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Calcium oxalate monohydrate crystals stimulate gene expression in renal epithelial cells

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Calcium oxalate monohydrate crystals stimulate gene expression in renal epithelial cells. Primary or secondary hyperoxaluria is associated with calcium oxalate nephrolithiasis, interstitial fibrosis and progressive renal insufficiency. Monolayer cultures of nontransformed monkey kidney epithelial cells (BSC-1 line) and calcium oxalate monohydrate (COM) crystals were used as a model system to study cell responses to crystal interactions that might occur in the nephrons of patients during periods of hyperoxaluria. To determine if COM crystals signal a change in gene expression, Northern blots were prepared from total renal cellular RNA after the cells were exposed to crystals. The immediate early genes c-*myc*, EGR-1, and Nur-77 were induced at one hour. At two to six hours stimulated expression of the genes encoding plasminogen activator inhibitor (PAI-1) and platelet-derived growth factor (PDGF)-A chain was detected, but constitutive expression of urokinase-type plasminogen activator (u-PA) was not altered. Expression of connective tissue growth factor (CTGF) was induced at one hour and persisted up to 24 hours. The stimulation of gene expression by COM crystals was relatively crystal- and renal cell-type specific. Thus the interaction of kidney epithelial cells with COM crystals alters expression of genes that encode three classes of proteins: transcriptional activators, a regulator of extracellular matrix (ECM), and growth factors. Activation of PAI-1 gene expression without a change in u-PA favors accumulation of ECM proteins, as does increased expression of PDGF and CTGF which can also stimulate fibroblast proliferation in a paracrine manner. These results suggest that COM crystal-mediated stimulation of specific genes in renal tubular cells may contribute to the development of interstitial fibrosis in hyperoxaluric states.

Hyperoxaluria occurs as a primary or secondary disease that in some cases is associated with the development of renal failure [1–14]. The mechanisms that mediate renal injury in this condition are largely unknown, although clues to pathogenesis may be drawn from descriptions of kidney biopsies obtained from specific patients. Interstitial and periglomerular fibrosis, as well as hyalinization of glomeruli, were the most consistent abnormalities observed in 16 of 18 biopsies of patients with hyperoxaluria secondary to intestinal bypass [14]. Numerous reports describe calcium oxalate crystals within tubular epithelial cells, extrusion of crystalline material into the interstitium, interstitial nephritis and fibrosis, and cell proliferation [1–16]. The association between intracellular calcium oxalate crystals and extracellular fibrosis in these renal biopsies prompted us to look for mechanisms that mediate the pathologic changes in hyperoxaluric states.

In the present study, monolayer cultures of nontransformed monkey kidney epithelial cells (BSC-1 line) and calcium oxalate monohydrate (COM) crystals were used as a model system to investigate the molecular events that ensue when a crystal interacts with a renal tubular cell. As interstitial fibrosis is characteristic of the renal injury associated with hyperoxaluria, genes which encode proteins that regulate the composition of extracellular matrix (ECM) were examined. The results indicate that the interaction between COM crystals and renal epithelial cells induces and stimulates expression of specific genes which could mediate interstitial fibrosis in patients with hyperoxaluria.

Methods

Cell culture

Renal epithelial cells of the nontransformed African green monkey line (BSC-1), whose site of origin within the nephron is uncertain, were used for study. Cells were grown in Dulbecco-Vogt modified Eagle's medium containing 25 mM glucose (DMEM), 1.6 μM biotin, and 1% calf serum at 38°C in a CO_2 incubator. Under these conditions, BSC-1 cells achieved confluence at 10^6 cells/60-mm plastic dish (Nunc, Naperville, IL, USA). High-density, quiescent cultures were prepared by plating 2×10^6 cells/dish. The spent medium was changed after three days so that there were 3 to 4×10^6 cells/plate six days later. Medium was then aspirated and replaced with fresh medium containing 16 μM biotin and 0.01% calf serum. The quiescent cells were ready for use three days later.

Madin-Darby canine kidney (MDCK) cells, which appear to originate from the distal tubule, were grown in DMEM containing 2% calf serum and 1.6 μM biotin as described previously [17]. To prepare high-density, quiescent cultures, 2×10^6 cells/60-mm dish were plated in DMEM containing 2% calf serum and 1.6 μM biotin. Two days later the medium was aspirated and replaced with fresh medium containing 0.5% calf serum and 1.6 μM biotin. One day later the cultures were used for study at a density of 4×10^6 cells/dish.

BALB/c3T3 fibroblasts were grown in DMEM containing 10% calf serum as described [17]. To prepare high-density, quiescent cultures, 7×10^5 cells were plated per 60-mm dish. Two days later the medium was aspirated and replaced with fresh medium containing 1% calf serum and 1.6 μM biotin; the cells were used one day later when a density of 17×10^6 cells/dish was reached.

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Materials

Crystals of COM, hydroxyapatite (HA) or brushite (BR) were each prepared from supersaturated solutions by Y. Nakagawa (University of Chicago) as previously described [17]. COM crystals were cuboidal to spindle shaped, and uniformly small at 1 to 2 μm in diameter. HA crystals were 2 to 5 μm in size and nearly spherical, whereas BR crystals were planar and irregularly shaped at 1 to 3 μm in diameter. Latex beads (1.1 μm diameter) (Sigma Chemical, St. Louis, MO, USA) were used as control particles of similar size. Crystals were sterilized by heating to 180°C overnight. X-ray crystallography (performed by S. Deganello, University of Chicago) demonstrated that heating did not alter the structure of COM crystals.

Northern blots

COM, HA, or BR crystals, or latex beads were each added directly from a suspension in sterile water, which was stirred rapidly to prevent aggregation, to the media of high-density, quiescent cultures to achieve a final concentration of 200 μg/ml (47.2 μg/cm^2) [17]. No additions were made to control cultures. Previous experiments suggest that COM crystals are nearly insoluble in DMEM [17]. At specified times after the crystals or latex beads were added, the medium was aspirated, cells were lysed in guanidinium isothiocyanate, scraped off the dish, and RNA was extracted as described [18]. Samples of total RNA (20 μg each) were electrophoresed through a 1.4 agarose-6% formaldehyde gel, and transferred to a nylon membrane (Nytran, Schleicher & Schuell, Keene, NH, USA). DNA probes were labeled with [α-^{32}P]dCTP by random hexamer priming [19], and hybridized to Northern blots at 42°C in a solution containing 1 M NaCl, 1% sodium dodecylsulfate (SDS), 50% formamide, and 10% dextran sulfate [20]. The blots were washed at 65°C in 2 × SSC buffer (0.3 M NaCl, 0.03 M Na citrate) containing 0.1% SDS. An autoradiogram of the blot was prepared at −70°C for 24 to 72 hours using X-ray film and two intensifying screens.

The following DNA probes were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA): human acidic fibroblast growth factor (2.2 kb insert in pUC18); human collagenase (2.1 kb insert of pBR322); human *gro* (0.84 kb insert in pGEM-3); human heat shock protein (HSP)-70 (1.6 kb insert in pBluescript SK-); human interleukin-1α (2.4 kb insert in pMG-5); human interleukin-1β (0.7 kb insert in pSM214); mouse interleukin-6 (5.2 kb insert in pBluescript SK+); rat stromelysin (1.7 kb insert from pUN121); mouse tissue-type plasminogen activator (2.519 kb insert in pBluescript KS+), and human urokinase-type plasminogen activator (1.5 kb insert in pEMBL8). The following probes were obtained from laboratories at the University of Chicago: human c-*sis* (1.0 kb in pAM 18), human platelet-derived growth factor (PDGF)-A chain (1.3 kb insert in PUC 13), and human transforming growth factor (TGF)-β1 (1.0 kb Nar1 fragment of human TGF-β1) were from G. Bell; mouse Nur-77 (2.5 kb insert in pGEM4Z) was from L. DeGroot; rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1.2 kb insert in pBR322) was from M. Favus; mouse c-*jun* (2.6 kb insert in pGEM) was from D. Hallahan, and mouse EGR-1 (2.2 kb insert in pUC13) from V. Sukhatme. The probe for rat fibronectin (0.27 kb Stul-EcoR1 fragment in pGEM2) was obtained from R. Hynes (Massachusetts Institute of Technology, Cambridge, MA, USA). Human c-*myc* (1.8 kb Eco R1 fragment of the third exon of c-*myc*) was purchased from Oncor (Gaithersburg, MD, USA). Probes for mouse β1-laminin (4.6 kb insert in pGEM 2) and mouse αIV-collagen (0.85 kb insert in pGEM2) were provided by Y. Yamada (NIH, Bethesda, MD, USA). Human plasminogen activator inhibitor (PAI-1) (2 kb insert in PAIB6) was obtained from D. Ginsburg (University of Michigan). Bovine basic fibroblast growth factor (4.2 kb insert in pGEM3Z) was obtained from R. Halaban (Yale University, New Haven, CT, USA). Human c-*fos* probe (6.1 kb Ambrobe plasmid containing a 3.1 kb Xho1-Nco1 *fos* gene fragment) was obtained from Amersham (Arlington Heights, IL, USA). Human connective tissue growth factor (CTGF) (1.1 kb in pRc/CMV) was provided by G. Grotendorst (University of Miami, Miami, FL, USA). TGF-β2 cDNA was generated with the polymerase chain reaction using two nucleotide primers: one obtained from position 402 to 421 and the other primer spanning 969 to 988 of the full-length monkey cDNA sequence of TGF-β2 [21].

No less than two blots were prepared and probed with each gene of interest. Blots were subsequently reprobed with GAPDH to verify that an equal amount of RNA was loaded in each lane of the gel.

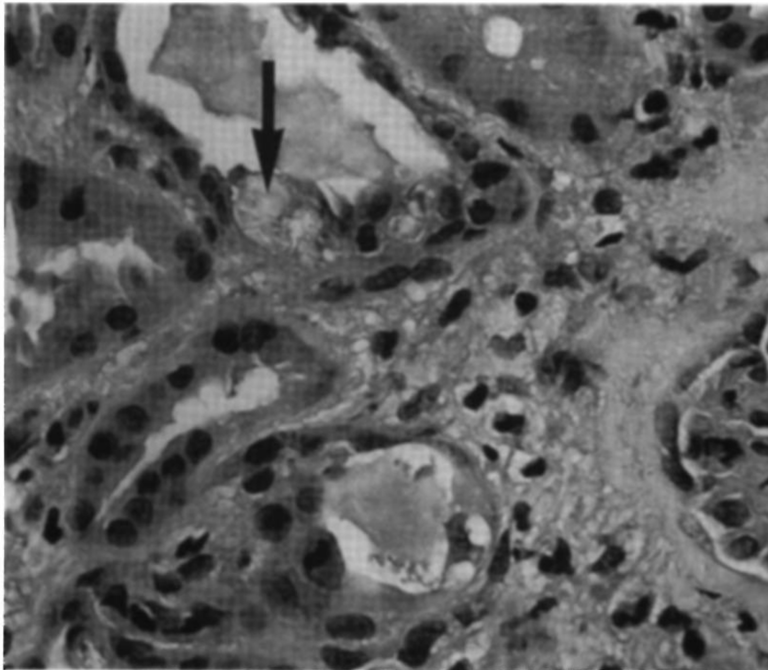
Results

Crystals and human renal tissue

Renal tissue was obtained at biopsy from a patient with an intestinal bypass who developed renal insufficiency. Crystals within reactive tubular epithelial cells, and adjacent interstitial fibrosis are seen (Fig. 1). The clinical history and histologic appearance suggested that the crystals were composed of calcium oxalate [22, 23]. Similar pathologic changes were seen in a renal biopsy obtained from a patient after a combined kidney-liver transplant for primary hyperoxaluria which we previously reported [15]. To determine if these crystals could mediate the observed pathologic changes, cultured kidney epithelial cells were used as a model to assess the effect of COM crystals on gene expression. High-density, quiescent monolayer cultures were used to simulate the tubular epithelium along the nephron.

Effect of COM crystals on gene expression in BSC-1 cells

Expression of immediate early genes (c-*myc*, EGR-1, c-*jun*, c-*fos*, and Nur-77) [24–26] was investigated in cells exposed to COM crystals (200 μg/ml) for specified periods of time. RNA was extracted from the cells and Northern blots were prepared and hybridized with specific [α-^{32}P]dCTP cDNA probes. The transcript for c-*myc* was induced as early as 15 minutes with maximal expression at one hour (Fig. 2). The transcript for EGR-1 was induced by 30 minutes with maximal expression at one to two hours (Fig. 3). Similarly, the transcript for Nur-77 was induced at two hours, and c-*jun*, (minimally expressed under control conditions), also showed a maximal increase at that time. The transcript for c-*fos* was not detected in control cells, nor was it induced by addition of crystals. However, the transcript was detected in RNA isolated from cells exposed for one hour to the mitogen adenosine diphosphate (200 μM) [25] which served as a positive control. GAPDH, an enzyme that mediates glycolytic metabolism, was constitutively expressed and was not altered by addition of crystals; it served to document equal loading of RNA in different lanes of Northern blots.



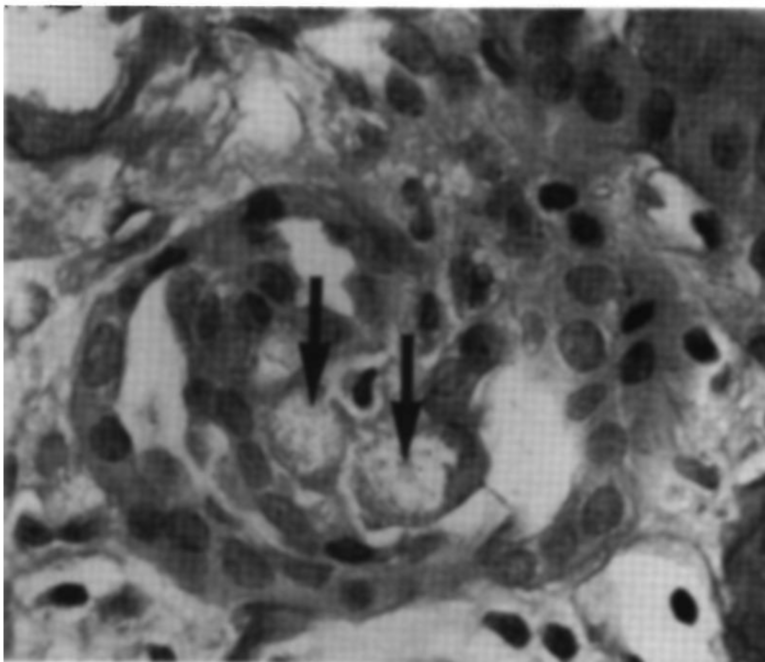


Fig. 1. *Tissue sections of a kidney biopsy from a patient with an intestinal bypass.* **Top.** Low-power view showing intracellular crystals (arrow) that are surrounded by proliferating reactive tubular cells containing hyperchromatic and enlarged nuclei. Note extensive interstitial fibrosis adjacent to crystals (H & E). **Bottom.** High-power view showing intracellular crystals (arrows) surrounded by reactive epithelial cells which project into the lumen of a dilated tubule (H & E).

Northern analysis was then performed using [α-^{32}P]cDNA probes to study proteins that regulate the ECM composition. Figure 4 shows that plasminogen activator inhibitor-1 (PAI-1) [27] was expressed constitutively in cells as a double transcript (2.4 and 3.4 kb in size). In the presence of COM crystals the message increased maximally between two and six hours, and returned towards the control level by 12 hours. When the same blot was hybridized with a probe for urokinase-type plasminogen activator (u-PA) [28], constitutive expression of the gene was detected that did not change significantly following exposure of the cells to crystals.

Northern blot analysis of mRNA encoding PDGF-A and -B chains [29, 30] is shown in Figure 5. Three transcripts encoding PDGF-A chain (2.8 kb, 2.3 kb, and 1.8 kb) are seen in control cells. Expression increased maximally between two to six hours after exposure to crystals, a time course similar to that observed for PAI-1. The gene encoding PDGF-B chain (c-*sis*), was constitutively expressed and changed little after exposure to crystals.

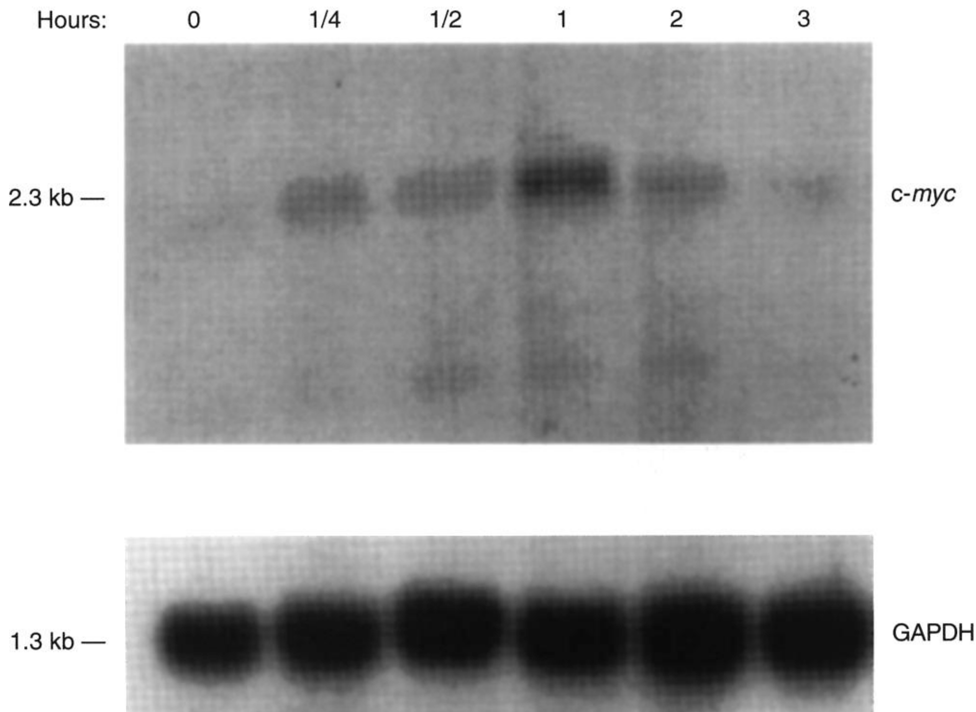


Fig. 2. *Northern blot analysis of total RNA from high-density, quiescent renal epithelial cells (BSC-1 line) after exposure to COM crystals (200 μg/ml) for specified periods of time.* Total cellular RNA (20 μg) was extracted and electrophoresed through a 1.4% agarose gel containing formaldehyde, and then transferred to a Nytran filter. The filter was hybridized with an [α-^{32}P]cDNA probe for c-*myc* or GAPDH. The transcript for c-*myc* was induced as early as 15 minutes, with maximal expression at 1 hour.

Connective tissue growth factor (CTGF) is a cysteine-rich protein that exhibits PDGF-like biological and immunologic activities [31]. Its transcript was not detected under control conditions, but was induced after one hour of exposure to crystals and thereafter was expressed continuously for up to 24 hours (Fig. 6).

Table 1 shows that six genes (left panel) which contribute to the composition and regulation of ECM were constitutively expressed by BSC-1 cells, but their expression was not altered by exposure to COM crystals. Nine genes (right panel) that could play a role in fibrogenesis and proliferation were not expressed in BSC-1 cells nor did crystals induce their expression.

Specificity of gene expression

To determine if the capacity of COM crystals to stimulate gene expression is crystal-type specific, the effect of two calcium-containing crystals, BR or HA, and a non-crystalline particulate, latex beads, were studied. Cells were exposed to each particulate (200 μg/ml) for specified periods of time, RNA was isolated, and Northern blots were prepared and probed with [α-^{32}P]PDGF-A chain cDNA. Only COM crystals induced gene expression (Fig. 7). To evaluate crystal-type specificity for PAI-1, cells were exposed to each particulate (200 μg/ml) for 1, 3, 6, 12, or 24 hours, RNA was isolated, and Northern blots were prepared and probed with [α-^{32}P]PAI-1 cDNA. The transcript for PAI-1 was detected under control conditions, whereas its expression was increased from 3 to 24 hours only after exposure to COM crystals (data not shown).

To determine whether stimulation of gene expression by COM crystals was cell-type specific, experiments were performed using cultures of canine renal epithelial cells (MDCK line) and BALB/3T3 fibroblasts. High-density, quiescent MDCK or 3T3 cells were exposed to COM crystals (200 μg/ml) for 1, 3, 6, 12, or 24 hours, and expression of PAI-1, a gene that regulates the ECM composition, and EGR-1 as a representative immediate-early gene were studied. Because preliminary experiments using 20 μg of total RNA showed no signal when blots prepared from 3T3 or MDCK cells were hybridized with [α-^{32}P]PAI-1 cDNA, a greater quantity (80 μg) of RNA was subsequently used. Induction of the PAI-1 transcript in MDCK cells was detected three hours after exposure to COM crystals, whereas expression in 3T3 fibroblasts was not detected under control conditions or in the presence of crystals (data not shown). To look for cell-type specificity of COM crystal-induced immediate-early gene expression, MDCK cells or 3T3 fibroblasts were exposed to crystals (200 μg/ml) for 1, 3, 6, 12 or 24 hours, and Northern blots (20 μg total RNA per lane) were prepared. The crystals stimulated expression of EGR-1 in MDCK cells (at six hr) but not in 3T3 fibroblasts (data not shown).

These results suggest that the capacity of COM crystals to stimulate gene expression in renal epithelial cells is relatively crystal- and cell-type specific.

Discussion

The results demonstrate that genes encoding diverse classes of proteins are activated in renal epithelial cells exposed to COM crystals. There is rapid and transient induction of c-*myc*, EGR-1 and Nur-77 transcripts which peaks at one to two hours, enhanced expression of PAI-1 and PDGF-A chain at two to six hours, and induction of CTGF at one hour that persists for 24 hours following the cell-crystal interaction. COM crystal-mediated stimulation of gene expression is relatively crystal- and renal cell-type specific. As far as we are aware, these findings represent the first evidence that the most common crystal in renal stones, COM, can activate gene and protooncogene expression in kidney cells.

Recent studies in this and other laboratories indicate that the interaction of COM crystals with kidney cells in culture can result in specific responses. The crystals can bind to the apical cell

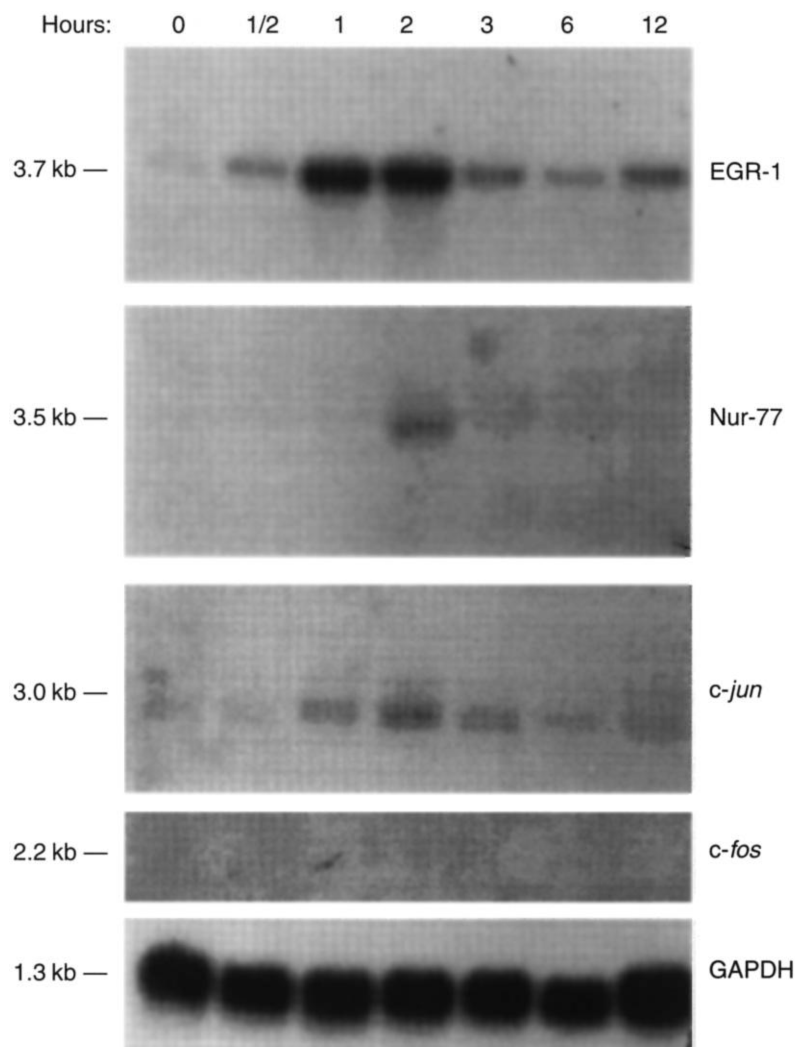


Fig. 3. *Northern blot analysis of total RNA from BSC-1 cells after exposure to COM crystals for EGR-1, Nur-77,* c-jun, c-fos, *and GAPDH.* Transcripts for EGR-1 and Nur-77 were induced and c-*jun* was stimulated rapidly and transiently for one to two hours, whereas c-*fos* was not expressed under control conditions nor stimulated by addition of crystals.

surface [32–34], undergo internalization [35], and in some cells initiate proliferation [17]. Each of these three responses appears to be under the control of a different set of extracellular factors [17, 35, 36]. Crystal binding to the apical plasma membrane can be blocked by diverse anions found in urine such as the glycoproteins nephrocalcin and uropontin, specific glycosaminoglycans, and citrate [36]. After crystals adhere they can be internalized by the cells, a process which can be stimulated (epidermal growth factor, adenosine diphosphate, calf serum), or inhibited [Tamm-Horsfall glycoprotein, heparin, transforming growth factor-$\beta 2$, tetrapeptide arginine-glycine-aspartate-serine (RGDS)] by diverse agents [35]. Uptake of COM crystals is associated with an increased probability of cell division [17]. The internalized crystals appear to be distributed to daughter cells at mitosis and can persist for at least two weeks within the cells [34], suggesting that they are not perceived as toxic.

Whether intracellular crystals *in vivo* serve as a nidus for additional crystal growth, are eventually exocytosed into the interstitium, or slowly dissolve inside the cell remains unknown. Previous experiments have shown that internalization is crystal-type specific. HA and BR crystals appear to be internalized by 7% and 3% of BSC-1 cells, respectively, whereas 42% of cells are able to engulf a COM crystal after one hour [17]. In the present study renal epithelial cells responded to an interaction with COM crystals, but not other particulates, by altering expression of specific genes (Fig. 7). It is possible that the extent of internalization of different crystals by these renal cells accounts for or contributes to the apparent crystal-type specificity. The results of the present study do not identify the genes whose expression is triggered by specific events such as adhesion of a crystal or its internalization by the cell.

Specific responses to crystals also occur in nonrenal cells. Basic calcium phosphate crystals induce c-*fos* and c-*myc* protooncogene expression [37] and initiate mitogenesis in BALB/3T3 fibroblasts [38–40]. A role for cytokines in cell-crystal interactions has also been reported [41–43]. Monosodium urate, calcium pyrophosphate dihydrate, and hydroxyapatite crystals each stimulated interleukin (IL)-6 production by synoviocytes and monocytes grown in culture [42], and monosodium urate crystals trigger release of IL-8 from cultured monocytes [43]. The plasminogen-activating system plays a key role in regulating the ECM composition [44]. As progressive accumulation of extracellular proteins is a central feature of interstitial fibrosis [45], genes which regulate

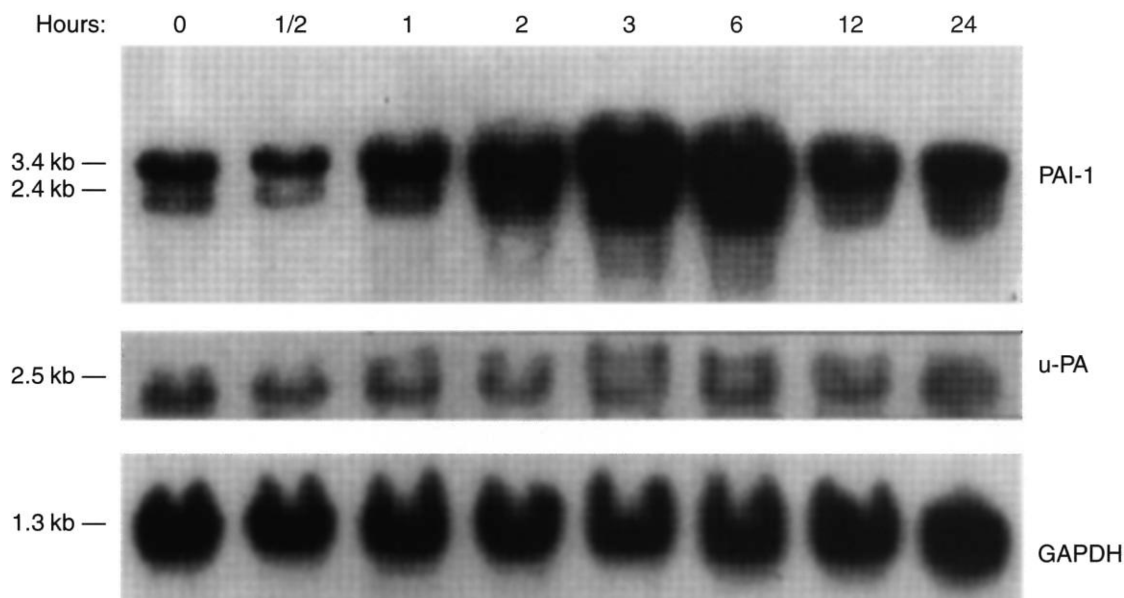


Fig. 4. *Effect of COM crystals on expression of PAI-1, u-PA, and GAPDH in BSC-1 cells.* PAI-1 was expressed constitutively and was stimulated maximally from 2 to 6 hours after exposure to crystals. Constitutive expression of u-PA was unchanged.

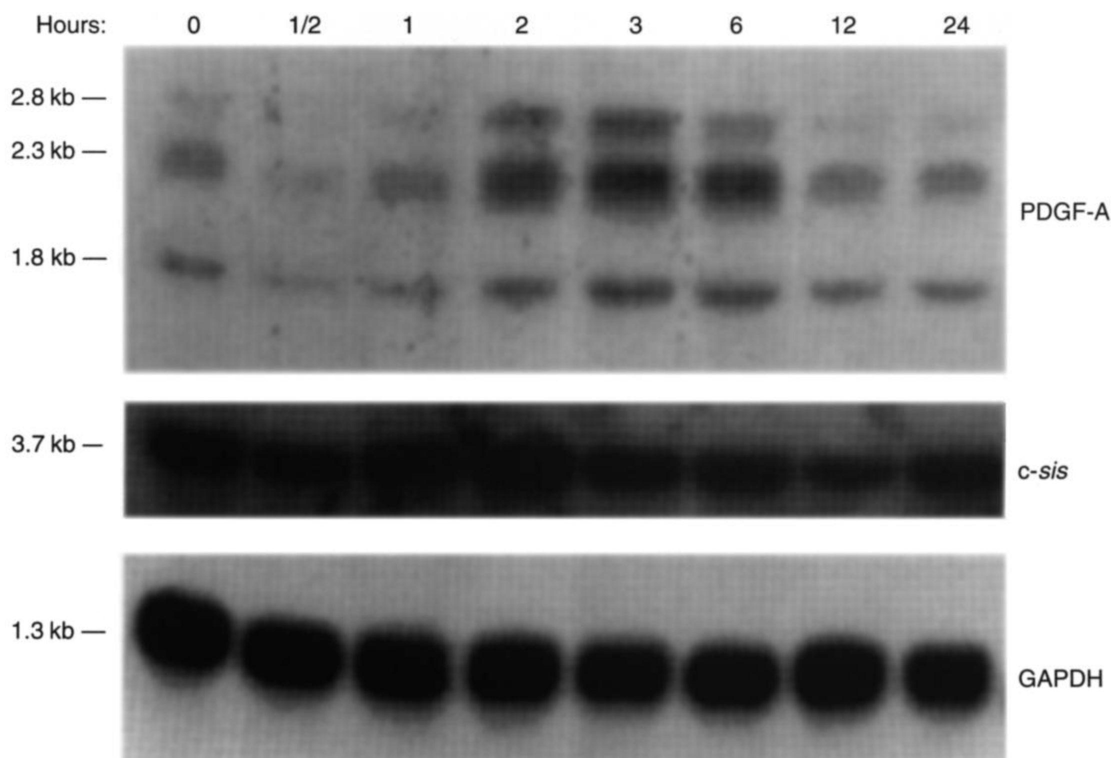


Fig. 5. *Northern blot analysis of RNA from BSC-1 cells after exposure to COM crystals for PDGF-A chain, c-*sis*, and GAPDH.* Expression of PDGF-A chain was constitutive under control conditions and increased from 2 to 6 hours after exposure to crystals, whereas expression of *c-sis* was unchanged.

the components of the plasminogen-activating system were studied. Plasmin is an extracellular broad spectrum protease that is activated when its precursor, plasminogen, is cleaved [46]. Plasminogen is the target of two other highly specific proteases, urokinase-type plasminogen activator (u-PA) and tissue-type plasminogen activator (t-PA) [46]. U-PA is primarily responsible for plasmin generation in processes involving degradation of ECM and basement membranes, whereas t-PA appears to mediate plasmin generation during fibrinolysis. Fast-acting plasminogen activator inhibitor (PAI-1) is an important component of this system. PAI-1 regulates plasmin activity by blocking the action of both tissue- and urokinase-type plasminogen activators which

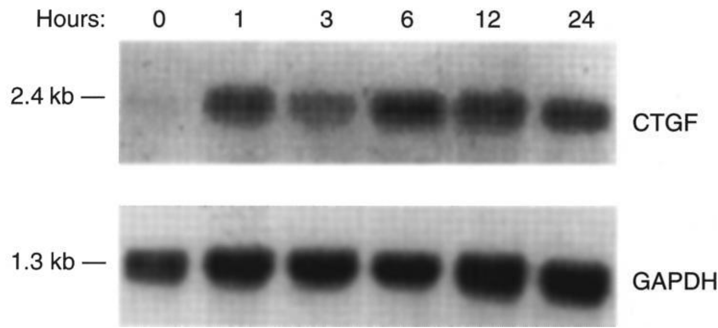


Fig. 6. *Effect of COM crystals on expression of CTGF in BSC-1 cells.* Expression of CTGF was not detected under control conditions, was induced after exposure to crystals for 1 hour, and persisted up to 24 hours.

Table 1. Effect of COM crystals on gene expression in BSC-1 cells

Constitutive expression not altered	Not expressed or induced
Laminin	Stromelysin
Collagen	Collagenase
Fibronectin	Interleukin-1β, -1α, -6
Transforming growth factor-β1, -β2	*gro*
Heat shock protein-70	Tissue-type plasminogen activator
	Basic fibroblast growth factor
	Acidic fibroblast growth factor

COM crystals were added to cultures of high-density, quiescent cells and RNA was extracted at eight different times (0 to 24 hours) thereafter. Northern blots were prepared and hybridized with each of the [α-^{32}P]cDNA probes listed in the table.

decreases formation of plasmin [46]. Reduced plasmin production could thereby permit accumulation of ECM proteins. In the present study, expression of PAI-1 was induced, u-PA was unchanged, and t-PA was not expressed in renal cells exposed to COM crystals. Increased expression of PAI-1 without a change in u-PA could result in decreased plasmin production; enhanced accumulation of ECM proteins and eventual fibrosis would be the predicted result. Augmented expression of the gene encoding PDGF-A chain was also detected. This growth factor is a potent mitogen for cells of mesodermal origin such as fibroblasts, stimulates connective-tissue forming cells to synthesize and release collagen, proteoglycans and elastic fiber proteins, and serves as a chemoattractant for fibroblasts and monocytes [47–49]. Increased availability of PDGF in the extracellular space would favor fibrosis. CTGF is a peptide originally identified as a secreted product of human vascular endothelial cells that has properties similar to PDGF; it is mitogenic and chemotactic for connective tissue cells such as fibroblasts and smooth muscle cells [31, 48]. Induction of the transcript for CTGF at one hour and its persistent expression for the next 23 hours suggests that secreted CTGF protein could stimulate fibroblast proliferation in a paracrine manner, as does PDGF [31, 50]. Of the 15 genes studied which regulate ECM composition, only three (PAI-1, PDGF-A chain, CTGF) exhibited increased expression after exposure of the cells to COM crystals (Table 1). These observations suggest that certain genes within specific classes are selectively induced or stimulated by exposure of the cell to COM crystals. The transcriptional control mechanisms that coordinate induction or stimulation of these specific genes remain to be explored. Studies to determine the effect of blockade of crystal adhesion and/or internalization on gene expression could provide additional insight into how the interaction of a crystal with a renal cell activates transcription.

Hyperoxaluria can be classified as either primary or secondary and is often associated with interstitial fibrosis and renal failure [1–14]. Primary hyperoxaluria is a genetically distinct inborn error of oxalate metabolism, whereas secondary hyperoxaluria occurs in several gastrointestinal malabsorptive states, during pyridoxine deficiency, and following ethylene glycol ingestion and methoxyflurane anesthesia [51]. Koten et al have suggested that calcium oxalate crystals deposited in the interstitium cause marked inflammation and fibrosis of the renal parenchyma [6]. An autopsy study of persons with normal kidney function, acute renal failure or chronic renal failure revealed that the incidence and severity of tubular and interstitial calcium oxalate deposition was a function of the duration of renal failure which in turn is correlated with an elevated plasma oxalate concentration [8]. Therefore calcium oxalate deposits in the kidney are associated with both interstitial fibrosis and loss of renal function [1–10]. Severe hyperoxaluria induced in rats by an intraperitoneal injection of sodium oxalate immediately produces intraluminal calcium oxalate crystals which appear to attach to the apical membrane of tubular epithelial cells and are subsequently deposited in the interstitium [52–54]. Therefore, the results of this and previous studies suggest that during periods of hyperoxaluria COM crystals can nucleate and grow within tubules, undergo endocytosis, and initiate release of factors from tubular cells that could stimulate fibroblast proliferation by a paracrine pathway, and ECM accumulation via the plasmin system. The end result of this scenario would be interstitial fibrosis and progressive kidney failure. This hypothesis could be tested in the human kidney by utilizing cDNA and antibody probes suggested by the results of the present study.

In summary, the response of renal epithelial cells to COM crystals is characterized by increased expression of specific genes which encode transcriptional activators (c-*myc*, EGR-1, Nur-77, and c-*jun*), a regulator of ECM composition (PAI-1), and growth factors (PDGF-A chain and CTGF). The protein products of these genes could contribute to interstitial fibrosis observed in kidneys of patients with primary or secondary hyperoxaluria.

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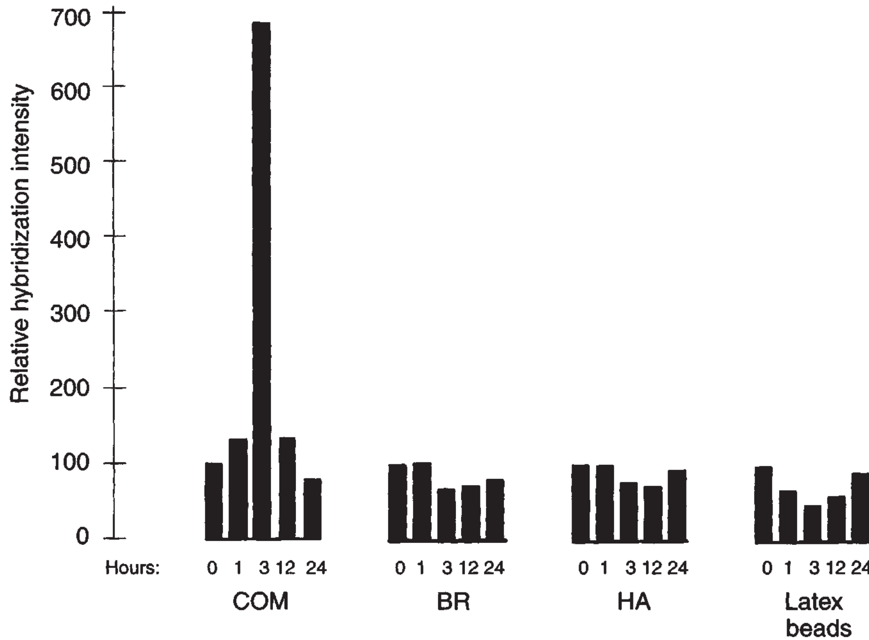

Fig. 7. *Effect of COM, brushite (BR) or hydroxyapatite (HA) crystals, or latex beads on expression of PDGF-A chain mRNA.* High-density, quiescent BSC-1 cells were exposed to crystals or latex beads at the specified times indicated on the abscissa. Values on the ordinate are relative hybridization intensity quantitated by a laser densitometer. Stimulation of PDGF-A chain gene expression was detected only in the presence of COM crystals.

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