25:199-210. 360
- [11] Chen G, Chen H, Wang C, Peng Y, Sun L, Liu H, et al. Rapamycin ameliorates kidney 361 fibrosis by inhibiting the activation of mTOR signaling in interstitial macrophages 362 and myofibroblasts. PLoS One 2012;7:e33626. 363
- [12] Demirbilek S, Emre MH, Aydin EN, Edali MN, Aksoy RT, Akin M, et al. Sulfasalazine 364 reduces inflammatory renal injury in unilateral ureteral obstruction. Pediatr Nephrol 365 2007;22:804-12. 366
- [13] Jiang D, Wu D, Zhang Y, Xu B, Sun X, Li Z. Protective effects of hydrogen rich saline 367 solution on experimental testicular ischemia-reperfusion injury in rats. J Urol 3682012:187:2249-53. 369
- [14] Liu Q, Shen WF, Sun HY, Fan DF, Nakao A, Cai JM, et al. Hydrogen-rich saline pro-370tects against liver injury in rats with obstructive jaundice. Liver Int 2010;30: 371 58-68
- [15] Wang F, Yu G, Liu SY, Li JB, Wang JF, Bo LL, et al. Hydrogen-rich saline protects 373 against renal ischemia/reperfusion injury in rats. | Surg Res 2011;167:e339-44. 374
- Cai J, Kang Z, Liu WW, Luo X, Qiang S, Zhang JH, et al. Hydrogen therapy reduces apotosis in neonatal hypoxia-ischemia rat model. Neurosci Lett 2008;441:167-376
- [17] Choi DE, Jeong JY, Lim BJ, Chang YK, Na KR, Shin YT, et al. Aliskiren ameliorates renal 377 inflammation and fibrosis induced by unilateral ureteral obstruction in mice. J Urol 378 2011;186:694-701. 379
- [18] Yeh CH, Chiang HS, Lai TY, Chien CT. Unilateral ureteral obstruction evokes renal 380 tubular apoptosis via the enhanced oxidative stress and endoplasmic reticulum 381 stress in the rat. Neurourol Urodyn 2011;30:472-9. 382

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033

6

390

398

415

ARTICLE IN PRESS

B. Xu et al. / International Immunopharmacology xxx (2013) xxx-xxx

- [19] Chevalier RL, Forbes MS, Thornhill BA. Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. Kidney Int 2009;75:1145–52.
- [20] Omori H, Kawada N, Inoue K, Ueda Y, Yamamoto R, Matsui I, et al. Use of xanthine oxidase inhibitor febuxostat inhibits renal interstitial inflammation and fibrosis in unilateral ureteral obstructive nephropathy. Clin Exp Nephrol 2012;16: 549–56.
 [21] Zecher M, Guichard C, Velasquez ML, Figueroa G, Rodrigo R, Implications of oxidative
 - [21] Zecher M, Guichard C, Velasquez MJ, Figueroa G, Rodrigo R. Implications of oxidative stress in the pathophysiology of obstructive uropathy. Urol Res 2009;37:19–26.
- [22] Ergur BU, Kiray M, Pekcetin C, Bagriyanik HA, Erbil G. Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. J Pediatr Surg 2008;43:722–8.
- [23] Chen H, Sun YP, Li Y, Liu WW, Xiang HG, Fan LY, et al. Hydrogen-rich saline ameliorates the severity of l-arginine-induced acute pancreatitis in rats. Biochem Biophys Res Commun 2010;393:308–13.
 [24] Sun H. Chen L Zhou W. Hu L, Li L Tu O, et al. The protective role of hydrogen-rich
 - [24] Sun H, Chen L, Zhou W, Hu L, Li L, Tu Q, et al. The protective role of hydrogen-rich saline in experimental liver injury in mice. J Hepatol 2011;54:471–80.
- [25] Nagata K, Nakashima-Kamimura N, Mikami T, Ohsawa I, Ohta S. Consumption of molecular hydrogen prevents the stress-induced impairments in hippocampus-dependent 400 learning tasks during chronic physical restraint in mice. Neuropsychopharmacology 401 2009;34:501–8.
 [26] Fuji K, Mapabo L Nami D, Panal collection durt with which all and the stress of the stress o
- [26] Fujiu K, Manabe I, Nagai R. Renal collecting duct epithelial cells regulate inflammation in tubulointerstitial damage in mice. J Clin Invest 2011;121:3425–41.
 [27] Klahr S, Morrissey J. Obstructive nephropathy and renal fibrosis. Am J Physiol Renal
- Physiol 2002;283:F861-75. 200 283 Xiao M, Zhu T, Wang T, Wen FQ. Hydrogen-rich saline reduces airway remodeling 407
- [20] Aldo W, Zhu L, Wang L, Wen PQ, Hydrogen-rich Saime reduces airway remodeling 407 via inactivation of NF-kB in a murine model of asthma. Eur Rev Med Pharmacol Sci 408 2013;17:1033–43.
 409
- [29] Cho MH, Jung KJ, Jang HS, Kim JI, Park KM. Orchiectomy attenuates kidney fibrosis 410 after ureteral obstruction by reduction of oxidative stress in mice. Am J Nephrol 411 2012;35:7–16.
 412
- [30] Dendooven A, Ishola Jr DA, Nguyen TQ, Van der Giezen DM, Kok RJ, Goldschmeding R, 413 et al. Oxidative stress in obstructive nephropathy. Int J Exp Pathol 2011;92:202–10. 414

Please cite this article as: Xu B, et al, Hydrogen-rich saline ameliorates renal injury induced by unilateral ureteral obstruction in rats, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.06.033