Urol Res (2007) 35:193–199
DOI 10.1007/s00240-007-0105-5

ORIGINAL PAPER

Pyrrolidine dithiocarbamate attenuate shock wave induced MDCK cells injury via inhibiting nuclear factor-kappa B activation

Xiang Li · Dalin He · Linlin Zhang · Yuquan Xue · Xinfa Cheng · Yong Luo

Received: 2 March 2007 / Accepted: 17 May 2007 / Published online: 12 June 2007

Abstract Shock wave lithotripsy (SWL)-induced renal damage appears to be multifactorial. Recent data indicated that the mechanism of renal tissue damage secondary to SWL is similar to that of ischemia reperfusion injury. Nuclear factor-kappa B (NFκB) and its target genes, inducible nitric oxide synthase (*iNOS*) and cyclooxygense-2 (*COX-2*), have been demonstrated to play a very important role in a variety of cells or tissues ischemia reperfusion injuries. Thus in the present study, using an in vitro model MDCK cells, we investigated the role of NFκB and its target cytotoxic enzyme in shock wave-induced renal cellular damage. We also examined whether inhibition this pathway by pyrrolidine dithiocarbamate (PDTC) is contributed to alleviate SWL-caused cell damage. Suspensions of MDCK cells were placed in containers for shock wave exposure. Three groups of six containers each were examined: control group, no shock wave treatment and SWL group, which received 100 shocks at 18 kV; 3 SWL + PDTC group. PDTC were added to the suspensions before shock wave exposure. After shock wave 0, 2, 4, 6 and 8 h, respectively, the cell supernatants were detected for the level of MDA and release of LDH. At post-shock wave 8 h, cells were harvested to detect the nuclear translocation of NFκBp65 by immunofluorescence staining. Degradation of IκB-a (an inhibitor protein of NFκB) and expression of *iNOS* and *COX-2* were also examined by western blotting. Our results indicated that shock wave initiated the apparent activation of NFκB, which in turn induced high expression of *iNOS* and *COX-2*. Blocking degradation of IκB-a by PDTC was contributed to decrease the expression of *iNOS*. And the level of MDA and the release of LDH were also significantly reduced by using PDTC. However, the degree of *COX-2* expression does not differ significantly between SWL and SWL + PDTC groups. Activation of NFκB and subsequent expression of its target cytotoxic enzyme have been demonstrated to be a potential and crucial mechanism in SWL-induced renal cell damage. Blocking this pathway by PDTC is contributed to protect against cellular damage from shock wave.

Keywords Shock wave lithotripsy · MDCK · NFκB

Introduction

Shock wave lithotripsy (SWL) is currently the first-line treatment for upper urinary tract calculi. However, this treatment is not completely free from side effects. Firstly, the adverse effects of SWL are attributed to renal damage resulting only from direct action of cavitation bubbles and shear stress. Recently, free radicals formation and its subsequent oxidative stress have also been considered to be another key element in shock wave-induced tissue damage [1, 2]. However, our previous study showed the expression of P-selectin, a pro-inflammatory cytokine, in the rabbit renal interstitium with severe inflammatory cells infiltration after shock wave treatment [3]. Apparently this phenomenon cannot be explained completely by the effect of mechanical trauma or free radicals oxidative stress. Thus, other potential mechanisms may be involved in shock wave-induced renal damage. In fact, shock wave has already been documented to induce generalized vasoconstriction in the kidney and lead to tissue hypoxia and ischemia in several

X. Li · D. He (✉) · L. Zhang · Y. Xue · X. Cheng · Y. Luo
Department of Urology, No. 1 Hospital,
Xi'an Jiaotong University,
Xi'an, Shaanxi Province 710061, China
e-mail: Urologistlx@yahoo.com.cn

animal models [4, 5]. Therefore, the mechanism of kidney damage by SWL is considered to be similar to that of ischemia reperfusion injury model. To our knowledge, inflammatory reaction with its release of several cytotoxic enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygense-2 (COX-2) have been demonstrated to play a very important role in a variety of cell or tissue ischemia reperfusion injuries [6–9].

Based on the results of our previous study, we hypothesized inflammatory reaction with release of cytotoxic enzymes to be another potential mechanism of renal damage secondary to SWL. It is well known that almost all inflammatory processes need to be regulated by transcriptional factors. Nuclear factor-kappaB (NFκB) is believed to be a predominantly transcriptional factor activated by a variety of signals, including reactive oxygen species (ROS), radiation, mechanical stress, ischemia/reperfusion (I/R) and several cytokines [10–13]. Inhibiting NFκB pathway activation has been demonstrated to alleviate different types of cells or tissues injuries via down-regulation its target cytotoxic gene expression in vivo and vitro [14–16]. Shear stress or free radicals production induced by SWL can activate NFκB pathway, and this activation may be playing a crucial role in initiation of inflammatory reaction and expression of cytotoxic enzymes iNOS and COX-2, which lead to severe renal cellular damage. In the present study, using Madin-Darby Canine kidney (MDCK) cell model, which is an established model to examine shock wave-induced cellular damage and its potential mechanism, we investigated whether NFκB and its target cytotoxic genes *iNOS* and *COX-2* are involved in shock wave-induced cell damage in vitro. We also aimed to explore whether inhibition of NFκB activation by pyrrolidine dithiocarbamate (PDTC) can contribute to protecting against shock wave-induced MDCK cells injury.

Materials and methods

Cell culture

MDCK cells are a cell line derived from dog kidney cortex (American Type Culture Collection). It is also an established in vitro model to study the mechanism of renal tubular injury induced by shock wave. Cells were grown in culture flasks containing Dulbecco's modified eagle's medium (DMEM) and 10% fetal calf serum at 37°C in 5% CO_2-containing humidified air.

SWL of cell suspension

Confluent cells in flasks were trypsinized, centrifuged and suspended in DMEM. The concentration of the cell suspension was adjusted to 10^6 cells/ml. For shock wave exposure, the cell suspension was placed in the polyethylene tubes, which were immersed in the custom-made water-filled plastic container. The cell-containing tubes were adjusted to the second focus of the ellipsoid by the specially designed holder. To avoid fluid–air interference, the tubes were filled completely [17]. The shock waves were generated by the Lithotripter (Huikang, China), which has been demonstrated to have approximately equal efficacy and resulting kidney injury compared with Siemens or Dornier machines. The impulse rate was 100 shock waves at a voltage of 18 kV. The control group cells were handled in the same way as those that received shock wave, except for the absence of shock wave exposure. Three groups of five containers each were examined: control group, SWL group and SWL + PDTC group. Thirty minutes before shock wave exposure, PDTC were added to the cell suspensions at the concentration of 100 μmol/L. The concentration of PDTC was based on reference value from our preliminary experiment.

Lipid peroxidation and cell viability analysis

After shock wave exposure 0, 2, 4, 6 and 8 h, respectively, the cell suspensions were centrifuged and collected. The supernatants were measured for MDA level and lactate dehydrogenase (LDH) release by biochemical spectrophotometric analysis according to the manufacturer's instructions (Nanjing Jiancheng Biological Techniques Institute, China). MDA is the marker of oxidative stress and LDH is the marker of cellular damage. The spectrophotometer was SUV 1200 (Sinco, Korea).

Measurement of NFκB activation by immunofluorescence staining

At post-shock wave 8 h, the adherent MDCK cells onto the glass microslides were fixed in 4% paraformaldehyde for 10 min, permeabilized in 0.2% TritonX 100 for 15 min and blocked with goat serum for 30 min. Cells were then incubated at 37°C for 2 h with mouse anti-human NFκBp65 monoclonal antibody (Santa Cruz, USA) at a dilution of 1:200. After washing three times with PBS (5 min each), cells were incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody at 37°C for 1 h. The fluorescence staining intensity and intercellular location were then examined by fluorescence-inverted microscope (Olympus IX 50, Japan). We scored with NFκB activation when the bright green fluorescent color (FITC) appeared in the entire nucleus of the cell, indicating nuclear location of activated NFκBp65. At least 200 cells were examined for each group.

Western blotting to determine expression of IκB-a, COX-2 and iNOS proteins

At post-shock wave 8 h, the cells were harvested to determine the expression of IκB-a, iNOS and COX-2 proteins. Cytoplasmic proteins were extracted by using the NE-PER Extraction Reagents Kit (Pierce, USA). Concentration of extracts was measured with a BCA kit. Cytoplasmic protein (50 μg) was separated by 12% SDS-PAGE for IκB-a, 10% SDS-PAGE for COX-2 and 5% for iNOS, respectively. Proteins were then transferred to nitrocellulose membranes (Bio-Rad, USA) and blocked with 5% non-fat milk in washing buffer (TBST) at room temperature for 1.5 h. The membranes were then incubated for 1.5 h with a dilution of 1:500 rabbit polyclonal primary antibody for iNOS (Upstate, USA), 1:300 for COX-2 and 1:300 for IκB-a (Santa Cruz, USA, respectively). After washing in TBST six times, the blot was incubated with horseradish peroxidase (HRP) labeled secondary antibody (Santa Cruz, USA) at the dilution of 1:5000. Immunodetection was performed using the enhanced chemiluminescent (ECL) western blot detection system (Pierce, USA). Protein loading equivalence was assessed by the expression of β-actin. The intensity of bands was assessed relative to their respective β-actin bands with Bandscan software (version 4.3 Glyco).

Statistical analysis

The results of MDA-level, release of LDH, and expression of iNOS and COX-2 were expressed as mean ± SD. Statistical analysis was performed using one way-ANOVA. $P < 0.05$ was considered significant. The results of p65 immunofluorescence staining were analyzed using chi-square test. $P < 0.05$ was considered significant.

Results

Figure 1 showed that after shock wave exposure, the level of MDA in MDCK cells supernatant was significantly elevated within 8 h ($P < 0.05$). Compared with SWL group, PDTC pretreatment could significantly decrease the level of MDA in cells supernatants especially at post 2, 4 and 6 h, respectively ($P < 0.05$). These results demonstrated that the degree of shock wave-induced oxidative stress was significantly inhibited by PDTC.

Shock wave significantly increased the release of LDH in MDCK cells supernatant compared to that in control group within 8 h ($P < 0.05$). At post-shock wave 6 and 8 h, the release of LDH in cell supernatants was significantly reduced by PDTC ($P < 0.05$, respectively) (Fig. 2). These results demonstrated that PDTC could significantly alleviate shock wave-induced cellular injury.

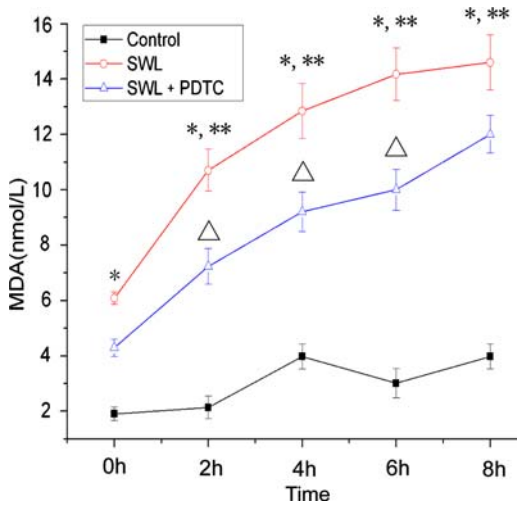


Fig. 1 Change of MDA level in MDCK cell supernatants at post-shock wave immediately (0 h), 2, 4, 6 and 8 h, respectively. *$P < 0.05$ indicates SWL group versus control group. **$P < 0.01$ indicates SWL group versus control group. Δ$P < 0.05$ indicates SWL group versus SWL + PDTC group

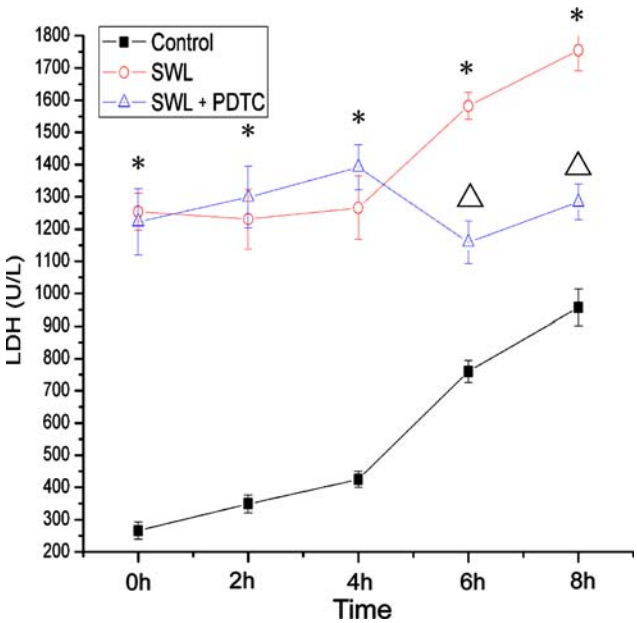


Fig. 2 Change of LDH release in MDCK cell supernatants at post-shock wave immediately (0 h), 2, 4, 6 and 8, respectively. *$P < 0.05$ indicates SWL group versus control group. Δ$P < 0.05$ indicates SWL group versus SWL + PDTC group

NFκB activation measured by NFκBP65 immunofluorescence staining method

At post-shock wave 8 h, approximately 53% of MDCK cells from SWL group showed nuclear location of NFκBP65; while only 13% of cells from control group without receiving shock wave treatment demonstrated nuclear location. The differences between the two groups

were significant ($P < 0.05$). Such findings indicated that activation of NFκB was caused by shock wave exposure. Nuclear location of P65 was detected in approximately 29% cells from SWL + PDTC group, which differed significantly from that in SWL group ($P < 0.05$). It indicated that PDTC could significantly inhibit shock wave-induced NFκB activation (Fig. 3).

NFκB activation measured by detecting degradation of Iκ B-a protein through western blotting method

At post-shock wave 8 h, cells were harvested to detect the expression of the inhibitory protein Iκ B-a, which physically masks the nuclear translocation sequences of NFκB and retains NFκB in cytoplasm. The results demonstrated that shock wave exposure could induce degradation of Iκ B-a protein significantly in MDCK cells cytoplasm ($P < 0.05$) and PDTC pretreatment significantly inhibited the degradation of Iκ B-a protein ($P < 0.05$) (Fig. 4).

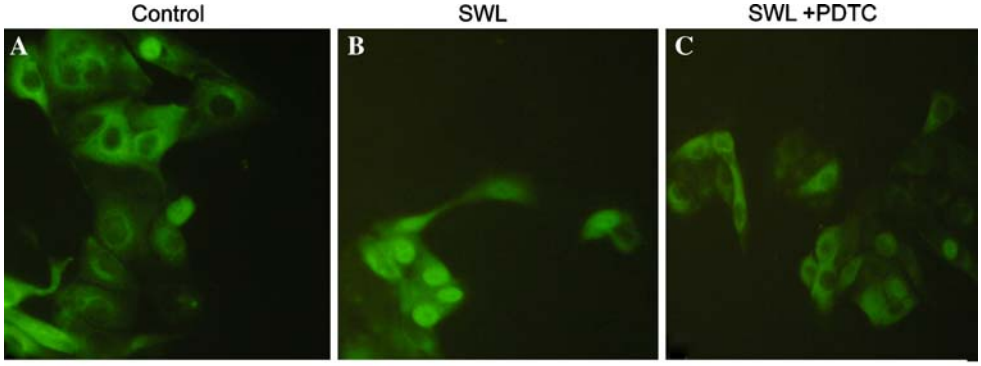
The expression of iNOS and COX-2 protein measured by western blotting method

The results demonstrated a significant increase of iNOS and COX-2 expression in shock wave-treated MDCK cells at post-shock wave 8 h ($P < 0.05$, respectively). PDTC pretreatment apparently decreased the expression of iNOS protein ($P < 0.05$); however, the expression of COX-2 protein did not significantly differ between SWL and SWL + PDTC groups (Fig. 5).

Discussion

In spite of the efficacy and relative safety, the adverse effects of SWL have been questioned by more and more investigators in the last two decades. Hematuria and postoperative pain or discomforts frequently occur in the patients undergoing shock wave treatment. Corticomedullary or interstitial hemorrhage and thrombosis of arteries or interlobular veins have also been documented in several animal experiments [18, 19].

Renal injury mediated by SWL appears to be complicated and multifactorial. Initially the mechanism of renal damage was believed to be mechanical trauma to renal vasculatures and tubules through the direct action of cavitation bubbles or shear stress [1]. Recently free radicals formation and the subsequent oxidative stress were considered to be another key element in shock wave-induced renal damage [2]. On the basis of the theory, several antioxidative agents such as melatonin [20], selenium [21], caffeic acid phenethyl ester [22], astragalosides [23] and vitamin E [24] have been demonstrated to have protective properties against short-term renal injury caused by SWL. However, we found severe inflammatory cells infiltration in the rabbit renal interstitium after shock wave treatment in our previous



D

Group	Positive cells	Negative cells	Total cells	Positive percentage
control	40	265	305	13%
SWL*	147	131	278	53%
SWL+PDTC△	63	157	219	29%

Fig. 3 Determination of NFκBp65 activation by immunofluorescence staining (×400) in MDCK cells at post-shock wave 8 h. **a** In control group, the green fluorescent color (FITC) was expressed in cytoplasm of most cells, indicating NFκBP65 was not activated in these cells. **b** Most of cells from SWL group showed bright green fluorescent color in the entire nuclear, indicating nuclear location of activated NFκBP65. **c** PDTC pretreatment reduced the degree of nuclear location of activated NFκBP65. **d** We scored with NFκB activation when the bright green fluorescent color appeared in the entire nucleus of the cells. At least 200 cells for each group from five independent experiments were examined. *Indicates SWL group versus control group, Pearson chi-square = 105.54, $P < 0.001$; Δ indicates SWL + PDTC group versus SWL group, Pearson Chi-square = 29.185, $P < 0.001$

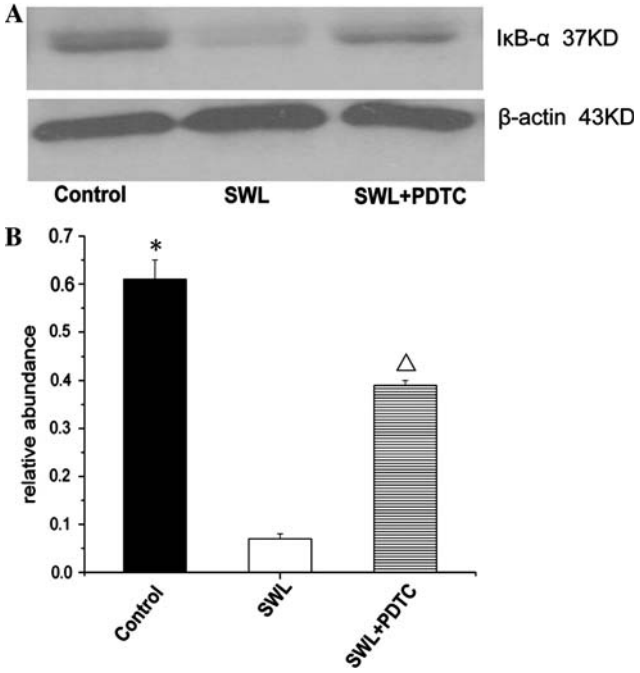


Fig. 4 Determination of IκB-aprotein in MDCK cells cytoplasm at post-shock wave 8 h through western blotting and densitometric analysis. (A) Using rabbit polyclonal primary antibody to detect the expression of IκB-a protein by ECL western blotting detection system. Using β-actin as the internal control to make loading equivalence. (B) The intensity of bands was assessed relative to their respective β-actin bands with Bandscan software (version 4.3 Glyco). Data were presented as mean ± SD ($n = 5$). *$P < 0.05$ indicates SWL group versus control group. $\Delta P < 0.05$ indicates SWL group versus SWL + PDTC group

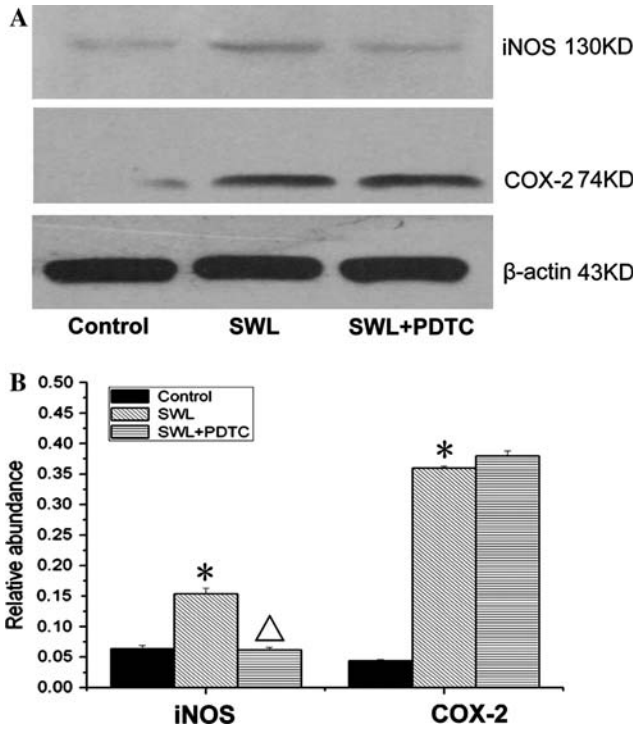


Fig. 5 Western blotting and densitometric analysis of iNOS and COX-2 protein in shock wave treated MDCK cells with or without PDTC pretreatment at post-shock wave 8 h. **a** Western blotting analysis of iNOS and COX-2 protein in each group, β-actin as the internal control to make loading equivalence. **b** Densitometry analysis of band intensity through Bandscan software 4.3. Data were presented as ratio of iNOS or COX-2 protein to their respective β-actin protein (mean ± SD, $n = 5$). *$P < 0.05$ indicates SWL group versus control group. $\Delta P < 0.05$ indicates SWL group versus SWL + PDTC group

study [3]. This phenomenon could not be explained by the effect of mechanical trauma or free radicals oxidative stress. Thus, other potential mechanisms are possibly involved in shock wave-induced renal damage. It has been demonstrated that shock wave could induce generalized vasoconstriction in the kidney and lead to renal hypoxia and ischemia in several animal models [4, 5]. Inflammatory reaction with the subsequent release of several cellular toxic enzymes, including iNOS and COX-2, have been documented to play a very important role in ischemia reperfusion injuries of different organs [7, 8, 25, 26]. And most inflammatory processes were regulated through NFκB signal transduction pathway. This pathway can be activated by a variety of signals such as mechanical stress, radiation, free radicals production and several cytokines. During or after SWL, NFκB pathway could be activated by shear stress or reactive free radicals. Inhibition of NFκB activation has been demonstrated to attenuate different types of tissue or cell injuries via down-regulating expression of its target cytotoxic enzymes iNOS and COX-2 [8, 27, 28]. Therefore, activation of NFκB with its target toxic enzymes expression may be a potential and important mechanism in shock wave-induced renal damage.

To our knowledge, this is the first report to investigate the role of NFκB signal transduction pathway in SWL-induced renal damage. NFκB is a ubiquitous transcriptional factor involved in almost all inflammatory processes. It consists of five known subunits in mammalian cells, including p50, p52, p65 (RelA), RelB and CRel. The most common active form is p50/p65 heterodimer and p50/p50 homodimer. NFκB protein exists as an inactive dimer in the cytoplasm bound to the inhibitory protein IκB, which physically masks the specific κB consensus sequences and retains active dimer in the cytoplasm [29, 30]. In response to different types of activating signals, NFκB could be activated via phosphorylation of IκB (mainly IκB-α), which is target for rapid degradation by the 26S proteasome. Degradation of IκB results in NFκB translocation to the nucleus, subsequently inducing expression of target genes [31]. In the present study, NFκB activation was determined by measuring NFκBp65 nuclear translocation via immunofluorescence staining and detecting IκB-a degradation by western blotting method. The results demonstrated significant activation of NFκB pathway after shock waves treatment, and this activation could be blocked by PDTC via its

ability to inhibit IκB-a degradation. How could shock waves trigger inflammatory reaction? Shear stress or free radicals are considered to play a key role in initiating inflammatory process. Vascular injuries caused by the direct action of shock waves could induce ischemia and hypoxia in renal tissues, which becomes more susceptible to free radical production as reperfusion occurs. Metabolic alterations caused by ischemia with reperfusion can result in abnormally high level of free radicals, which coupled with shear stress could in turn make IκB-α protein phosphorylation and degradation, this then causing p50/p65 heterodimer to be translocated from the cytoplasm to the nucleus. This translocation would activate a series of target genes expression, which in turn triggers inflammatory reaction. However, the exact mechanism remains to be illuminated. Our data also showed a significant increase of MDA level and a release of LDH in shock wave-treated MDCK cells supernatants. MDA is believed to be a reliable marker of free radical-mediated lipid peroxidation. As a breakdown product of cellular lipids, MDA could reflect the degree of cellular oxidative stress [32]. The release of LDH reflects the cell viability. Therefore, our results demonstrated oxidative stress and cellular damage after shock wave treatment, which is consistent with numerous previous reports.

Furthermore, the results also showed that PDTC pretreatment could significantly reduce cellular oxidative damage. PDTC is one of the most effective potent NFκB inhibitors because of its ability to transverse into the cell and prolonged stability in solution at physiological pH and its antioxidant ability to suppress free radicals production [33]. PDTC have been demonstrated to play a protective role in different types of cell or tissue injuries with its antioxidant properties and ability of inhibiting NFκB activation in vitro and vivo [34–36]. For the current study the data suggested that besides antioxidant properties, PDTC appears to play a protective effect against shock waves-induced MDCK cells injury through inhibiting NFκB activation, which in turn down-regulating *iNOS* expression. *iNOS* has been considered to be one of the important cytotoxic target genes regulated by NFκB. Under certain pathological conditions, nitric oxide (NO) produced by iNOS reacts with superoxide radicals to form the extremely destructive ROS, $ONOO^-$ [37]. The cytotoxic processes triggered by $ONOO^-$ included initiation of lipid peroxidation and induction of DNA strand breaks, which causes cellular damage or death [37, 38]. After shock wave treatment, inhibiting NFκB by PDTC could decrease *iNOS* expression and alleviate cells oxidative injury. Our results also examined the expression of *COX-2*. As a rate-limiting enzyme in generating prostaglandins, COX-2 is regarded as one of the crucial enzyme in supporting and sustaining the inflammatory response. Blocking overexpression of COX-2 using a specific inhibitor has been demonstrated to protect against different types of cell or tissue injuries [39, 40]. Our results also showed high expression of COX-2 after shock wave treatment. However, this overexpression could not be inhibited by PDTC. The precise mechanism of this phenomenon remains unclear. A possible explanation is that regulation of COX-2 requires other signal pathways such as activator protein-1 (AP-1) or hypoxia inducible factor-1 (HIF1-α) [41].

Conclusion

This is the first report to investigate the role of NFκB signal transduction pathway in shock wave-induced cellular damage. Using MDCK cell model, the present study demonstrated that NFκB activation and its target cytotoxic genes expression (*iNOS* and *COX-2*) were involved in shock wave-induced cells damage. Inhibiting this pathway by using PDTC could decrease iNOS protein expression and resultantly alleviate cells damage. The precise mechanism is required to be illuminated by further studies.

References

1. Williams JC Jr, Stonehill MA, Colmenares K, Evan AP, Andreoli SP, Cleveland RO, Bailey MR, Crum LA, McAteer JA (1999) Effect of macroscopic air bubbles on cell lysis by shock wave lithotripsy in vitro. Ultrasound Med Biol 25:473–479
2. Munver R, Delvecchio F, Kuo RL, Brown SA, Zhong P, Preminger GM (2002) In vivo assessment of free radical activity during shock wave lithotripsy using a microdialysis system: the renoprotective action of allopurinol. J Urol 167:327–334
3. Xiang L, He DL, Cheng XF, Zhang LL, Yu LH, Li JJ (2005) Effects of components isolated from *Astragalus mengholicus* on expression of P-selectin in shock wave induced kidney injury in rabbit model. Zhongguo Zhong Yao Za Zhi 20:1609–1609
4. Delvecchio F, Auge Bk, Munver R, Brown SA, Brizuela R, Zhong P, Preminger GM (2003) Shock wave lithotripsy causes ipsilateral renal injury remote from the focal point: the role of regional vasoconstriction. J Urol 169:1526–1529
5. Willis LR, Evan AP, Connor BA, Reed G, Fineberg NS, Lingeman JA (1996) Effects of extracorporeal shock wave lithotripsy to one kidney on bilateral glomerular filtration rate and PAH clearance in mini pigs. J Urol 156:1502–1507
6. Suzuki A, Kudoh S, Mori K, Takahashi N, Suzuki T (2004) Expression of nitric oxide inducible nitric oxide synthase in acute renal allograft refection in the rat. Int J Urol 11:837–844
7. Chien CH, Hwu Cm, Liou TL, Huang ZL, Shen AR, Yang VH, Lee CW, Chien EJ (2006) Inducible nitric oxide synthase expression and plasma bilirubin changes in rats under intermitten hypoxia treatment. Chin J Physiol 49:275–280
8. Lahde M, Korhonen R, Moilanen E (2000) Regulation of nitric oxide production in cultured human T84 intestinal epithelial cells by nuclear factor-κB dependent induction of inducible nitric oxide synthase after exposure to bacterial endotoxin. Aliment Pharmacol Ther 14:945–954
9. Ito Y, Katagiri H, Ishii K, Kakita A, Hayashi I, Majima M (2003) Effects of selective cyclooxygenase inhibitors on ischemia/reper-

fusion-induced hepatic microcirculatory dysfunction in mice. Eur Surg Res 35:408–416
10. Dimayuga FO, Wang C, Clark JM, Dimayuga ER, Dimayuga VM, Bruce Keller AJ (2007) SOD1 over expression alters ROS production and reduces neurotoxic inflammatory signaling in microglial cells. J Neruoimmunol 182:189–199
11. Eliseev RA, Zuscik MJ, Schwarz EM, O'Keefe RJ, Drissi H, Rosier RN (2005) Increased radiation-induced apoptosis of Saos2 cells via inhibition of NF-kappaB: a role for c-Jun N-terminal kinase. J Cell Biochem 96:1262–1273
12. Davis ME, Grumbach IM, Fukai T, Cutchins A, Harrison DG (2004) Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding. J Biol Chem 279:163–168
13. Suetsugu H, Iimuro Y, Uehara T, Nishio T, Harada N, Yoshida M, Hatano E, Son G, Fujimoto J, Yamaoka Y (2005) Nuclear factor-kappaB inactivation in the rat liver ameliorates short term total warm ischemia/reperfusion injury. Gut 54:835–842
14. Adams V, Nehrhoff B, Spate U, Linke A, Schulze PC, Baur A, Gielen S, Hambrecht R, Schuler G (2002) Induction of iNOS expression in skeletal muscle by IL-1beta and NF-kappaB activation: an in vitro and in vivo study. Cardiovasc Res 54:95–104
15. Nakao S, Ogtata Y, Shimizu E, Yamazaki M, Furuyama S, Sugiya H (2002) Tumor necrosis factor alpha (TNF-alpha)-induced prostaglandin E2 release is mediated by the activation of cyclooxygenase-2 (COX-2) transcription via NF-kappaB in human gingival fibroblasts. Mol Cell Biochem 238:11–18
16. Lin CI, Chen CN, Chen JH, Lee H (2006) Lysophospholipids increase IL-8 and MCP-1 expressions in human umbilical cord vein endothelial cells through an IL-1-dependent mechanism. J Cell Biochem 99:1216–1232
17. Jan CR, Chen WC, Lee YH, Huang JK, Ou HC, Tseng CJ (1997) Allopurinol blocks shock-wave-induced rises in cytosolic calcium levels in MDCD cell. Urol Res 25:427–432
18. Jaeger P, Redha F, Marquardt K, Uhlschmid G, Hauri D (1995) Morphological and functional changes in canine kidneys following extracorporeal shock wave treatment. Urol Int 54:48–58
19. Evan AP, Wills LR, Mc Ateer JA, Bailey MR, Connors BA, Shao Y, Lingeman JE, Williams JC Jr, Finberg NS, Crum LA (2002) Kidney damage and renal functional changes are minimized by waveform control that suppresses cavitation in shock wave lithotripsy. J Urol 168:1556–1562
20. Ogiste JS, Nejat RJ, Rashid HH, Greene T, Gupta M (2003) The role of mannitol in alleviating renal injury during extracorporeal shock wave lithotripsy. J Urol 169:875–877
21. Strohmaier WL, Billes IC, Abelius A, Lahme S, Bichler KH (2002) Selenium reduces high energy shock wave induced renal injury in rats. Urol Res 30:31–34
22. Ozguner F, Armagan A, Koyu A, Caliskan S, Koylu H (2005) A novel antioxidant agent caffeic acid phenethyl ester prevents shock wave-induced renal tubular oxidative stress. Urol Res 33:239–243
23. Li X, He D, Zhang L, Cheng X, Sheng B, Luo Y (2006) A novel antioxidant agent, astragalosides, prevents shock wave-induced renal oxidative injury in rabbits. Urol Res 34:277–282
24. Delvecchio FC, Brizuela RM, Khan SR, Byer K, Li Z, Zhong P, Preminger GM (2005) Citrate and vitamin E blunt the shock wave-induced free radical surge in an in vitro cell culture model. Urol Res 33:448–452
25. Midwest Urologic Stone Unit HennepinCounty Medical Center, Minneapolis. MN (1994) Renal function after extracorporeal shock wave lithotripsy to a solitary kidney. J Endourol 8:15–19
26. Chanani NK, Cowan DB, Takeuchi K, Poutias DN, Garcia LM, del Nido PJ, McGowan FX Jr (2002) Differential effects of amrinone and milrinone upon myocardial inflammatory signaling. Circulation 106:284–289
27. Candelario-Jalil E, Gonzalez-Falcon A, Garcia-Cabrera M, Leon OS, Fiebich BL (2007) Post-ischaemic treatment with the cyclooxygenase-2 inhibitor nimesulide reduces blood-brain barrier disruption and leukocyte infiltration following transient focal cerebral ischaemia in rats. J Neurochem 100:1108–1120
28. Li G, Labruto F, Sirsjo A, Chen F, Vaage J, Valen G (2004) Myocardial protection by remote preconditioning: the role of nuclear factor kappa-B p105 and inducible nitric oxide synthase. Eur J Cardiothorac Surg 26:968–973
29. McDonald MC, Mota-Filipe H, Paul A, Cuzzocrea S, Abdelrahman M, Harwood S, Plevin R, Chatterjee PK, Yaqoob MM, Thiemermann C (2001) Calpain inhibitor I reduces the activation of nuclear factor-kappaB and organ injury/dysfunction in hemorrhagic shock. FASEB J 15:171–186
30. Tran K, Merika M, Thanos D (1997) Distinct functional properties of I-kappaB alpha and I-kappaB beta. Mol Cell Biol 17:5386–5399
31. Karin M (1999) How NF-kappaB is activated: the role of the I-kappaB kinase (IKK) complex. Oncogene 18:6867–6874
32. Boaz M, Matas Z, Biro A, Katzir Z, Green M, Fainaru M, Smetana S (1999) Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. Kidney Int 56:1078–1083
33. Muia C, Mazzon E, Maiere D, Zito D, Di Paola R, Domenico S, Crisafulli C, Britti D, Cuzzocrea S (2006) Pyrrolidine dithiocarbamate reduced experimental periodontitis. Eur J Pharmacol 539:205–210
34. Krunkosky TM, Martin LD, Fischer BM, Voynow JA, Adler KB (2003) Effects of TNF-alpha on expression of ICAM-1 in human airway epithelial cells in vitro: oxidant-mediated pathways and transcription factors. Free Radic Biol Med 35:1158–1167
35. Ferreira ZS, Fernandes PA, Duma D, Assreuy J, Avellar MC, Markus RP (2005) Corticosterone modulates noradrenaline-induced melatonin synthesis through inhibition of nuclear factor kappa B. J Pineal Res 38:182–188
36. Ha T, Li Y, Gao X, McMullen JR, Shioi T, Izumo S, Kelley JL, Zhao A, Haddad GE, Williams DL, Browder IW, Kao RL, Li C (2005) Attenuation of cardiac hypertrophy by inhibiting both mTOR and NF-kappaB activation in vivo. Free Radic Biol Med 39:1570–1580
37. Bank N, Kiroycheva M, Singhal PC, Anthony GM, Southan GJ, Szabo C (2000) Inhibition of nitric oxide synthase ameliorates cellular injury in sickle cell mouse kidney. Kidney Int 58:82–89
38. Szabo C, Salzman AL (1996) Inhibition of terminal calcium overload protects against peroxynitrite-induced cellular injury in macrophages. Immunol Lett 51:163–167
39. Moberly JB, Harris SI, Riff DS, Dale JC, Breese T, McLaughlin P, Lawson J, Wan Y, Xu J, Truitt KE (2007) A randomized, double-blind, one-week study comparing effects of a novel COX-2 inhibitor and naproxen on the gastric mucosa. Dig Dis Sci 52:442–450
40. Cheng H, Wang S, Jo YI, Hao CM, Zhang M, Fan X, Kennedy C, Breyer MD, Moeckel GW, Harris RC (2007) Overexpression of cyclooxygenase-2 predisposes to podocyte injury. J Am Soc Nephrol 18:551–559
41. Hierholzer C, Harbrecht BG, Billiar TR, Tweardy DJ (2001) Hypoxia-inducible factor-1 activation and cyclo-oxygenase-2 induction are early reperfusion-independent inflammatory events in hemorrhagic shock. Arch Orthop Trauma Surg 121:219–222