Urol Res (2005) 33: 440–447
DOI 10.1007/s00240-005-0505-3

ORIGINAL PAPER

Pouran Habibzadegah-Tari · Karen Byer
Saeed R. Khan

Oxalate induced expression of monocyte chemoattractant protein-1 (MCP-1) in HK-2 cells involves reactive oxygen species

Received: 27 July 2004 / Accepted: 26 May 2005 / Published online: 24 November 2005

Abstract Oxalate is a toxic end product of metabolism largely because of its propensity to crystallize and form calcium oxalate, which is insoluble at physiologic pH and often deposits at very unfortunate sites, notably the kidneys. In the current study, we investigated the oxalate-induced injury and up-regulation of monocyte-chemoattractant protein-1 (MCP-1) in HK-2 cells, a proximal tubular epithelial cell line derived from normal human kidney. The cells were exposed to oxalate ions for different lengths of time. The culture media was tested for LDH release, a cell injury marker. mRNA was isolated from the cells and subjected to reverse transcriptase-polymerase chain reaction. The data showed that oxalate exposure resulted in cell injury in a time and concentration dependent manner. The MCP-1 mRNA increased following exposure to oxalate and was reduced upon treatment with free radical scavengers, catalase and superoxide dismutase. These data support the importance of reactive oxygen species in the induction of expression of MCP-1 in renal epithelial cells. To our knowledge, this is the first report of MCP-1 expression and its upregulation by oxalate exposure in HK-2 cells.

Keywords Oxalate · Reactive oxygen species · Oxidative stress · MCP-1 · Inflammation · Kidney stones · Nephrolithiasis

Introduction

Oxalate (Ox) is a naturally occurring, toxic byproduct of metabolism and a common constituent of most diets [1]. Research in the past few years has shown that epithelial as well as non-epithelial cells are activated by Ox ions and calcium oxalate (CaOx) crystals [2–15]. The response is biphasic and concentration dependent [4, 5]. Ox is mitogenic at low concentrations and toxic at higher concentrations and in association with CaOx crystals. At high concentrations, Ox promotes hyperoxalemia, hyperoxaluria [2] and deposition of CaOx crystals in the kidneys [2, 3] producing diverse pathological disorders such as CaOx urolithiasis, renal fibrosis and, in extreme cases, renal failure [7].

Injury to the renal epithelial cells results in cellular degradation and the production of membranous vesicles, which promote crystal nucleation at reduced supersaturation [3, 6, 8]. These crystals are either passed as crystalluria particles [9, 10], or retained inside the kidneys by attaching to tubular epithelial cells [3, 11–14], or they are endocytosed by the epithelial cells [15] to be processed by their lysosomal system or transported to the interstitium, where they become surrounded by monocytes and macrophages [3, 16]. Results of investigations in animals and in LLC-PK1, MDCK and NRK52E cells in culture have demonstrated that Ox toxicity (caused by either Ox ions and/or CaOx crystals) is associated with the generation of free radicals [17, 18]. It is our hypothesis that, as a reaction to the Ox induced injury, kidneys respond by inflammation and repair [19]. Renal epithelial cells produce various mediators of inflammation leading to mononuclear infiltration of the interstitium. Mononuclear phagocytes become activated, which then release their contents around interstitial crystals. These contents contain enzymes and other factors needed as a defense against unwanted invaders, but when released into the tissue cause serious damage.

Since inflammation is the result of the injury, it is important to investigate the inflammatory processes during renal epithelial exposure to Ox, specifically the upregulation of monocyte chemoattractant protein-1 (MCP-1) in renal epithelial cells. MCP-1 is a potent chemoattractant for monocytes and lymphocytes, and plays a crucial role in the pathogenesis of a variety of

P. Habibzadegah-Tari · K. Byer · S. R. Khan (✉)
Department of Pathology and Laboratory Medicine,
University of Florida College of Medicine, Box 100275 Gainesville,
Florida 32610-0275, USA
E-mail: Khan@pathology.ufl.edu
Tel.: +1-352-3923574
Fax: +1-352-3928177

crystal deposition diseases. Silica stimulates alveolar macrophages and alveolar type II cells to produce host cytokines and chemokines including MCP-1 [20]. The cellular response is mediated in part by oxidant stress and can be attenuated by antioxidant treatment. Both crocidolite and chrysotile asbestos as well as talc stimulate mesothelial cells lining the pleura to produce of MCP-1 [21, 22]. Upon interaction with monosodium urate, synovial lining cells produce MCP-1 [23]. Research in our laboratory has shown that both Ox ions and CaOx crystals stimulate NRK52 E cells, a line obtained from normal rat kidneys, to produce MCP-1. The response is mediated, at least in part, by reactive oxygen species (ROS) [24, 25].

The toxicity of Ox to HK-2 cells, a line of proximal tubular epithelial cells from normal human kidney, has already been demonstrated [26]. It has been suggested that the increased urinary Ox levels may play a role in crystal retention and, therefore, in the pathogenesis of urolithiasis by inducing damage to renal epithelial cells. In the current study, the cells were exposed to hydrogen peroxide, H_2O_2, a naturally occurring ROS and an established mediator of cell injury. We also evaluated the effect of Ox ions on MCP-1 expression in HK-2 cells, and the involvement of ROS in the process. We further examined whether catalase and superoxide dismutase (SOD), the two endogenous free radical scavengers and part of cellular antioxidant defenses, can inhibit MCP-1 gene expression following exposure to Ox ions.

Materials and methods

Cell culture

HK-2 cells were purchased from the American Type Culture Collection (CRL-2190; Manassa, Va., USA). HK-2 is the only human renal tubular cell line available, and has previously been used for similar studies [26]. Both clinical and animal model studies have shown crystal deposition in the renal proximal tubules during increased urinary excretion of Ox associated with primary hyperoxaluria and many types of enteric hyperoxalurias [1].

For experimental studies, cells were maintained in 75 cm^2 Falcon (Fisher) T-flasks in Dulbecco's modified essential medium and F-12 (DEMM/F-12, Gibco BRL) containing 10% fetal calf serum, and penicillin and streptomycin at 37°C in a 5% CO_2 atmosphere. The media was changed every 2–3 days. The cells were seeded at 10^6 in six well plates and at 10^5 for 96-well plates unless otherwise stated. Cell cultures were grown to confluence unless otherwise stated. To reduce the variability of the results caused by differences in seeding density and/or plating efficiency, the cells were grown to confluence to allow the formation of a tight junction and well defined cell-to-cell contacts, as reported earlier using scanning electron microscopy [6]. Confluence monolayers (95%) of HK-2 cells were used on days 3–4 after plating. Once the cells became confluent, the media was removed and the cells washed with phosphate buffered saline (PBS). The cells were placed in serum and sodium-pyruvate free media (Gibco BRL) supplemented with an insulin/transferin/selenium mix (Gibco BRL), hydrocortisone (Sigma), triiodo-L-thyronine (Sigma) and prostaglandin E1 (Sigma) overnight to arrest growth.

Oxalate studies

The duration of cell exposure and concentration of Ox to which the cells were exposed were selected based on the results of earlier studies from our laboratory [17, 24, 25] as well as other laboratories [4, 5, 18, 26], and on the likelihood of the concentration occurring inside the kidneys. The concentration of Ox in the urine changes as it courses through the nephron and is 0.22 mM in the normal excreted urine, 0.44 mM in conditions of mild hyperoxaluria and 1.5 mM in primary and various enteric hyperoxalurias. Various studies mentioned above have used 0.1–4 mM Ox for exposure of renal epithelial cells in vitro. Ox concentrations used here create metastable conditions without overt crystallization of CaOx in the medium. Culture medium was analyzed by light and scanning electron microscopy to determine the presence of any crystals. A stock solution (10 mM) of Ox as sodium Ox was made in PBS and used within 24 h. Following washing with PBS, the cells were exposed to the serum and sodium-pyruvate free media supplemented with NaOx (0.5, 0.8, 1 and 2 mM) for 0.5, 1, 3, 6 and 12 h at 37°C unless otherwise stated. The pyruvate was removed because previous studies have shown that it interferes with the production of hydrogen peroxide by hypoxanthine/xanthine oxidase or glucose/glucose oxidase [17, 29]. Each treatment was done in triplicate unless otherwise stated. The culture media was collected in 2 ml microfuge tubes and stored at −20°C till further analysis, and total RNA was isolated from the cells using TRIZOL reagent (Life Technologies) according to the manufacturer's procedure and stored at −80°C.

A similar experiment was performed to look at the effect of various concentrations of H_2O_2 (0.5, 0.8 and 1 mM) for 3 and 6 h. Concentrations chosen here are similar to those published elsewhere [26].

Superoxide generation

A modified version of a previously described assay for the conversion of nitro blue tetrazolium (NBT) to formazan by superoxide anions was used to measure the generation of ROS [30]. For this set of experiments, the HK-2 cells were grown in 96-well plates, using one row (12 wells) per concentration per time point. Once they reached confluence (95%), the media was removed and the cells washed and placed in serum free media as

described previously. The cells were then exposed to the serum free media (0.2 ml) supplemented with 1 mg/ml NBT. Ox (0, 0.5, 1 and 2 mM) was also added, and incubated at 37°C for 0, 2, 4, 6 and 12 h. At specified times, the supernatant NBT solution was aspirated from the wells and the wells thoroughly washed with 75% methanol to stop the reaction. The wells were then washed four times with 100% methanol to remove unreduced NBT dye and allowed to air-dry. The reduced formazan precipitate remained visible as purple granules on the bottom of the wells. After air drying, 70 µl of 2 M KOH was added to each well to lyse the cells. The formazan was then solubilized by the addition of 82 µl dimethyl sulfoxide to the KOH (at a ratio of 1:1.17 volume per volume). The content of the wells was then mixed by pipetting to complete solubilisation. The $O.D._{655}$ of the solution was read on an ELISA reader (BioRad 3550 microplate reader). The blank for this experiment consisted of wells with no cells which were incubated with NBT solution and subjected to the same processing (fixing, washing and solubilisation steps), which remained colorless.

LDH release

Lactate dehydrogenase (LDH) is a stable cytosolic enzyme that is released when the cell is lysed or the cell membrane is injured. On the day the experiments were carried out, conditioned media were removed from the experimental wells and assayed for LDH activity or stored at −20°C for later use. A colorimetric assay kit (Proteins International) was used to measure the LDH. The samples were centrifuged to remove crystals and cellular debris. Aliquots of the conditioned media (50 µl) in duplicate were transferred to 96 well flat plates. A positive control (cells lysed with lysis buffer supplied with kit) and blank (media) were also aliquoted to the designated wells. The substrate, 50 µl (provided with the kit), was added to all the samples and incubated at room temperature in the dark for half an hour. Following incubation, stop solution was added and the plate read at 490 nm on a microplate reader (BioRad 3550 microplate reader). The comparisons were made against appropriate blanks.

RNA isolation and PCR quantification of MCP-1 mRNA

To determine MCP-1 mRNA expression in HK-2 cells, total cellular RNA was isolated from treated and untreated cells (1×10^8 cells) using TRIZOL reagent (Gibco BRL) according to the manufacturer's instructions. This was subjected to reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, a 20-µl reaction volume was used for 2 µg of total RNA. The following components: 1 µl of 10-mmol/l dNTP mix (Eppendorf), 1 µl of oligo d $(T)_{12–18}$ (Invitrogen) and mRNA were added to a nuclease free microfuge tube. Enough DNase/RNase-free H_20 was added to bring the final volume to 10 µl. The sample was mixed by pipetting up and down, placed at 65°C for 5 min and chilled on ice. The tubes were briefly centrifuged to collect the contents to which the following were added: 4 µl of 5× first-strand buffer (Invitrogen), 2 µl 100 mmole/l DTT (Invitrogen), 1 µl RNasin (Promega), 2 µl acetylated BSA (Promega) and 1.0 µl reverse transcriptase (Invitrogen). The sample was mixed gently and incubated at 42°C for 5 min, then at 70°C for 15 min in the thermocycler. PCR was carried out in a reaction mixture consisting of 1 µl of RT product, 5 µl of 10×PCR reaction buffer (Qiagen), 2.5 µl of 10 mmol/l dNTP (Eppendorf) mix, 2.5 µl of 25 mmol/l MgCl2 (Qiagen), 0.5 U of recombinant Taq polymerase (Qiagen) and 2 µl of each of the 5′ and 3′ primers (50 pm/µl) of human MCP-1 and β-actin. The primers from the published cDNA sequence are hMCP-1:5′-AGTCTCTGCCGCCCTTCTGTG-3′ and 5′-TT-GGGTTGTGGAGTGAGTGTT-3′, amplifying a 315 bp product; and β-actin: 5′-GTGGGGCGCCCC-AGGCACCA-3′ and 5′-CTCCTTAATGTCACGCAC-GATTTC-3′, amplifying a 590 bp product. MCP-1 and β-actin were amplified for 36 cycles at 94°C for 1 min, annealing at 70°C for 45 s, followed by extension at 72°C for 1 min. The product (10 µl) was resolved on a 2% agarose gel plus ethidium bromide using a 100 bp DNA ladder (Promega) as the molecular weight marker. The PCR band intensity was analyzed using quantitative densitometry.

Effect of free radical scavengers

To evaluate the protective effect of free radical scavengers, cells were exposed to NaOx (1 mM) following pretreatment with catalase (2,000u/ml, Sigma) or SOD (400 μ/ml, Sigma) for 8–12 h. Briefly, after treatment the media was removed and replaced with fresh serum free media containing NaOx (1 mM) alone and/or in combination with catalase (2,000 U/ml) or SOD (400 U/ml) for 3 or 6 h, unless otherwise stated. The conditions, such as concentration of SOD and catalase and time of exposure were chosen from previously published values [17, 25]. The exposure time (8–12 h) was shorter than that reported earlier (24 h) and had no effect on cell viability.

Enzyme linked-immunosorbent assay

The content of MCP-1 in the liquid culture conditioned media was determined using a human specific enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's instructions (Biosource, Camarillo, Calif., USA). Total protein content of the culture media from treatments and control were determined using the BCA Protein Assay Kit (Pierce, Rockford, Ill., USA). The experiment was done twice and data are presented as means ± SED.

Statistical analysis

The data analyzed here consisted of LDH concentration, superoxide production, and free radical scavengers. There are three factors: cell line, HK-2 cells, treatment, (control, Ox and/or H_2O_2 alone or in combination with catalase and SOD and treatment time: 0, 3, 6, and 12 h). Each experiment was done at least three times. The analysis was done using one-way analysis of variance (ANOVA) with Boneferri/Dunn tests to determine the variability within an experiment (intra) and among the experiments (inter). $P < 0.05$ was considered significant.

Results

The addition of Ox to the culture medium did not produce overt crystallization visible under the light microscope. However, when culture medium to which 1 mM Ox was added was examined by scanning electron microscopy a few crystals could be identified.

LDH release

Figure 1A and B show LDH release following exposure to Ox. Experiments were carried out using Ox at a final concentration of 0.2, 0.5 and 1 mM for 3, 6 or 12 h. In general, there was a time and concentration dependent increase in LDH release by HK-2 cells. A significant release of LDH was observed following exposure to 1 mM Ox (Fig. 1), which reached its peak in 12 h (30%) (data for 0.2 and 0.5 mM Ox over time are not shown). When cells were exposed to H_2O_2, a concentration dependent release of LDH was observed after 6 h of exposure (Fig. 2). Our results are comparable to those reported by Koul and colleagues [26], which showed that exposure of HK-2 cells to higher levels of H_2O_2 resulted in cell loss. At 0.8 mM H_2O_2 the maximum LDH release was observed.

Since Ox is known to induce the production of free radicals, we examined the effect of SOD, a scavenger of free radicals, on Ox-induced LDH release (Fig. 3). There was a significant increase in LDH release at 6 h following exposure to 1 mM Ox in the absence of SOD. When cells were exposed to Ox in the presence of SOD, the level of LDH declined to that of the control levels. The level of LDH release by cells exposed to SOD alone was comparable to that of the control. The LDH release at 6 h of exposure to 1 mM H_2O_2 in the absence of SOD was similar to Ox exposure and highly significant.

Effect of oxalate on the production of superoxide anion

We measured the production of superoxide anion in HK-2 cells following exposure to Ox (0.5, 1 or 2 mM)

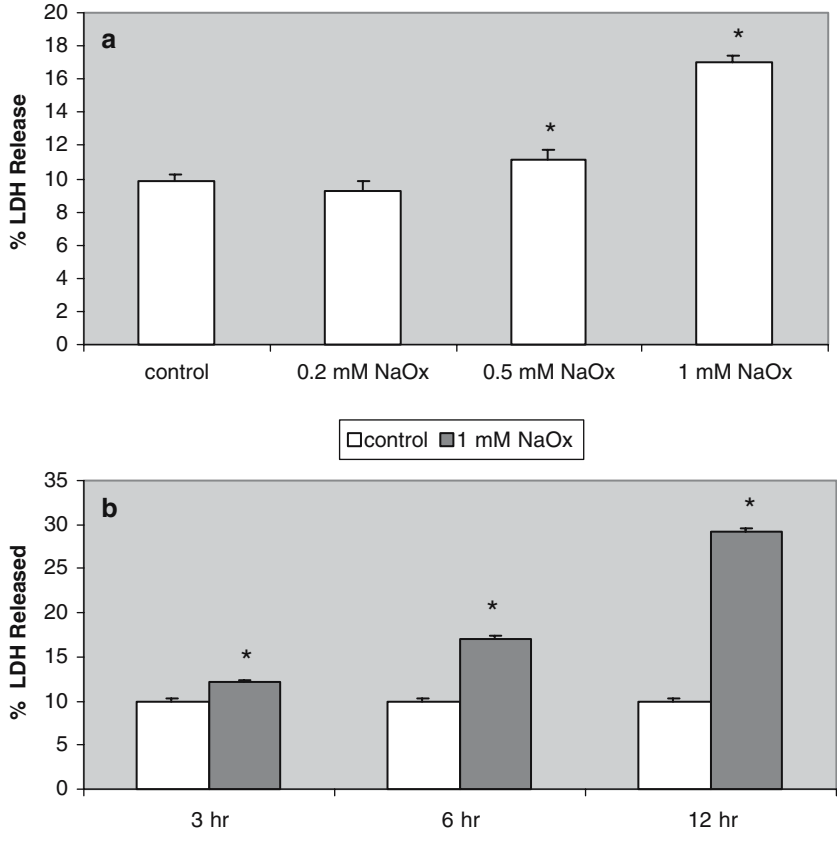

Fig. 1 Percent increase in lactate dehydrogenase (*LDH*) release by HK-2 cells exposed to Ox. A positive control (cells lysed with lysis buffer supplied with kit) and blank (media) were also aliquoted to the designated wells. **A** Confluent cells were exposed to 0.2, 0.5 and 1 mM of oxalate ions for 6 h.**B** Confluent cells were exposed to 1 mM Ox ions for 3, 6 or 12 h. Three independent experiments showed similar results. Data shown are mean ± SEM; $n = 9$. An *asterisk* indicates $P < 0.05$ versus 3, 6 and 12 h

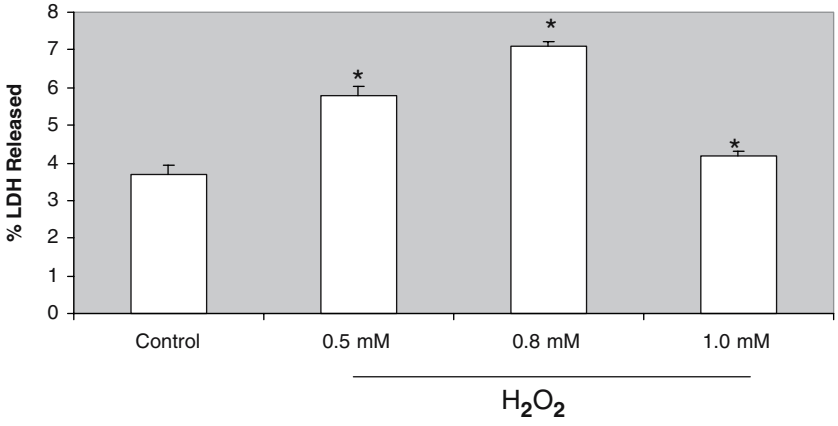

Fig. 2 Effect of various concentrations of H_2O_2 (0.5, 0.8 and 1 mM) on LDH release after exposures for 6 h. Data shown are mean ± SEM; $n = 9$. An *asterisk* indicates $P < 0.05$ versus 0.5, 0.8 and 1 mM OX, 6 h

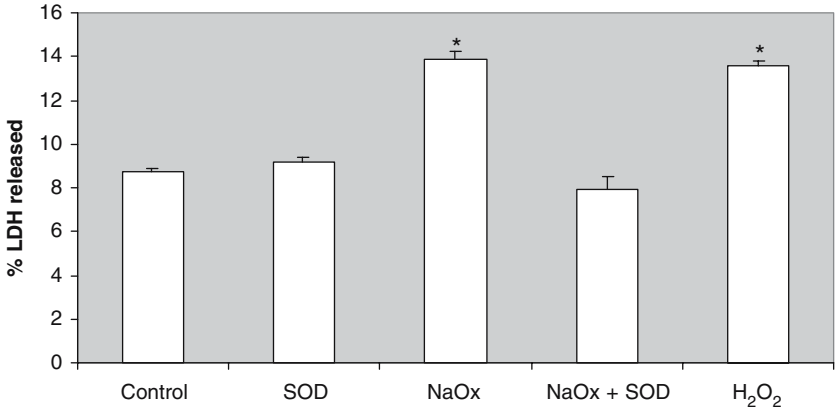

Fig. 3 Percent increase in lactate dehydrogenase (LDH) release against control by HK-2 cells exposed to 1.0 mM NaOx with or without superoxide dismutase (*SOD*) and H_2O_2 (1 mM) for 6 h. The *bar graphs* show the mean ± SEM; $n = 9$. An *asterisk* indicates $P < 0.05$ versus control

using the nitro blue tetrazolium assay. This experiment was done three times and ANOVA was used to look at the variability within an experiment (intra) and among the experiments (inter). Furthermore, ANOVA was used to test for main effects. There was a significant increase in superoxide production in the cells exposed to Ox compared to the unexposed control cells (Fig. 4). The increase was time and concentration dependent for up to 12 h. From 12–48 h, superoxide production remained constant (results not shown).

Expression of MCP-1 mRNA

RT-PCR was used to determine the MCP-1 mRNA expression in HK-2 cells (Fig. 5A, B). Results show that normal unexposed HK2 cells express MCP-1 mRNA. The expression increased when the cells were exposed to Ox. We also investigated the effect of an externally supplied inorganic H_2O_2 on the expression of MCP-1 mRNA in HK2 cells. H_2O_2 is a normally occurring ROS which is formed by the dismutation of superoxide [31, 32]. H_2O_2

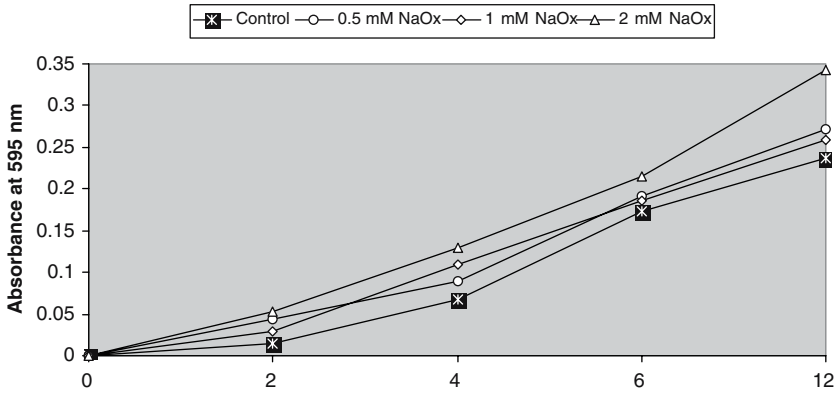

Fig. 4 Effect of Ox exposure on production of superoxide anion. Confluent cells were exposed to 0.5, 1 mM and 2 mM of oxalate ions for 0, 2, 4, 6, 12, 24, and 48 h. There was a time and concentration dependent increase. Production plateaued after 12 h (data not shown); $n = 12$, data shown are mean ± SEM. $P < 0.05$ versus 0.5, 1.0 and 2 mM OX, 2, 4, 6 and 12 h

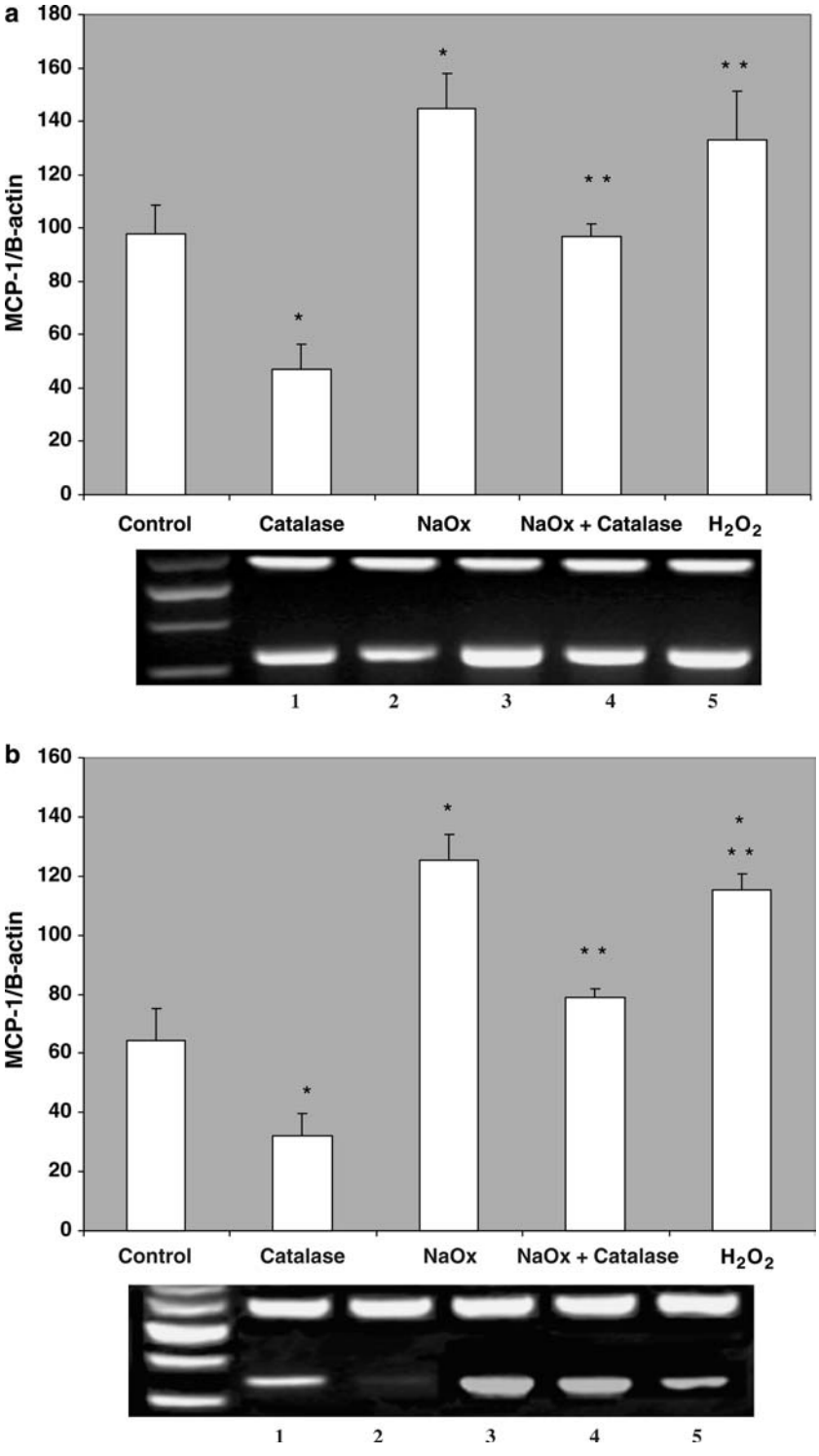

Fig. 5 Expression of MCP-1 mRNA by RT-PCR in HK-2 cells exposed to NaOx (1 mM) and/or H2O2 (1 mM) with or without catalase (**A**) or superoxide dismutase (*SOD*) (**B**) for 6 h. Samples are as follow: *1* Control, *2* catalase/SOD, *3* NaOx, *4* NaOx + catalase/SOD, *5* H_2O_2. The *lower band* is the predicted 315 bp fragment of MCP-1 and the *upper band* is the predicted 590 bp fragment of β-actin. The *bar graphs* show the MCP-1/β-actin ratio. Significance determined $P < 0.05$. An *asterisk* indicates significance against the control; a *double asterisk* indicates significance between treated groups; $n = 6$

exposure, similarly to Ox exposure, up-regulated the expression of MCP-1 mRNA in the HK2 cells.

Since ROS have been shown to induce MCP-1, we also examined the effect of antioxidants on Ox-induced MCP-1 expression. The cells were exposed to Ox alone or in combination with catalase or SOD (Fig. 5A, B). Catalase is a peroxisomal enzyme which breaks down H_2O_2 into oxygen and water. The MCP-1 mRNA levels were reduced when cells were exposed to Ox in the presence of catalase (Fig. 5A). Endogenous (constitutive) MCP-1 mRNA was also reduced when unexposed cells were incubated with catalase as compared to the control cells that received no catalase.

The pattern of inhibition of MCP-1 mRNA was similar when SOD was used (Fig. 5B). The level of MCP-1 mRNA was reduced when cells were exposed to

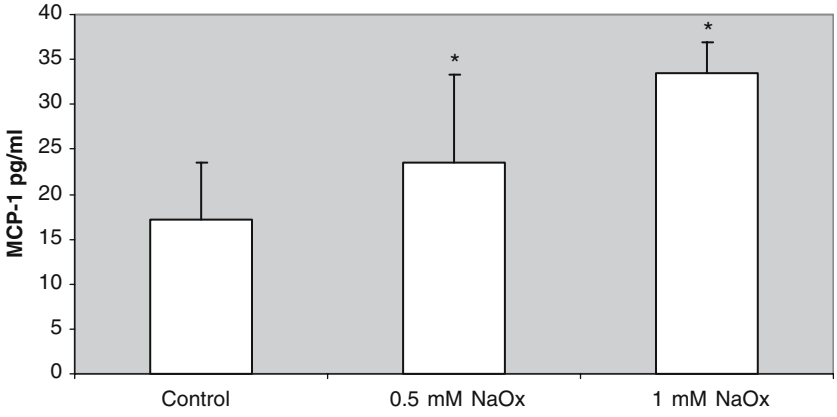

Fig. 6 Dose dependent effect of Ox on MCP-1 production released into the culture media of HK-2 cells after 6 h of exposure. The data are presented as mean ± SED; $n = 6$

Ox in the presence of SOD. SOD also reduced the expression of constitutive MCP-1 mRNA.

Figure 6 shows the release of MCP-1 protein into the culture medium. Normal HK-2 cells produced and secreted MCP-1. Exposure of HK-2 cells to Ox for 6 h increased the release of MCP-1 in a dose dependent manner.

Discussion

This study demonstrates that exposure to Ox ions injures HK-2 cells, a line of human proximal renal epithelial cells. The cell injury, demonstrated by the release of LDH into the culture media, is shown to be a time and concentration dependent response. These results confirm previous observations of Ox induced injury of HK2 cells [26, 33]. Exposure of HK-2 cells to Ox produces a time and concentration dependent increase in membrane permeability. Injurious effects of high concentrations of Ox may also result from the presence of CaOx crystals, which can form at concentrations of 1 mM and above.

The current study shows that Ox exposure of HK2 cells results in the production of superoxide in a time and concentration dependent manner (Fig. 4). Similar results were obtained when LLC-PK1, MDCK and NRK52E cells were exposed to Ox ions [6, 17, 24, 25]. Exposure of these diverse renal epithelial cells to Ox resulted in injury and release of LDH, γ-glutamyltranspeptidase and N-acetyl-β-glucosaminidase. Ox exposure also led to membrane permeability and DNA fragmentation, which was time and concentration dependent [34].

Results of the current study also demonstrate that HK-2 cells expresses MCP-1 mRNA and that this expression is increased following exposure to Ox ions. In addition, the exposure of epithelial cells to Ox increases the secretion of MCP-1 protein. To the best of our knowledge this is the first report of MCP-1 gene expression and its upregulation by Ox exposure in a human renal epithelial cell line. Our earlier studies have shown that a rat renal epithelial cell line, NRK52E, expresses MCP-1 mRNA and protein, the levels of which are increased following Ox exposure.

Furthermore, when HK-2 cells were exposed to Ox in the presence of catalase and/or SOD, MCP-1 gene expression was suppressed, providing evidence that ROS and oxidative stress are involved in the regulation of MCP-1 gene expression. Interestingly, exposure to exogenous H_2O_2, a known oxidant, also resulted in the upregulation of MCP-1 mRNA as determined by RT-PCR. Both catalase and superoxide dismutase caused a decrease in Ox induced MCP-1 gene expression.

'Oxidative stress is a result of either an increase in ROS production or a reduction in endogenous antioxidant defenses. Normal metabolic pathways such as mitochondrial respiration generate ROS. The increased production of ROS by cells exposed to CaOx crystals also originates in the mitochondria [35]. Superoxide anion ($O_2^{\cdot -}$), hydroxy radical ($HO^{\cdot}$) and H_2O_2 are the most prominent ROS. Antioxidant enzymes include SOD, glutathione peroxidase (GPX) and catalase. Dismutation of $O_2^{\cdot -}$ by SOD produces H_2O_2, which is a precursor of the highly reactive $HO^{\cdot}$. H_2O_2 is more stable than other ROS. The superoxide or hydroxyl radicals can go through the cell membrane and have been shown to cause lipid peroxidation, DNA damage and in the end, cell death. H_2O_2 converts to water and oxygen in the presence of catalase. In addition to the antioxidant enzymes, cells also contain compounds such as GSH, ascorbic acid, NADPH and α-tocopherol, which donate hydrogen to neutralize ROS. Reduced GSH donates hydrogen to convert H_2O_2 to water via GPX or acts as a non-enzymatic scavenger of $O_2^{\cdot -}$ or $HO^{\cdot}$.

As mentioned earlier, both animal model studies and investigations of patients with primary hyperoxaluria show the movement of monocytes and macrophages into the renal interstitium [16, 19]. This happens in association with the deposition of CaOx crystals in the kidneys. Infiltration of monocytes and macrophages to the site of the injury is a very important step in the inflammatory response. On the basis of results obtained from this study and earlier observations, we suggest that exposure to Ox ions causes oxidative stress, which eventually leads to the increased expression and synthesis of MCP-1. Chemokines such as MCP-1 play an important role in cell migration and activation during inflammatory

processes. However, this increase in migration and activation of monocytes and macrophages could also be secondary to an increased expression of chemokines other than MCP-1. These inflammatory cells, monocytes and macrophages, can in turn secrete MCP-1 or other chemokines and cytokines at the site of the injury which would lead to more infiltration and damage. The underlying mechanism for Ox-induced upregulation is still not fully understood. Current studies in our laboratory are focused on understanding the molecular mechanism involved in regulation of MCP-1 and other chemokines.

Acknowledgement Supported by NIH grants # RO1 DK 053962, 059765, and 065658.

References

1. Danpure CJ, Purdue PE (1995) Primary hyperoxaluria. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th edn, McGraw-Hill, New York, p 2385
2. Mitwalli A, Oreopoulos DG (1985) Hyperoxaluria and hyperoxalemia: one more concern for the nephrologist. Int J Artif Organs 8: 71
3. Khan SR (1995) CaOx crystal interaction with renal epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res 23: 71
4. Koul H, Kennington L, Nair G, Honeyman T, Menon M, Scheid C (1994) Oxalate-induced initiation of DNA synthesis in LLC-PK1 cells, a line of renal epithelial cells. Biocehm Biophys Res Commun 205: 1632
5. Koul H, Kennington L, Honeyman T, Jonassen J, Menon M, Scheid C (1996) Activation of c-myc gene mediates the mitogenic effects of oxalate in LLC-PK1 cells, a line of renal epithelial cells. Kidney Int 50: 1525
6. Hackett RL, Shevock PN, Khan SR (1995) Alterations in MDCK and LLC-PK1 cells exposed to oxalate and calcium oxalate monohydrate crystals. Scanning Microsc 9: 587
7. Lieske JC, Spargo BH, Toback FG (1992) Endocytosis of calcium oxalate crystals and proliferation of renal tubular epithelial cells in a patient with type 1 primary hyperoxaluria. J Urol 148: 1517
8. Hackett RL, Shevock PN, Khan SR (1990) Cell injury associated calcium oxalate crystalluria J Urol 144: 1535
9. Finlayson B, Reid F (1978) The expectation of free and fixed particles in urinary stone disease. Invest Urol 15: 442
10. Kok DJ, Khan SR (1994) Calcium oxalate nephrolithiasis, a free or fixed particle disease. Kidney Int 46: 847
11. Wiessner JH, Hasegawa AT, Hung LY, Mandel NS (1999) Oxalate-induced exposure of PS on surface of renal epithelial cells in culture. J Am Soc Nephrol 10: S441
12. Mandel NS (1994) Crystal-membrane interaction in kidney stone disease. J Am Soc Nephrol 5: S37
13. Verkoelen CF, Van der Broom BG, Kok DJ, Schroeder FH, Romijn JC (1999) Attachment sites for particles in the urinary tract. J Am Soc Nephrol 10: S430
14. Lieske JC, Leonard R, Swift H, Toback FG (1996) Adhesion of CaOx monohydrate crystals to anionic sites on surface of renal epithelial cells. Am J Physiol 270: F192
15. Lieske JC, Swift H, Martin T, Patterson B, Toback FG (1994) Renal epithelial cells rapidly bind and internalize CaOx monohydrate crystals. Proc Natl Acad Sci U S A 91: 6987
16. DeWater R, Noordermeer C, Houitsmuller AB, Nigg AL, Stinjen T, Schroeder FH, Kok DJ (2000) Role of macrophages in nephrolithiasis in rats: an analysis of the renal interstitium. Am J Kid Dis 36: 615
17. Thamilselvan S, Byer KJ, Hackett RL, Khan SR (2000) Free radical scavengers, catalase and superoxide dismutase provide protection from oxalate-associated injury to LLC-PK1 and MDCK cells. J Urol 164: 224
18. Scheid CR, Koul H, Hill WA, Luber-Narod J, Kennington L, Honeyman T, Jonassen J, Menon M (1996) Oxalate toxicity in LLC-PK1 cells: role of free radicals. Kidney Int 49: 413
19. Khan SR, Thamilselvan S (2000) Nephrolithiasis: a consequence of renal epithelial cell exposure to oxalate and calcium oxalate crystals. Mol Urol 4: 305
20. Barrett EG, Johnston C, Oberdorster G, Finkelstein JN (1999) Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-alpha-induced oxidant stress. Am J Physiol. 276: L979
21. Nasreen N, Hartman DL, Mohammed KA, Antony VB (1998) Talc-induced expression of C-C and C-X-C chemokines and intercellular adhesion molecule-1 in mesothelial cells. Am J Respir Crit Care Med 158: 971
22. Tanka S, Choe n, Iwagaki A, hemenway DR, Kagan E (2000) Asbestos exposure induces MCP-1 secretion by pleural mesothelial cells. Exp Cell Res 26: 241
23. Matsukawa A, Miyazaki S, Maeda T, Tanase S, Feng L, Ohkawara S, Yoshinaga M, Youshimura T (1998) Production and regulation of monocyte chemoattractant protein-1 in lipopolysaccharide or monosodium urate crystal-induced arthritis in rabbits: role of tumor necrosis factor alpha, interleuken-1, and interleuken-8. Lab Invest 78: 973
24. Umekawa T, Chegini N, Khan SR (2002) Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate phosphate and uric acid crystals. Nephrol Dial Transplant 18: 664
25. Umekawa T, Chegini N, Khan SR (2002) Oxalate ions and calcium oxalate crystals stimulate MCP-1 expression by renal epithelial cells. Kidney Int 61: 105
26. Bhandari A, Koul S, Sekhon A, Pramanik SK, Chaturvedi LS, Huang M, Menon M, Koul HK (2002) Effects of oxalate on HK-2 cells, a line of proximal tubular epithelial cells from normal human kidney. J Urol 168: 253
27. De Water R, Leenen PJ, Noordermeer C, Nigg AL, Houtsmuller AB, Kok DJ, Schroder FH (2001) Cytokine production induced by binding and processing of calcium oxalate crystals in cultured macrophages. Am J Kidney Dis 38: 331
28. Hackett RL, Shevock PN, Khan SR (1994) Madin-Darby canine kidney cells are injured by exposure to oxalate and to calcium oxalate crystals. Urol Res 22: 197
29. Salahudeen AK, Clark EC, Nath KA (1991) Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo. J Clin Invest 88: 1886
30. Rook GAW, Steele J, Umar S, Dockrell HM (1985) A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ-interferon. J Immunol Methods 82: 161
31. Chen, Xi-Lin, Zhang Q, Zhao R, Medford RM (2004) Superoxide, H2O2 and iron are required for TNF-α-induced MCP-1 gene expression in endothelial cells: role of Rac1 and NADPH oxidase. Am J Physiol Heart Circ Physiol 286: H1001
32. Baud L, Ardaillou R (1986) Reactive oxygen species: production and role in the kidney. Am J Phsiol 251: F765
33. Koul S, Fu S, Koul H (2003) Oxalate exposure promotes re-initiation of the DNA synthesis and apoptosis of KH-2 cell, a line of human renal epithelial cells. Ann N Y Acad Sci 1010: 292
34. Scheid C, Koul H, Hill W, Luber-Narod J, Jonassen J, Honeyman T, Kennington L, Kohli R, Hodapp J, Ayvazian P, Menon M (1996) Oxalate toxicity in LLC-PK1 cells, a line of renal epithelial cells. J Urol 155: 1112
35. Khand FD, Gordge MP, Robertson WG, Noronha-Dutra AA, Hothersall JS (2002) Mitochondrial superoxide production during oxalate mediated oxidative stress in renal epithelial cells. Free Radic Biol Med 32: 1339