Differential Activation of Signaling Pathways Involved in Cell Death, Survival and Inflammation by Radiocontrast Media in Human Renal Proximal Tubular Cells

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Radiocontrast media (RCM) are widely used in clinical medicine but may lead to radiocontrast-induced nephropathy (RCIN). The pathogenesis of acute renal failure secondary to RCM is not fully understood, but direct toxic effects are believed to be a major cause of RCIN. We have investigated the effect of different types of RCM on signaling pathways known to play a role in cell death, survival, and inflammation. HK-2 cells were incubated with sodium diatrizoate and iomeprol (IOM) at a concentration of 75 mg I/ml for 2 h. Both RCM caused an increase in phosphorylation of p38 mitogen-activated protein kinase (MAPK) (p38) and c-Jun N-terminal kinases (JNKs) and NF-κB (at Ser 276), with sodium diatrizoate having a more drastic effect. Although cell viability was reduced significantly by both RCM, in cells pretreated with IOM the cell viability recovered over a 22-h time period after removal of the RCM. However, viability of diatrizoate-treated cells rose at 5 h but then fell at 22 h after removal of the RCM. The decrease in cell viability in diatrizoate-treated cells corresponded with an increase in phosphorylation of p38 mitogen-activated protein kinase (MAPK) (p38) and c-Jun N-terminal kinases (JNKs) and NF-κB (at Ser 276), with sodium diatrizoate having a more drastic effect. Although cell viability was reduced significantly by both RCM, in cells pretreated with IOM the cell viability recovered over a 22-h time period after removal of the RCM. However, viability of diatrizoate-treated cells rose at 5 h but then fell at 22 h after removal of the RCM. The decrease in cell viability in diatrizoate-treated cells corresponded with an increase in phosphorylation of JNKs, p38, and NF-κB and a decrease in phosphorylation of Akt, signal transducer and activator of transcription 3, and forkhead box O3a, as well as poly (ADP-ribose) polymerase and caspase-3 cleavage. The recovery in viability of IOM-treated cells corresponded most notably with an increase in STAT3 phosphorylation and induction of Pim-1 kinase. There was also an increase in interleukin-8 release by diatrizoate-treated cells indicating the possibility of proinflammatory effects of RCM. A knowledge of the signaling pathways by which RCM exert their cytotoxic actions may help in finding future therapies for RCIN.

Key Words: signal transduction; renal cell; contrast media; toxicity.

Radiographic contrast media (RCM) are widely used in clinical practice, and the use of contrast procedures continues to grow. However, radiocontrast-induced nephropathy (RCIN) may occur especially in patients with preexisting renal insufficiency and in particular those with diabetes (Barrett et al., 1992; Byrd and Sherman, 1979; Lin and Bonventre, 2005; Rich and Creecius, 1990; Rudnick et al., 1995).

Given the high incidence of acute renal failure (ARF) associated with the wide use of contrast media (Briguori et al., 2005; Katzberg, 2005; Marenzi et al., 2004; McCullough and Soman, 2005; Persson et al., 2005; Rezkalla, 2003; Solomon, 2005; Weisbord and Palevsky, 2005), several strategies have been suggested to prevent RCIN, such as avoiding dehydration or performing fluid infusion before contrast injection.

The pathogenesis of ARF secondary to contrast media is still not completely understood, although reduction of renal blood flow and direct toxic effects on renal tubular epithelial cells have been postulated as major causes of RCIN (Heyman et al., 1991; Tervahartiala et al., 1997).

Radiocontrast agents have been reported to induce apoptosis both in glomerular cells and in renal tubular epithelial cells (Heyman et al., 1997; Hizoh and Haller, 2002; Yano et al., 2003; Zhang et al., 1999). Previous work carried out in our laboratory (Andreucci et al., 2006) demonstrated the effects of different RCM on various signaling pathways known to play a role in cellular survival, growth, and proliferation. In particular, it was observed that incubation of human renal proximal tubule cells with RCM caused a dephosphorylation of Akt, a kinase known to play a role in cell survival (Datta et al., 1999), and of many of its downstream targets. Transfection of the renal cells with a plasmid encoding a constitutively active form of Akt kinase substantially, but not completely, alleviated the toxic effect of the RCM. This suggested that there might be other pathways that may be affected by RCM that may determine cellular death/survival.

In this paper, we report the activation of signaling molecules that are known to be involved in cell death, namely, the JNK and p38 mitogen-activated protein kinases (MAPKs) (Kyriakis and Avruch, 2001; Weston and Davis, 2007; Zarubin and Han, 2005), in renal cells incubated with different RCM for up to...
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MATERIALS AND METHODS

MATERIALS. The RCM used in our study were iomeprol (IOM) (Iomeron 400, Bracco S.p.A, Milan, Italy) and sodium diatrizoate (Sigma-Aldrich Co., St Louis, MO). Mannitol was obtained from Sigma-Aldrich Co. and SB203580 from Alexis Corporation. The dose of RCM used in our studies was 75 mg I/ml, as previously done by our group (Andreucci et al., 2006), based on the dosage commonly used in clinical practice of 1.5–2.5 mg I/kg body weight. This leads to plasma concentrations of 15–20 mg I/ml; because in the kidney 70–80% of the ultrafiltrate is reabsorbed in the proximal convoluted tubule, the RCM concentration will range between 75 and 100 mg/ml (Hardick et al., 2001).

CELL CULTURE. In our experiments, we have used HK-2 cells (a human renal proximal tubular epithelial cell line) obtained from the American Type Culture Collection and grown in 100-mm culture dishes (Corning, NY) as described previously (Andreucci et al., 2006). In brief, they were cultured in Dulbecco’s modified Eagle’s medium containing Glutamax ( Gibco, Invitrogen, San Giuliano Milanese, Milano, Italy) supplemented with 10% fetal calf serum and 100 units/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich Co.) in an atmosphere of 5% CO2 in air at 37°C up to a confluence of approximately 90%.

CELL VIABILITY. Cell viability was measured by the ability of viable cells to reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma-Aldrich Co.) ( Mosmann, 1983 ). As previously described reduce 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (Sigma-Aldrich Co.) ( Mosmann, 1983 ). As previously described

RESULTS

Viability of HK-2 Cells after Exposure to RCM for 2 h

HK-2 cells were incubated with sodium diatrizoate (referred to from now on as simply “diatrizoate”) and IOM at a final concentration of 75 mg I/ml for a period of 2 h, after which the RCM was removed and cell viability determined using the MTT assay. The results shown in Figure 1A reveal that although both RCM caused a significant decrease in cell survival with respect to control (nontreated) cells (p < 0.001), diatrizoate (64% in cell viability) had a significantly greater effect than IOM (80% in cell viability) (p < 0.001). Incubation of HK-2 cells with 400 and 200mM mannitol corresponding to the osmolarities of diatrizoate and IOM, respectively, resulted in a statistically significant decrease in cell viability with respect to control cells (p < 0.001). However, the decrease in cell viability was significantly greater in cells treated with diatrizoate than cells treated with 400mM mannitol (p < 0.001) (Fig. 1B); similarly, the decrease in cell viability was greater in cells treated with IOM than cells treated with 200mM mannitol (p < 0.001) (Fig. 1C).

Long-term Viability of HK-2 Cells after Initial Exposure to RCM for a 2-h Period

HK-2 cells were exposed to 75 mg I/ml of each of the two RCM diatrizoate and IOM for 2 h, after which time the medium containing the RCM was removed and replaced with fresh
serum-free medium. Cell viability was measured at 5 and 22 h after removal of the RCM stimulus (i.e., 7 and 24 h after the initial incubation of the cells with each RCM); the data obtained are shown in Figure 1D. It can be seen that 5 h after removal of the RCM, the viability of cells incubated with diatrizoate (83% viability with respect to control cells at \( t = 5\) h) was significantly less than that of both control nontreated cells and cells incubated with IOM (92% viability with respect to control at \( t = 5\) h) \( (p < 0.001\) and \( p < 0.05\), respectively). The viability of diatrizoate-treated cells (74% viability with respect to control at \( t = 5\) h) remained subdued 22 h after removal of the RCM stimulus, again less than that of control at \( t = 22\) h \( (p < 0.0005\) and IOM-treated (113% viability with respect to control at \( t = 5\) h) cells \( (p < 0.001\) over the same time period, and also significantly less than the viability of diatrizoate-treated cells 5 h after removal of the RCM \( (p < 0.05\) ). However, the viability of IOM-treated cells at 22 h after removal of the RCM was significantly higher than that of IOM-treated cells at 5 h after removal of the RCM \( (p < 0.01\) ).

**RCM Cause Phosphorylation of p38, JNKs, and NF-κB in HK-2 Cells**

Incubation of HK-2 cells with both diatrizoate and IOM at a concentration of 75 mg I/ml caused an increase in the phosphorylation of the p38 and JNK subfamilies of MAPKs and also of the transcription factor NF-κB at Ser276 (Fig. 2). Within 5 min of addition of the RCM to HK-2 cells, an increase in JNK phosphorylation was observed. With IOM, there was a gradual increase in phosphorylation, peaking at 60 min and then decreasing after 120 min, but still remaining higher than in control (nontreated) cells. Diatrizoate caused a greater phosphorylation of JNKs at all time points studied, and phospho-JNK (pJNK) levels remained at maximal levels 120 min after addition of the RCM (Fig. 2). Diatrizoate also caused a dramatic increase in p38 phosphorylation within 5 min, maintaining this level of phosphorylation for 120 min. After addition of IOM to HK-2 cells, an increase in p38 versus 400 mannitol-treated cells \( (p < 0.001\), shown with *) (B). Mannitol (200mM) (indicated as “Mannitol 200”) significantly decreased \( (p < 0.001\), shown with *) cell viability versus the untreated HK-2 cells (control, indicated as C). Iomeprol (indicated as IOM) significantly decreased cell viability versus 200 mannitol-treated cells \( (p < 0.001\), shown with *) (C). Viability of HK-2 cells significantly decreased versus untreated HK-2 cells 5 h after removal of sodium diatrizoate (“N5”) (indicated as “N5”; \( p < 0.001\), shown as *). Viability of HK-2 cells significantly decreased versus untreated HK-2 cells 5 h after removal of IOM (indicated as “I5”) \( (C5; p < 0.05\), shown as #) (D). Viability of HK-2 cells significantly decreased not only versus untreated HK-2 cells (indicated as “I22”; \( p < 0.0005\), shown as §) but also versus N5,22 h after removal of sodium diatrizoate (“N22”) \( (p < 0.05\), shown as ~). Viability of HK-2 cells did not change versus untreated HK-2 cells (C22) but significantly increased versus I5 22 h after removal of IOM (indicated as “I22”) \( (p < 0.01\), shown as *) and significantly increased versus N22 \( (p < 0.01\), shown as §) (D). HK-2 cell viability at the time point C22 was significantly higher versus C5 \( (p < 0.01\), shown as +) (D). The data are mean ± SE of three experiments.
phosphorylation was noticeable after 20–30 min, which then decreased to control levels after 120 min. Phosphorylation of NF-κB at Ser276 was also increased 10 min after addition of diatrizoate, increasing dramatically after 30 min and continuing to increase after 120 min. With IOM, however, the increase in phosphorylation was observed at 30 min after addition of the RCM and remained at this level for up to 120 min after RCM addition.

Changes in the Phosphorylation Status of JNKs, p38, and NF-κB in HK-2 Cells after Removal of the RCM Stimulus

After incubating HK-2 cells with the RCM for 2 h, the medium containing the RCM was replaced with fresh serum-free medium, and the phosphorylation/activation of numerous molecules important in cell death/survival and inflammatory signaling pathways were assessed by Western blotting. It was observed that from 1 to 5 h after removal of the RCM from HK-2 cells, the levels of pJNK, phospho-p38, and phospho-NF-κB (Ser276) were higher in the cells exposed to diatrizoate than in those exposed to IOM (Fig. 3A). In fact, IOM-treated cells did not exhibit any levels of pJNKS after removal of the stimulus, whereas pJNK levels in diatrizoate-treated cells were higher up to 5 h after removal of RCM and were still evident 22 h after RCM removal. Phospho-p38 levels in IOM-treated cells were comparable with those of control cells even up to 22 h after removal of the RCM, whereas they were evidently higher in diatrizoate-treated cells and even increased 22 h after removal of the diatrizoate (Fig. 3A). IOM-treated cells did show increased levels of phospho-NF-κB (Ser276) with respect to control cells up to 1 h after removal of the RCM, but this was not evident in later time points. In contrast, levels of phospho-NF-κB (Ser276) in diatrizoate-treated cells were elevated well above those in control cells for up to 5 h after removal of the RCM.

Effects on Prosurvival Signaling Molecules in HK-2 Cells after Removal of RCM Stimulus

An increase in phosphorylation of Akt (Ser473) was observed 1–2 h after removal of diatrizoate, which was greater than in control cells (Fig. 3B) but decreased to lower levels than in control at 3–22 h after removal of diatrizoate. However, in IOM-treated cells, phospho-Akt (Ser473) levels were slightly higher 1 h after removal of the RCM, and then remained at levels comparable with those of control cells thereafter, up to the 22-h time point (Fig. 3B). After removal of RCM, levels of phospho-ERK1/2 were found to be higher in diatrizoate-treated than in IOM-treated cells at 1–5 h after removal of the RCM but lower at 22 h after RCM removal. Furthermore, the phospho-ERK levels were higher than in control cells at 1–5 h but less at 22 h after removal of diatrizoate. The levels of phospho-ERKs in IOM-treated cells were comparable with those of control cells at 1–5 h after removal of IOM, but were higher 22 h after removal of the RCM.

Phospho-STAT3 (Tyr705) levels were also observed to increase 3–5 h after removal of the RCM in IOM-treated cells and remained at levels higher than in control cells for up to 22 h after removal of IOM. However, in diatrizoate-treated cells, levels of phospho-STAT3 appeared to decrease slightly with time after removal of the RCM, the decrease becoming more evident 5 h after removal of the RCM and then diminishing at 22 h after RCM removal. Expression of the kinase Pim-1 was also observed in IOM-treated cells, but not in diatrizoate-treated cells.

Effects on Caspase-3, PARP, and FOXO3a in HK-2 Cells after Removal of the RCM

It was also observed that caspase-3 levels were reduced in diatrizoate-treated cells at 22 h after removal of the diatrizoate (Fig. 3C), although no cleavage product was detected. However, the 89-kDa PARP cleavage product was observed in diatrizoate-treated cells within 1 h after removal of diatrizoate, peaking at 5 h and was still obvious at 22 h after removal of the RCM (Fig. 3C).

The phosphorylation status of the transcription factor FOXO3a at Thr32 in diatrizoate-treated cells gradually decreased with time after removal of the RCM, whereas it did not change drastically in IOM-treated cells (Fig. 3C).

Effect of SB203580 on Long-term Viability of HK-2 Cells after Initial Exposure to Diatrizoate for a 2-h Period

Given the increase in p38 phosphorylation with time after removal of diatrizoate (Fig. 3A), we decided to investigate the possible role of p38 in the decrease in cell viability of diatrizoate-treated cells using the commercially available inhibitor of p38, SB203580. HK-2 cells were preincubated with SB203580 (5μM) prior to treatment with diatrizoate for 2 h. Then, the
inhibitor was maintained in the cell culture medium at the concentration of 5μM even after removal of the diatrizoate stimulus for 22 h. HK-2 cells pretreated and incubated with SB203580 showed a small (but significant) increase in cell viability (approximately 5%) measured 22 h after the removal of diatrizoate (Fig. 4).

Treatment of HK-2 Cells with Sodium Diatrizoate Causes an Increase in IL-8 Release

HK-2 cells treated with RCM for 2 h were then left to incubate for a further 22 h in serum-free medium without RCM. Samples of the culture medium were removed, and the levels of IL-8 released by the cells were determined using ELISA. Figure 5 shows the significant increase in IL-8 production for diatrizoate-treated cells compared with control (nontreated) cells ($p < 0.05$) and IOM-treated cells ($p < 0.05$). There was no significant difference between control and IOM-treated cells.

**DISCUSSION**

In a previous paper (Andreucci et al., 2006), we demonstrated that incubation of HK-2 cells with the RCM diatrizoate, iopromide, and IOM caused the dramatic dephosphorylation of Akt over a period of 2 h. This was accompanied by a decrease in cell viability, as determined by the chemical reduction of MTT. Transfection of HK-2 cells with plasmids encoding the constitutively active form of Akt only partially recovered cell viability (Andreucci et al., 2006). We also observed a decrease in phosphorylation of the ERK1/2 kinases that may also have contributed to lowered cell viability because these kinases are known to play an important role in cell proliferation and survival (Yoon and Seger, 2006). In the present work, we have demonstrated that RCM caused the phosphorylation (and hence activation) of the JNK and p38 MAPK subfamilies as well as of the transcription factor NF-κB. The degree of phosphorylation was significantly greater with diatrizoate than with IOM, corresponding with a greater increase in phosphorylation (activation) of JNKs, p38, and NF-κB (Ser276). Given the proposed role of these molecules in cell death and apoptosis (Kyriakis and Avruch, 2001; Weston and Davis, 2007; Zarubin and Han, 2005), the activation of these molecules by RCM may have also contributed to the decrease in cell viability as reported previously by our group (Andreucci et al., 2006). Two recent papers both suggested that the JNK and p38 MAP kinases may be involved in RCM-induced apoptosis in

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**FIG. 3.** Modification of signaling molecules in HK-2 cells after initial exposure to RCM. HK-2 cells were incubated with RCM at a final concentration of 75 mg I/ml for 2 h. The medium containing the RCM was then removed and replaced with fresh serum-free medium; cell lysates were prepared at each of the time points indicated (1, 2, 3, 5, and 22 h after removal of each RCM) and then subjected to SDS-PAGE followed by Western blotting using antibodies against signaling molecules known to play a role in cell death/inflammation (A and C) and cell growth/survival/proliferation (B). “N” and “I” refer to prior treatment with sodium diatrizoate and iomeprol, respectively.

In (A), phospho-NF-κB is indicated as “pNF-κB,” phospho-p38 is indicated as “p P38,” and phospho-JNK1/2 is indicated as “pJNK1/2.” In (B), phospho-Akt is indicated as “pAkt,” phospho-ERK1/2 is indicated as “pERK1/2,” and phospho-STAT3 is indicated as “pSTAT3.” In (C), phospho-FOXO3a is indicated as “pFOXO3a.” The shown data are representative of blots of three experiments.
We have observed that incubation of HK-2 cells with diatrizoate for 2 h caused a greater decrease in cell viability than with IOM. Furthermore, we observed that, 5 h after the removal of the RCM, the cell viability in diatrizoate-treated cells was significantly lower than in control and IOM-treated cells. At 22 h after removal of the RCM, the cell viability of diatrizoate-treated cells was still less than that of control and IOM-treated cells and also less than diatrizoate-treated cells at the 5-h time point (following RCM removal) (Fig. 1D). However, the viability of both control and IOM-treated cells was significantly higher at 22 than at 5 h. These results suggest that whereas IOM-treated HK-2 cells are able to make a full recovery after removal of the RCM, diatrizoate-treated cells continue to lose viability. Also, the percentage of viable cells in diatrizoate-treated cells was higher 5 h after removal of diatrizoate than at the end of the initial 2-h incubation but then decreased again at 22 h after removal of RCM. Likewise, the viability of IOM-treated cells increased in the 5-h period following removal of the RCM, but unlike the diatrizoate-treated cells, these cells were able to recover like the control untreated cells (Figs. 1A and 1D). We therefore turned our attention to the components of signaling pathways involved in cell death/survival in order to explain the differences in effects on cell viability between the two types of RCM.

After removal of diatrizoate, levels of pJNKs, phospho-p38, and phospho-NF-κB (Ser276) were all at considerably higher levels than in control and IOM-treated cells. Their role in cell death (Kyriakis and Avruch, 2001; Weston and Davis, 2007; Zarubin and Han, 2005) may partially explain the greater decrease in cell viability with diatrizoate-treated cells compared with IOM-treated cells even after removal of the RCM. Interestingly, whereas levels of pJNKs and phospho-NF-κB (Ser276) gradually decreased with time after removal of diatrizoate from HK-2 cells (although remaining significantly higher than in control cells), the elevated levels of phospho-p38 were maintained 5 and 22 h after RCM removal. This suggests that p38 may play a more central role in long-term cell death/survival in HK-2 cells treated with RCM. However, use of the SB203580 inhibitor only slightly improved cell survival in diatrizoate-treated cells (Fig. 4). Because this inhibitor acts on the α and β isoforms of p38, it is possible that the γ and δ isoforms may be playing a role in cell death in HK-2 cells as it has been reported for other cell types (Efimova et al., 2004). Interestingly, despite the initial increase in phosphorylation of JNKs, p38, and NF-κB (at Ser276), there was also a marked increase in phosphorylation of Akt (at Ser473) and ERK1/2 in diatrizoate-treated cells. In fact, the increase in phospho-ERK1/2 was greater in diatrizoate-treated cells (1–3 h after the removal of diatrizoate) than in IOM-treated cells and control cells.

The prosurvival kinase Akt was phosphorylated (at Ser473) at levels higher or equal to control for 2 h after removal of diatrizoate but began to decrease 3–5 h after removal of the RCM. This increase in activity of these prosurvival kinases may have been significantly great enough to lead to a partial recovery in cell viability observed in diatrizoate-treated cells at 5 h after the removal of diatrizoate with respect to the cell viability at 2 h after removal of diatrizoate. In addition to its role in cell survival, Akt has also been implicated in endothelium-dependent vasorelaxation (Kobayashi et al., 2005); the long-term decrease in phospho-Akt levels may therefore contribute to vasoconstriction of blood vessels. Our previous study (Andreucci et al., 2006) together with the present data have demonstrated a decrease in
Akt phosphorylation caused by RCM. In this way, RCM may exacerbate vascular dysfunction in diabetic patients where it is believed that Akt signaling is already abnormally regulated (Kobayashi et al., 2005).

Another signaling molecule that has been implicated in cell proliferation is the transcription factor STAT3 (Horiguchi et al., 2002). Phosphorylation of STAT3 at Tyr705 causes it to dimerize, enter the nucleus, and to bind to DNA (Levy and Lee, 2002). The levels of phospho-STAT3 in IOM-treated cells increased with time after removal of the RCM, suggesting that this molecule may contribute to the proliferation and recovery of cell numbers in culture. In contrast, cells treated with diatrizoate showed decreased phospho-STAT3 levels 5 h after removal of the RCM, whereas 22 h after RCM removal phospho-STAT3 levels had diminished (Fig. 3B).

One target of STAT3 transcription is the gene encoding the prosurvival kinase Pim-1 (Hirano et al., 2000), a Ser/Thr kinase that may phosphorylate substrates involved in apoptosis and metabolism also recognized by Akt (Amaravadi and Thompson, 2005). We observed that the 34-kDa Pim-1 was induced in IOM-treated cells, but its induction preceded the significant increase in STAT3 phosphorylation, suggesting that other factors may be responsible for its expression at least at the earlier time points after removal of RCM. It has also been suggested that Pim-1 may be induced by Akt (Muraski et al., 2007), but again its absence in diatrizoate-treated cells even when high phospho-Akt (Ser473) levels were evident suggests otherwise. Nonetheless, Akt, STAT3, and Pim-1 may contribute independently to the recovery and survival of renal cells to RCM stress.

Diatrizoate-treated cells also demonstrated PARP cleavage, demonstrated by the presence of the 89-kDa cleavage protein (Fig. 3C), indicating that the cells may have been undergoing caspase-dependent apoptosis. However, there was only evidence of caspase-3 cleavage (Fig. 3C) at the last time point observed, but because PARP is also a substrate for caspase-7 (Germain et al., 1999), activation of this caspase may be responsible for the PARP cleavage observed at the earlier time points. Phosphorylation of the transcription factor FOXO3a at Thr32 ensures its sequestration in the cytoplasm. Its dephosphorylation enables it to reach its target genes such as that encoding the Fas ligand (Brunet et al., 1999), thereby triggering apoptosis. The changes observed in the phosphorylation status of this transcription factor may well be a contributing factor to the loss in cell viability as well.

Another major difference between the different RCM treatments was observed with respect to the release of IL-8 (Fig. 5). The increase in IL-8 release in diatrizoate-treated cells may be attributed in part to the upregulation of kinases and transcription factors known to play a role in inflammation. All three main subfamilies of the MAPKs have been implicated in regulation of IL-8 expression (Kim et al., 2006; Li and Nord, 2002), though it appears that the stimulus and cell type may determine which pathways are involved. The transcription factor NF-κB has also been implicated in IL-8 upregulation, particularly when phosphorylated at Ser276 (Nowak et al., 2008). Thus, it is feasible that in diatrizoate-treated cells, the persistent activation of these molecules may lead to increased expression of IL-8 and thereby predispose the kidney to an inflammatory state.

In summary, we have observed a differential activation of signaling molecules in renal tubular cells exposed to different types of RCM that suggests that high osmolar RCM (like diatrizoate) are more cytotoxic as it has been suggested by clinical studies. It has been reported that patients with renal insufficiency and receiving diatrizoate were 3.3 times more likely to develop acute nephrotoxicity than those receiving iohexol (a monomeric, nonionic RCM, like IOM) (Rudnick et al., 1995). In addition, it is widely agreed that high osmolar contrast media are more nephrotoxic than low or iso-osmolar contrast media (Morcos, 2009). The differences shown by our study may enable us not only to find ways of overcoming the harmful and common side effects of RCM but also gives an insight into which signaling pathways may be involved in determining whether the cell survives or not when subjected to a particular stress. This is important as renal tubular cells have a great capacity to proliferate, and understanding the signaling molecules that underpin this may also help in projecting new specific therapies to facilitate recovery, after injury, of the kidney in humans (Bonventre, 2003). Our experimental design may be extended to other RCM and other conditions. We chose an exposure time of 2 h and a concentration of 75 mg I/ml. However, some RCM are more viscous than others and may be retained longer in the kidney (Jost et al., 2010), whereas in patients with preexisting renal problems, the elimination of the RCM may also be prolonged, thereby causing a longer contact with renal tubules. The use of such a cell culture system may give an indication as to the degree of toxicity of a given RCM and under a given set of conditions and also enable the study of the underlying mechanisms of toxicity with respect to the signaling pathways that may be involved.

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