Cytotoxicity of radiocontrast agents on polarized renal epithelial cell monolayers

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Abstract

Objective: Radiocontrast-induced nephropathy is a clinically important complication of coronary angiography. The cellular mechanisms of radiocontrast-induced renal dysfunction are not clear. Since tubular transport functions depend on the polarity of renal epithelial cells, we investigated the effects of radiocontrast agents on polarized tubular cells in vitro. Methods: We studied the effects of iso-iodine concentrations (37 and 74 mg iodine/ml) of an ionic (diatrizoate) and a non-ionic (iopamidol) monomeric radiocontrast agent and of hyperosmolal mannitol control solutions on filter-grown renal epithelial cell (MDCK, LLCPK) monolayers in vitro. The cytotoxicity was assayed by measurement of cell viability, transepithelial resistance, inulin permeability and (polarized) cellular enzyme release. The polarized MDCK cell phenotype was assessed by transmission electron microscopy and indirect immunofluorescence microscopy using monoclonal antibodies against specific apical (gp135) and basal (gp60, uvomorulin) MDCK surface markers. Results: The radiocontrast agents reduced cell viability to a greater extent than hyperosmolal mannitol solutions in both cell lines; diatrizoate was more toxic than iopamidol. LLCPK cells were more susceptible to radiocontrast cytotoxicity than MDCK cells. This cytotoxicity was associated with an alteration of MDCK cell polarity as assessed by the redistribution of surface marker proteins. Conclusions: Diatrizoate is more toxic than iopamidol, which is partly related to its higher osmolality. The cytotoxicity of radiocontrast agents induces a redistribution of polarized membrane proteins which could contribute to the pathophysiology of radiocontrast-induced nephropathy.

Keywords: MDCK, LLCPK; Polarity; Permeability; Osmolality; Nephrotoxicity

1. Introduction

Radiocontrast-induced nephropathy contributes significantly to the morbidity of coronary angiography [1–3], particularly since patients undergoing coronary angiographic procedures often have chronic renal insufficiency due to generalized arteriosclerotic cardiovascular disease, hypertension, diabetes mellitus and other co-morbid conditions. Moreover, they may be subjected to large radiocontrast volumes, especially during coronary interventions, and/or (transient) high renal arterial concentrations during left ventricular and aortic angiography.

Renal dysfunction due to radiocontrast agents has been attributed to hemodynamically mediated ischemic/hypoxic renal cell damage due to radiocontrast-induced vasoconstriction [4,5]. In addition to a diminution in renal blood flow, direct cytotoxic effects of radiocontrast agents on renal tubular cells have been described [4,6–8], but their contribution to the clinical syndrome of radiocontrast-induced nephropathy is not clear, since in vivo cellular toxicity and hemodynamic/neurohumoral effects can hardly be differentiated. To exclude systemic alterations we analyzed the direct cytotoxicity of radiocontrast agents in vitro.

Renal tubular cells perform a variety of vectorial transport functions, which underlie the secretion and absorption of solutes in the kidney. In addition, they separate the internal from the external (urine) environment. In order to perform their physiological functions, renal tubular cells exhibit apical plasma membrane domains facing the urine space and basolateral membrane domains anchoring the...
cells to the basal membrane. The apical and basolateral membranes differ in their protein and lipid composition and are separated by specialized intercellular junctions resulting in a polarized cell phenotype. The generation and maintenance of renal tubular cell polarity require the regulated interplay of protein sorting, membrane trafficking, intercellular junctions and cytoskeletal anchorage [9,10]. Consequently, the disorganization of tubular cell polarity may disrupt vectorial transport functions which, for example, could prevent the effective reabsorption of filtered solutes from the urine. Indeed, a disturbance of cell polarity has important pathophysiological implications in acute renal failure and other pathophysiological conditions [11].

We hypothesized that the cytotoxicity of radiocontrast agents induces a disturbance of polarized renal cell functions which could contribute to the radiocontrast-induced renal failure. Therefore we examined the cytotoxicity of radiocontrast agents on renal epithelial cell monolayers grown on permeable supports which separate an apical from a basal tissue culture compartment. We used primarily the permanent renal epithelial MDCK cell line, which has been isolated from dog kidney and represents the best-characterized model system for the study of cell polarity. The plasmalemmal surfaces of MDCK cells have been analyzed in considerable detail and monoclonal antibodies have been generated for several marker proteins of the apical and basolateral MDCK cell surface. MDCK cells display mostly distal tubular features [12], but their precise tubular origin is not known. For comparison, we studied the cytotoxicity of radiocontrast agents on LLCPK cells (isolated from pig kidney) which exhibit more proximal tubular characteristics [13]. Since the contribution of hyperosmolality to the cytotoxicity of radiocontrast agents is unclear, we compared the cytotoxicity of clinically relevant concentrations (37 and 74 mg iodine/ml) of the monomeric ionic and non-ionic contrast agents diatrizoate and iopamidol, which have widely different osmolalities (diatrizoate 2000 mosmol/kg, iopamidol 800 mosmol/kg). In addition, we studied the effects of hyperosmolar mannitol control solutions.

This is the first study of radiocontrast cytotoxicity in the context of renal tubular cell polarity. Diatrizoate was more toxic on polarized renal cell monolayers in vitro than iopamidol, which was partly explained by its pronounced hyperoncoticity. The radiocontrast cytotoxicity was associated with a disturbance of cell polarity which could contribute to the pathophysiology of radiocontrast-induced nephropathy.

2. Methods

2.1. Cell culture

Low-resistance MDCK(II) cells were provided by Dr. K. Simons (European Molecular Biology Laboratory, Heidelberg), LLCPK cells by Dr. S.L. Alper (Harvard University, Boston, MA). MDCK cells were maintained in MEM, LLCPK cells in DMEM. Both media were supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml). All cell culture reagents were obtained from GIBCO. All other reagents were from Sigma, unless stated otherwise. Radiochemicals were from New England Nuclear/Du Pont. 5 × 10^5 cells were plated onto 24 mm polycarbonate filters (Costar) with a pore size of 0.4 µm and fed daily for 3–4 days before experiments. LLCPK cells were larger than MDCK cells, resulting in a lower cell density of confluent LLCPK monolayers than MDCK monolayers.

2.2. Cytotoxicity assays

2.2.1. Cell viability

Confluent cell monolayers grown on tissue culture plastic were exposed to iso-iodine concentrations (37 or 74 mg iodine/ml) of the radiocontrast agents diatrizoate or iopamidol for up to 12 h. Isohydric, hyperosmolal control media were prepared by adding mannitol solutions to the incubation media to achieve the same degree of hyperosmolality and media dilution as in the radiocontrast media. The osmolality of the radiocontrast and mannitol media was measured with a vapor pressure osmometer (Wescor, Logan, UT, USA). Table 1 shows the osmolality of the cell culture media used in the present study. For cell counting and viability assays the cells were washed with osmolality-adjusted Ca²⁺-free solutions and completely dissociated by trypsinization. Live and dead cells were distinguished microscopically after incubation in a trypan blue solution (10% in Hanks' balanced salt solution, 10 min): dead cells were stained intensely blue, while viable cells were able to exclude the dye and remained unstained. Cells were counted using a hemocytometer.

2.2.2. Monolayer integrity

Since the polycarbonate filters are opaque, direct visualization of the filter-grown monolayers was not feasible. The integrity of the monolayers was operationally determined by their transepithelial electrical resistance and their impermeability to inulin. The confluence of the cells was checked after fixation by epifluorescence microscopy. The transepithelial resistance of the filter-grown cell monolayers.
ers was measured according to the manufacturer's instructions using chopstick electrodes connected to a voltohmeter (WPI, Sarasota, FL, USA). Briefly, the resistance of the cell monolayers was determined by subtracting the resistance of the cell-free filter assembly from the resistance measured across cell-containing filters. The permeability of the cell monolayers to inulin was assessed by adding either $[^3]$Hinulin (2.5 µCi/filter, LLCPK monolayers) or $[^5]$C-inulin (0.4 µCi/filter, MDCK monolayers) to the apical tissue culture compartment followed by timed sampling and liquid scintillation counting of fluid from the basal compartment.

2.2.3. Tubular marker enzymes

Filter-grown monolayers of MDCK or LLC PK cells were incubated in serum-free control, radiocontrast or hypertonic mannitol media for 12 h. Then the apical and basal media were collected and stored at $-80^\circ$C until further analysis of the steady-state distribution of tubular enzyme activities by photometric tests at $25^\circ$C according to the German Society of Clinical Chemistry standards. The activity of the renal tubular enzymes lactate dehydrogenase (LDH, reagent kit: Rolf Greiner GmbH, Germany), γ-glutamyltranspeptidase (GGT, reagent kit: Bayer Diagnostics), and alkaline phosphatase (AP, reagent kit: Bayer Diag-

![Graphs showing cell monolayer density](image)

Fig. 1. MDCK and LLC PK monolayer density. Cells were counted after a 6 h incubation with diatrizoate or iopamidol (10 and 20%, i.e. 37 and 74 mg iodine/ml) or the corresponding hyperosmolal mannitol media (mannitol L, matching 10%; mannitol H, matching 20% radiocontrast agent). For osmolalities, see Table 1. Mean ± s.e.m.; n = 12; * P < 0.05.
MDCK-Cells

LLCPK-1-Cells

Fig. 2. Transepithelial resistance (TER) before (black bars) and after (hatched bars) incubating MDCK or LLCPK monolayers with either radiocontrast (20%) or the corresponding hyperosmolar mannitol media for 12 h (mannitol L, 415 mosmol/kg; mannitol H, 575 mosmol/kg). At each level of hyperosmolality the decrease in TER was greater in radiocontrast-treated monolayers than in the mannitol controls. Mean ± s.e.m., n = 14; * P < 0.05.

nostics) and β-N-acetylglucosaminidase (β-NAG, reagent kit: Boehringer Mannheim) was measured in the apical and basal tissue culture compartments.

2.3. Electron microscopy

Filter-grown cell monolayers were washed with PBS, pH 7.4, containing 1 mM CaCl₂ and 1 mM MgCl₂ (PBS C/M) with the osmolality adjusted to each experimental incubation condition by the addition of hypertonic mannitol solutions to minimize changes in cell volume during fixation. After fixation with 1% glutaraldehyde/3.3% paraformaldehyde in 0.07 M cacodylate buffer, pH 7.3, the filters were excised from their supports and cut into small pieces which were embedded in Epon 812. Special care was taken to avoid curling of the filters. Using an OmU3 ultramicrotome (Reichert and Jung, Wien/Heidelberg) 0.12 μm sections were cut. After counterstaining with

Fig. 3. Radioactivity in the basal tissue culture compartment after addition of radiolabeled inulin to the apical compartment. After a 12 h incubation with radiocontrast (20%) or hyperosmolar mannitol media (mannitol L, 415 mosmol/kg; mannitol H, 575 mosmol/kg), radiolabeled inulin was added to the apical compartment (MDCK monolayers, 0.4 μCi/filter [¹⁴C]inulin; LLCPK monolayers, 2.5 μCi/filter [¹³H]inulin) followed by sampling of basal media at the indicated times. Mean ± s.e.m., n = 10 (control monolayers); n = 3 (radiocontrast, mannitol monolayers); n = 6 (filters without cells).
uranyl acetate/lead citrate the sections were mounted on
copper grids and examined using an EM 10A electron
microscope (Carl Zeiss, Oberkochen, Germany).

2.4. Fluorescence microscopy

MDCK monolayers were washed with osmolality-ad-
justed PBS C/M and fixed with 3.7% formaldehyde in
PBS C/M for 1 h. The fixation was quenched in 50 mM
glycine in PBS, pH 8. The fixed MDCK cell monolayers
were blocked with 1% bovine serum albumin in PBS
containing 0.05% saponin for 30 min prior to incubation
for 1 h in 1/10 diluted murine hybridoma supernatants
containing monoclonal antibodies against the MDCK cell
surface glycoproteins gp135 (apical MDCK membrane
surface marker [14]) or gp60 (basolateral MDCK mem-
brane surface marker [15]). The hybridoma supernatants
were kindly provided by Dr. G.K. Ojakian (State Univer-
sity of New York, Brooklyn, NY). In addition, a rat
monoclonal antibody against the lateral epithelial cell ad-
herence junction protein uvomorulin/E-cadherin (DE-
CMA, Sigma) was used. Cy3-conjugated anti-rat and anti-
mouse IgGs (Dianova) were used as secondary antibodies
at a 1:200 dilution. All incubations were at room tempera-
ture.

2.5. Statistics

Results are given as mean ± standard error. Statistical
analyses were performed using Student’s t-test and/or
ANOVA as appropriate. A level of P < 0.05 was consid-
ered significant.

3. Results

3.1. Cell viability

Fig. 1 shows the effect of 2 concentrations of diatri-
zotate and iopamidol (10 and 20%, 37 and 74 mg iodine/ml,
respectively) and the corresponding hyperosmolar, isohy-
dric mannitol media on confluent renal epithelial cell
monolayers. The number of viable MDCK cells (panels
a + b) decreased from (70 ± 1.7) × 10⁴ (mean value of

![MDCK-Cells](image)

Fig. 4. Activity of tubular marker enzymes in MDCK cell monolayers. Steady-state enzyme activities measured in conditioned media of the apical (A,
black bars) and basal (B, hatched bars) tissue culture compartments. Samples were taken after a 12 h incubation with radiocontrast or hyperosmolar
mannitol media (mannitol L, 415 mosmol/kg; mannitol H, 575 mosmol/kg). Compared with iopamidol, diatrizoate (20%) induced a significantly greater
increase in the activity of all tubular marker enzymes which was restricted to the apical tissue culture compartment. Mean ± s.e.m.; n = 10.
panels a and b) with increasing osmolality of the incubation media to \((30 \pm 1.8) \times 10^4\) at 20% diatrizoate. This reduction in cell number is not due to hypotonic lysis of hyperosmolality-adapted cells during washes/trypsinization, since harvesting the cells under hypertonic conditions did not alter the results. The diatrizoate- and 20% iopamidol-treated monolayers contained significantly fewer viable cells than the corresponding hyperosmolal mannitol controls; the toxicity of 10% iopamidol solutions and of the mannitol controls was similar.

Diatrizoate and iopamidol also reduced the cell density of LLCPK monolayers (Fig. 1, panels c and d) from \((10.3 \pm 0.7) \times 10^4\) to \((5.3 \pm 0.7) \times 10^4\) (20% iopamidol), and from \((10.2 \pm 0.6) \times 10^4\) to \((3.4 \pm 0.5) \times 10^4\) (20% diatrizoate), respectively. At both concentrations of either contrast agent the relative decrease of the cell count was more pronounced in LLCPK monolayers than in MDCK monolayers, suggesting that LLCPK cells may be more susceptible to radiocontrast cytotoxicity than MDCK cells. As in MDCK cells, the radiocontrast agents reduced the number of viable cells to a greater extent than mannitol-induced hyperosmolality. Therefore hyperosmolality by itself cannot fully account for the toxicity of radiocontrast agents, although the pronounced hyperosmolality of diatrizoate may contribute to its higher cytotoxicity compared to iopamidol.

3.2. Transepithelial resistance

Fig. 2 shows the results of the TER measurements in MDCK and LLCPK monolayers before (black bars) and after (hatched bars) a 12 h incubation with diatrizoate or iopamidol (74 mg iodine/ml) or the corresponding hyperosmolal mannitol media. In MDCK cell monolayers 20% diatrizoate significantly reduced the TER from \(208.5 \pm 10.4\) to \(93.8 \pm 13.3\ \Omega\text{cm}^2\); 20% iopamidol also diminished the TER significantly, albeit to a lesser degree (from \(207.6 \pm 13.5\) to \(156.4 \pm 9.6\ \Omega\text{cm}^2\)). In LLCPK cell monolayers the cytotoxicity of diatrizoate was so pronounced as to abolish the TER consistent with the markedly decreased cell density. Compared with the effects of the radiocontrast agents, hyperosmolal mannitol media only caused

Fig. 5. Activity of tubular marker enzymes in LLCPK cell monolayers. Steady state enzyme activities measured in conditioned media of the apical (A, black bars) and basal (B, hatched bars) tissue culture compartment. Samples were taken after a 12 h incubation with radiocontrast or hyperosmolal mannitol media (mannitol L, 415 mosmol/kg; mannitol H, 575 mosmol/kg). High osmolality (mannitol H) induced a non-polarised increase in LDH activity; iopamidol had no significant effects. In contrast, diatrizoate induced a significant increase in the activity of all 4 tubular marker enzymes. The apical predominance of GGT and AP persisted during incubation with diatrizoate, the apical predominance of β-NAG was reduced though still significant, while the activity of LDH was higher in the basal compartment of diatrizoate-treated monolayers. Mean ± s.e.m.; \(n = 15\).
small/insignificant reductions of the TER. These results suggest a higher cytotoxicity of diatrizoate than iopamidol in vitro and a greater radiocontrast sensitivity of LLCPK than MDCK cells. The decrease in TER by the radiocontrast agents is consistent with an opening of intercellular tight junctions and/or a disruption of the monolayers (diatrizoate).

3.3. Inulin permeability

Fig. 3 shows the activity of radiolabeled inulin in the basal tissue culture compartment of filter-grown MDCK (a) and LLCPK (b) cell monolayers during a 24 h incubation period. Since inulin was exclusively added to the apical compartment, the basal radioactivity is a measure of the passive monolayer permeability for inulin, as this molecule does not undergo cellular transport. In MDCK cell monolayers (a) the permeability to inulin was largely unaffected by mannitol-induced hyperosmolality and by iopamidol (74 mg iodine/ml). This indicates the continued presence of functional intercellular tight junctions even during prolonged incubations with hyperosmolar solutions which may reduce cell viability and TER. In contrast, an iso-iodine concentration of diatrizoate induced a rapid increase in the monolayer permeability for inulin, indicating the disruption of the monolayer and/or opening of tight junctions.

In LLCPK cell monolayers (b) both iopamidol and
pronounced hyperosmolality (580 mosmol/kg) increased inulin permeability significantly compared to control monolayers, consistent with the cytopathic effects seen in the other assessments of monolayer function and cell viability. Diatrizoate raised the inulin permeability of LL-CPK monolayers to cell-free filter levels. This indicates disruption of the monolayers, and the abolition of tight junctions by diatrizoate.

3.4. Tubular marker enzymes

Fig. 4 shows the steady-state activities of AP, LDH, β-NAG and GGT in apical (black bars) and basal (hatched bars) tissue culture media of control-, mannitol-, and radiocontrast-treated MDCK cell monolayers. Diatrizoate (20%), but not mannitol-induced hyperosmolality, significantly increased the activity of all tested tubular marker enzymes exclusively in the apical tissue culture compartment. In iopamidol (20%)-treated monolayers the steady-state activity of AP, LDH and β-NAG was greater in the apical than in the basal compartment. These results are consistent with the presence of an intercellular barrier for the diffusion of enzymes, although more sensitive assays of tight junctional integrity, such as TER and permeability to inulin (which is a relatively small molecule compared to the enzymes), indicated a disturbance of barrier functions in radiocontrast-treated MDCK monolayers.

Fig. 5 shows the steady-state activities of the same enzymes measured in the apical (black bars) and basal (hatched bars) compartments of control-, radiocontrast- or hyperosmolality-treated filter-grown LLCPK cell monolayers. In control, as well as in mannitol- or iopamidol-treated monolayers the steady-state activity of AP, GGT and β-NAG was significantly higher in the apical than in the basal compartment; neither treatment induced a significant change in enzyme activities compared to control values. The activity of LDH was significantly increased by pronounced mannitol-induced hyperosmolality ('Mannitol H') without apical predominance. In contrast, diatrizoate led to a significant increase in all enzyme activities which was confined to the apical compartment for AP, GGT and β-NAG. The activity of LDH was more prominent in the basal compartment. These results are also consistent with a largely preserved barrier function of the LLCPK monolayers for the diffusion of the relatively large enzyme molecules.

Fig. 7. Indirect immunofluorescence of filter-grown MDCK-cell monolayers. The control panels show respectively the typical microvillar apical staining of gp135 and the distinctive basolateral staining pattern of gp60 and uvomorulin. In the iopamidol-treated monolayers the apical staining of gp135 is largely unchanged; in diatrizoate-treated monolayers there is pronounced coarsening with discrete staining of the cell borders. In radiocontrast-treated monolayers the basolateral staining of gp60 is reduced with increased intracellular staining in a vacuolar pattern. This redistribution was more pronounced in diatrizoate- than in iopamidol-treated monolayers. The lateral membrane marker, uvomorulin, shows a similar redistribution with an increased intracellular signal. While the redistribution of the apical marker gp135 is consistent with the rarefaction of apical microvilli, the reduction of membrane staining and increased intracellular fluorescence signal suggest basolateral membrane internalization in the radiocontrast-treated monolayers. All panels same magnification; bar = 10 μm.
3.5. Electron microscopy

Fig. 6 shows the ultrastructural appearance of filter-grown MDCK cells during control conditions (a) and after a 12 h incubation with 20% (74 mg iodine/ml) diatrizoate (b). Vertical sections of control monolayers show typical cuboidal epithelial cells (cell height 11 μm) with prominent apical microvilli. The radiocontrast-treated MDCK cells (b) are flattened (cell height 7.5 μm) with many intracytoplasmic vacuoles and display a marked reduction of apical microvilli as ultrastructural evidence of the cytotoxicity.

3.6. Fluorescence microscopy

Fig. 7 shows the distribution of the apical marker protein gp135 and of the basolateral marker proteins gp60 and uvomorulin in confluent MDCK monolayers that had been incubated with regular growth medium or radiocontrast agents (74 mg iodine/ml) for 12 h. The gp135 staining in iopamidol-treated monolayers remained largely unchanged. Diatrizoate induced a coarsening of the apical gp135 staining pattern with evidence of gp135 staining at the cell borders.

The radiocontrast-induced changes in the distribution pattern of the basolateral marker proteins gp60 and uvomorulin are particularly prominent: during control conditions the MDCK monolayers display the distinctive basolateral staining pattern of gp60 and uvomorulin/E-cadherin with bright staining of the cell borders and little fluorescence signal in the cell interior; after incubation with radiocontrast the lateral fluorescence signal was attenuated and changed from a (linear) plasma membrane to a predominantly intracellular (vesicular) localization, suggesting basolateral membrane internalization, particularly in diatrizoate-treated monolayers. The redistribution of plasmalemmal marker proteins was less pronounced in iopamidol-treated monolayers; mannitol-induced hyperosmolality had no consistent effects on the immunofluorescence staining of either the apical MDCK surface glycoprotein gp135 or the basolateral marker proteins gp60 or uvomorulin (not shown). These results suggest that the mechanisms which generate and/or maintain the polarized phenotype of renal epithelial cells may be disturbed by the toxic effects of radiocontrast agents, in particular diatrizoate.

4. Discussion

The present results provide clear evidence of direct cytotoxic effects of an ionic and a non-ionic monomeric radiocontrast agent on renal tubular cells in vitro. In both LLC-PK1 and MDCK cell monolayers the radiocontrast agents induced a concentration-dependent decrease in cell viability and a disruption of monolayer integrity. The main and novel finding of the present study is the alteration of polarized monolayer features as evidenced by the redistribution of polarized plasmalemmal marker proteins.

4.1. Ionic versus non-ionic radiocontrast agents

Many of the clinical side-effects of radiocontrast agents are attributed to their hyperosmolality. However, the role of hyperosmolality in the pathogenesis of the radiocontrast-induced renal dysfunction is not clear, since the acute renal failure may be precipitated by both ionic and non-ionic radiocontrast agents with markedly different osmolalities [1,16]. In order to address the effect of hyperosmolality versus molecular toxicity we compared an ionic and a non-ionic monomeric radiocontrast agent and used hyperosmolar mannitol solutions as controls. Our data show that hyperosmolality may indeed decrease cell numbers and can interfere with monolayer integrity (transepithelial resistance, inulin permeability). Thus the higher osmolality of diatrizoate may partly explain its greater toxicity compared to the non-ionic radiocontrast agent, iopamidol. Other in-vitro data [7,8], as well as experience in patients [17] and in experimental animals [18,19], have indicated a greater nephrotoxicity of hyperosmolar ionic than of non-ionic radiocontrast agents.

Hyperosmolar concentrations of impermeable solutes exert their biological effects via their hypertonicity. In contrast, isotonic hyperosmolality induced by urea failed to cause significant cell death of MDCK cells (Haller et al., unpublished results). Hypertonicity could mediate cytotoxicity in renal cells by a variety of mechanisms, including the expression of heat shock proteins and the activation of immediate early genes [20]. However, at each level of hyperosmolality mannitol was less toxic than the corresponding radiocontrast agents. In particular, hypertonicity alone did not induce the redistribution of plasmalemmal marker proteins observed in radiocontrast-treated cells. Therefore, while osmolality-dependent cell damage can contribute to the toxic effects of radiocontrast agents, the cytotoxicity of radiocontrast agents is not fully explained by their hypertonicity.

4.2. Radiocontrast-induced cytotoxicity and acute renal failure

In vitro investigations provide an opportunity to study the cytotoxicity of radiocontrast agents without the interference of hemodynamic and/or neurohumoral mechanisms. Several in vitro studies of radiocontrast agents have demonstrated direct cytotoxic effects [6–8]. Studies by Messana and co-workers [7] using freshly isolated proximal tubule segments in suspension showed radiocontrast-induced changes in intracellular electrolytes and energy metabolism which were less pronounced with iopamidol than with diatrizoate; in the in vitro toxicity of the radiocontrast agents was aggravated by hypoxia. Andersen et al. [8]
compared the cytotoxicity of the ionic monomer metrizoate, the ionic dimer ioxaglate and the non-ionic monomer iohexol on plastic-grown LLCPK and MDCK cells which did not afford the study of polarized cell functions. However, these authors demonstrated a concentration-dependent cytotoxicity in both cell lines including cell death, release of tubular marker enzymes and ultrastructural alterations similar to the present results.

The incubation time and iodine concentrations used in the present experiments are pathophysiologically relevant, since they may occur in the kidney in clinical settings: a persisting nephrogram (even over 24 h) is a characteristic finding in radiocontrast-induced nephropathy [21]. Urine concentrations of up to 140 mg iodine/ml have been measured in rabbits following the intravenous administration of radiocontrast at a dose of 450 mg iodine/kg body weight [22]. In patients this radiocontrast dose may be exceeded, especially during coronary angioplasty, where radiocontrast volumes > 500 ml (> 150 g iodine, > 2 g iodine/kg body weight) may be injected.

LLCPK cells were more susceptible to radiocontrast-induced cell injury than MDCK cells. While the exact reason for this difference is not known, we speculate that this could be related to their more proximal tubular cell phenotype in vitro, their larger size with an attendant lower osmotic resistance and/or their origin from a different species. LLCPK and MDCK cells are permanent cell lines which have undergone phenotypic alterations since their original isolation. Such a dedifferentiation could explain the present finding of the release of GGT and β-NAG, which are traditionally considered to be proximal tubule brush-border markers, from MDCK cells despite their mostly distal phenotype. Moreover, LLCPK and MDCK cells originate from different species (LLCPK, pig; MDCK, dog) which may differ in their susceptibility to radiocontrast cytotoxicity. However, in both LLCPK and MDCK cell monolayers diatrizoate was more toxic than iopamidol.

Direct cytotoxic effects on renal tubular cells and hemodynamic/neurohumoral mechanisms complement each other as mediators of the acute renal dysfunction, since hemodynamic alterations may set the stage for the toxic effects of radiocontrast agents on tubular cells: although during left ventricular angiography, aortography and selective renal angiography a very high renal arterial concentration of radiocontrast may occur instantaneously, this high local concentration is normally rapidly dissipated. However, when the regulation of renal blood flow is compromised by radiocontrast-induced vasoconstriction [4,5] which may be mediated by endothelial dysfunction [23,24], it is conceivable that renal tubular cells may be exposed to high concentrations of radiocontrast agents for prolonged periods of time. Low-flow conditions prevail in the renal medulla which operates on the brink of hypoxia even under physiological conditions [25]. Indeed, it is this nephron segment which shows the most severe injury from radiocontrast administration [26]. In vitro studies in isolated proximal tubule segments have shown that hypoxia can aggravate the cytotoxicity of radiocontrast agents [7].

4.3. Cell polarity

To our knowledge, this is the first study in which radiocontrast cytotoxicity has been examined in the context of cell polarity. The redistribution of MDCK membrane proteins and the disruption of the monolayers represent a disturbance of key mechanisms for the generation and/or maintenance of cell polarity. The intracellular, instead of plasmalemmal, staining of gp60 and uvomorulin suggests that radiocontrast agents may induce basolateral membrane internalization in MDCK cells. This is consistent with the increased vacuolization of radiocontrast-exposed renal epithelial cells observed by electron microscopy. In addition, the apical cell membrane is also altered as evidenced by the coarsening and discrete redistribution of the gp135 fluorescence signal. The coarsening of the apical gp135 staining pattern may relate to the loss of apical microvilli observed by electron microscopy. In pilot experiments we observed a coarsening of the apical staining pattern of the lectin wheat germ agglutinin and intercellular gaps in diatrizoate-treated LLCPK cell monolayers (not shown) suggesting that the toxic effects on LLCPK cells also include an alteration of cell polarity.

The role of a loss of tubular cell polarity in the pathogenesis of acute renal failure has recently been reviewed [27]. In acute ischemic tubular necrosis the normally basolaterally located sodium-potassium ATPase may be redistributed to the apical cell membrane [28]. This could result in futile cycling of sodium between the urine and the interstitial compartment, thereby interfering with the effective reabsorption of sodium from the tubular lumen. Since the reabsorption of sodium provides the driving force for other vectorial transport processes in the nephron, the non-polarized distribution of this key enzyme may account for many of the functional alterations observed in acute tubular necrosis. Besides ischemic acute tubular necrosis [28] changes in tubular cell polarity have also been reported in other pathological conditions such as polycystic kidney disease [29] and oncogenesis [11]. Based on the present data, radiocontrast-induced nephropathy may be another example of a clinical condition in which the polarized phenotype of tubular cells is altered. The redistribution/internalization of polarized plasma membrane proteins and the opening of intercellular junctions could contribute to the pathogenesis of radiocontrast-induced nephropathy.

4.4. Summary

Radiocontrast agents induce direct cytotoxic effects on renal tubular cells in vitro which are partly related to their hyperosmolality. The markedly hyperosmolar ionic radiocontrast agent, diatrizoate, is more cytotoxic than the
moderately hyperosmolal non-ionic compound, iopamidol. The cytotoxicity was associated with changes in renal epithelial cell polarity which could contribute to the clinical syndrome of radiocontrast-induced nephropathy by interfering with vectorial transport functions in the kidney.

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