

## Histomorphological Changes after Renal X-Ray Arteriography Using Iodine and Gadolinium Contrast Media in an Ischemic Porcine Model

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Elmståhl B, Leander P, Grant D, Doughty RW, Chai C.-M, Björk J, Almén T, Nyman U. Histomorphological changes after renal x-ray arteriography using iodine and gadolinium contrast media in an ischemic porcine model. *Acta Radiol* 2007;48:1109–1119.

**Background:** Gadolinium contrast media (Gd-CM) are regarded as non-nephrotoxic or considerably less nephrotoxic than iodine contrast media (I-CM), and have therefore come to be used as a substitute for I-CM in patients with renal insufficiency in a variety of radiographic examinations.

**Purpose:** To investigate renal histomorphological changes caused by Gd-CM in comparison with I-CM after renal X-ray arteriography in an ischemic porcine model, and to evaluate these changes in relation to the nephrotoxicity of the CM used.

**Material and Methods:** Test solutions: gadopentetate, gadodiamide, iohexol, gadobutrol, iopromide, iodixanol, mannitol, and saline. The experiments were performed on 152 animals. Each pig was randomized to receive one test solution injected into the balloon-occluded (10 min) right renal artery. The kidneys were evaluated histomorphologically. The severity of histomorphological changes was graded subjectively: 1=minimal, 2=mild, 3=moderate, and 4=marked.

**Results:** The main histological changes were 1) proximal tubular and glomerular necrosis, 2) hemorrhage/congestion of the cortex, medulla, and glomeruli, 3) proximal tubular vacuolation, and 4) protein-filled tubules in the cortex and medulla. Necrosis and hemorrhage/congestion were more frequent after injections with gadopentetate, mannitol solution iso-osmotic to gadopentetate, and gadobutrol compared to all other groups ( $P < 0.001$ ). The degree of necrosis and hemorrhage/congestion was related to the degree of impairment of renal function, but inversely related to vacuolation and tubular protein filling.

**Conclusion:** In ischemic porcine kidneys, the histomorphological changes caused by Gd-CM are similar to those caused by I-CM. Vacuolation appears to be independent of the osmolality and viscosity of the CM, and does not seem to be an indicator of renal impairment. "High-osmolal" Gd-CM are more nephrotoxic than "low- and iso-osmolal" I-CM when compared in equal volumes of concentrations, resulting in equal X-ray attenuation.

**Key words:** Contrast media; experimental model; nephrotoxicity; tubular necrosis; vacuolation

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Accepted for publication July 28, 2007

Gadolinium (Gd) contrast media (CM) are similar to iodine (I) CM with regard to such properties as high water solubility, low binding to plasma proteins, and body clearance dominated by glomerular filtration. They also possess the physics of attenuating X-ray beams, and may therefore be used as CM

in radiography. Commercially available Gd-CM have *relatively high* osmolalities, ranging from 2.2 (gadoteridol) to 7 times (gadopentetate) that of plasma. The highest osmolalities are comparable with the osmolalities of the so-called "high-osmolal" ionic monomeric iodine contrast media

(HOCM). It is acknowledged that HOCM are more nephrotoxic than “low-osmolal” iodine contrast media (LOCM) (8, 41). Thus, Gd-CM with the highest osmolality may have similar nephrotoxicity as iodine HOCM.

CM-induced nephropathy (CIN) is almost exclusively associated with I-CM, although occasional cases of renal failure after administration of Gd-CM have been reported (19, 42, 44, 52). It is noteworthy that Gd-CM are still generally considered to be non-nephrotoxic, and have therefore been used as a substitute for I-CM in a variety of X-ray arteriography (XRA) and computed tomography (CT) examinations in patients with renal impairment (1, 11, 17, 38, 43, 46, 47). According to some authors, Gd-CM may even *improve* glomerular filtration rate (GFR) in patients with impaired renal function (23). However, such claims are not substantiated by studies that compare the nephrotoxicity of Gd- and I-CM in equal volumes or concentrations that result in the same X-ray attenuation.

We have investigated the nephrotoxicity of Gd-CM compared to I-CM after renal arteriography of transiently ischemic porcine kidneys by studying the effect on glomerular filtration rate (GFR) (14–16). The impairment of renal function after administration of the “high-osmolal” Gd-CM was higher than that of an equimolar molecular concentration of the “low-osmolal” I-CM. It was also shown that I-CM iso-osmotic to plasma at concentration equi-attenuating with 0.5 M Gd-CM at 70–90 kVp XRA had no effect on renal function (14). Furthermore, it could be concluded that renal impairment of the CM was correlated to their degree of hyperosmolality relative to plasma (16). Thus, it seems reasonable to assume that increased risk of CIN may also be of relevance to Gd-CM in a clinical setting.

The diagnosis of CIN in humans is generally based on changes in serum creatinine levels, and very little is known about CM-induced histomorphological renal changes. This is not surprising since CM administration does not normally induce CIN and morbidity justifying renal biopsy. Thus, most of the morphological description of these changes is based on experimental *in vitro* and *in vivo* animal studies (6, 7, 9, 12, 30, 34, 35, 37, 50, 51, 53–55). Furthermore, there is only scant literature describing histomorphological renal changes after administration of Gd-CM (2, 22, 50, 53).

The aim of the study was to investigate renal histomorphological changes caused by Gd-CM in comparison with I-CM following renal arteriography in an ischemic porcine model, and to evaluate these changes in relation to the nephrotoxicity of the CM used (14–16).

## Material and Methods

### Animals

The experiments were performed on 152 healthy Swedish landrace male pigs, Swedish University of Agricultural Sciences (SLU), with a mean weight of 21 kg (range 16–29 kg). The local ethics committee approved the study. The histomorphological analysis included the right kidney of all pigs in the three different experimental studies on the evaluation of renal function and were reported separately (14–16). The pigs were acclimatized at the Department of Experimental Research, Malmö University Hospital, for 4–6 days prior to surgery. They were deprived of food for 15 hours before the experiment but had free access to water. After the experiment, all animals were killed by an overdose of pentobarbital administered intravenously.

### Anesthesia and surgery

The anesthesiological and monitoring procedures have been described in detail previously (14, 16). A left-sided nephrectomy was performed through a subcostal incision. After surgery, a 60-min calibration period was used to monitor the pigs for hemodynamic stability. Subsequently, a 4F Cobra catheter (Cordis, Oostende, The Netherlands) was inserted, via the right femoral artery introducer, into the right renal artery. The catheter was then exchanged over a guidewire for a 5F balloon occlusion catheter (Boston Scientific MediTech, Watertown, Mass., USA). Heparin (200 IU/kg b.w.; Leo, Ballerup, Denmark) was given to prevent thrombotic occlusion of the renal artery during the subsequent balloon occlusion. The occlusion balloon was inflated for a period of 10 min to produce a transient renal ischemia.

### Test solutions

Details of the test solutions are given in Table 1, which also includes separately reported median plasma half-life elimination time of the CM, functioning as a measure of their effect on GFR (14–16). Each pig was randomized to receive one test solution, at a dose of 3 ml/kg b.w. and at a rate of 20 ml/min, into the right renal artery through the occlusion catheter during the first 3 min of a 10-min ischemic period. In the animals subjected to saline in the NaCl-1 group, a total dose of 3 ml iohexol (300 mg I/ml) was injected intravenously as a GFR marker. In the NaCl-2 and NaCl-3 groups, a total of 3 ml iohexol (300 mg I/ml) was added as a GFR marker to the saline dose injected into the right renal artery.

Table 1. Molarity and osmolality of contrast medium (CM) molecules and gadolinium (Gd) and iodine (I) atoms in the various test solutions. All test solutions were injected during a 10-min period of ischemia except "NaCl-3." Plasma half-life elimination time of the CM (functioning as GFR markers) is given. The longer the half-life, the more severe was the depression of glomerular filtration rate

Test solutions (injected at a dose of 3 ml/kg b.w.)	CM molecules, molarity (M)	Gd or I atoms, molarity (M) (mg/ml)	Osmolality 37°C, Osm/kg H <sub>2</sub> O	Median (range) plasma half life, hours‡	Comments
Dimeglumine gadopentetate (Magnevist; Schering AG, Berlin, Germany)	0.5 M	0.5 M (79 mg Gd/ml)	1.96*	28.8 (16.8–∞)§	
Mannitol/io 1.96	0.153 M iohexol	0.460 M (58 mg I/ml)	1.96†	46.4 (20.1–∞)	Mannitol dissolved in 0.18 M iohexol (70 mg I/ml)
Gadobutrol (Gadovist; Schering AG)	1.0 M	1.0 M (158 mg Gd/ml)	1.60*	18.4 (6.8–∞)	
Gadodiamide (Omniscan; Amersham Healthcare AS, Oslo, Norway)	0.5 M	0.5 M (79 mg Gd/ml)	0.78*	3.8 (1.9–6.8)§	
Mannitol/io 0.82	0.179 M iohexol	0.538 M (68 mg I/ml)	0.82†	2.8 (2.3–4.3)	Mannitol dissolved in 0.18 M iohexol (70 mg I/ml)
Iohexol 190 (Omnipaque 350 mg I/ml [Amersham Healthcare AS] diluted with H <sub>2</sub> O)	0.5 M	1.50 M (190 mg I/ml)	0.42	3.0 (2.6–3.5)§	
Mannitol/io 0.43	0.182 M iohexol	0.547 M (69 mg I/ml)	0.43†	2.5 (2.0–2.5)	Mannitol dissolved in 0.18 M iohexol (70 mg I/ml)
Iopromide 150 (Ultravist; Schering AG)	0.39 M	1.18 M (150 mg I/ml)	0.34*	2.3 (1.5–3.7)	
Iodixanol 150 (Visipaque; Amersham Healthcare AS)	0.20 M	1.18 M (150 mg I/ml)	0.29*	2.3 (1.7–6.5)	
Iodixanol 320 (Visipaque)	0.42	2.5 M (320 mg I/ml)	0.29*	2.4 (1.7–3.1)	
Iohexol 70 (Omnipaque 140 mg I/ml [Amersham Healthcare AS] diluted with saline)	0.18 M	0.55 M (70 mg I/ml)	0.29	2.7 (2.0–3.5)	
NaCl-1			0.29*	2.2 (1.5–2.8)	Total of 3 ml of iohexol 300 mg I/ml injected as GFR marker into the jugular vein
NaCl-2			≈0.29	3.3 (2.7–5.2)	Total of 3 ml of iohexol 300 mg I/ml added as GFR marker to the saline test solution
NaCl-3			≈0.29	3.3 (2.1–4.2)	Without ischemic period; total of 3 ml of iohexol 300 mg I/ml added as GFR marker to the saline test solution

\* According to manufacturer.

† According to measurement using a Vapor Pressure Osmometer 5500 XR.

‡ Data from studies separately reported (14–16)

§ Pooled data from two separately reported studies (14, 16)

Note—osmolality: osmoles per kg water (the solvent); molarity: moles per liter solution (solute+solvent).

Data from the same CM appearing in more than one study have been pooled. The longer the half-life, the more severe was the depression of GFR. The preparations of the mannitol/iohexol solutions have been described previously (14, 16).

Each pig was randomized to receive one test solution, at a dose of 3 ml/kg b.w., a rate of 20 ml/min, and at room temperature. They were all injected into the right renal artery through the occlusion catheter during the first 3 min of the 10-min ischemic period, except for one group of eight pigs that received saline injections without ischemia, i.e., group "NaCl-3" (Table 1) (14).

#### *Histomorphological evaluation*

Two samples of the right kidney, i.e., one slice of the dorsal and one of the ventral part, were collected and immersion fixed in 10% neutral buffered formalin for histomorphological evaluation. After fixation, the tissue samples were trimmed, processed into paraffin wax, sectioned at a nominal thickness of 5  $\mu$ m, stained with hematoxylin and eosin, mounted under cover slips on glass slides, and then examined on a Leitz DMRD light microscope.

In the evaluation procedure, both sections from the same animal were examined sequentially and all slides were read on at least two separate occasions (one original read and one review). The primary pathologist (D.G.) was blinded to the test solutions except for the control groups (saline). A second pathologist (R.D.) peer reviewed the slides in a blinded manner. Any differences between the primary reading and the peer review were resolved by discussion between the two pathologists to reach a single final diagnosis.

The severity of histomorphological changes was subjectively graded as follows: 1=minimal, 2=mild, 3=moderate, and 4=marked.

#### *Statistics*

To evaluate any statistically significant difference of histomorphological changes between the various test solutions, the nonparametric Mann-Whitney U test was used. In order to reduce the risk of false-positive findings, only *P* values <0.01 (two-tailed tests) were considered significant.

#### **Results**

All the kidneys from pigs injected with gadopentetate, mannitol/io 1.96, or gadobutrol were swollen and discolored macroscopically (Fig. 1). The kidneys injected with all other CM and saline appeared macroscopically normal (Figs. 2–4).



Fig. 1. Macroscopic photograph of a kidney after administration of gadopentetate. The kidney is swollen and discolored as a result of hemorrhage/congestion and necrosis.



Fig. 2. Macroscopic photograph of a kidney after administration of 0.5 M gadodiamide. Normal appearance.

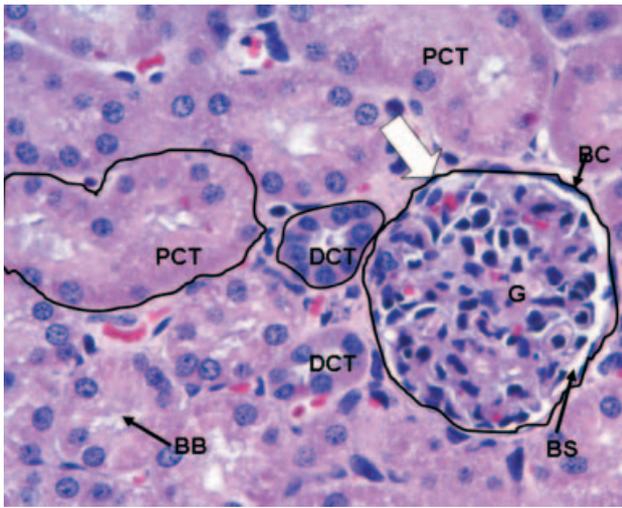


Fig. 3. Saline-treated animals (control). Light micrograph of histological sections of immersion-fixed kidney demonstrates a normal cortex. H&E,  $\times 400$ . White arrow indicates renal corpuscle (combination of two structures: Bowman's capsule [BC] and glomerulus [G]). The space between the visceral and parietal layers of the BC is known as a Bowman's space (BS; urinary space). PCT: proximal convoluted tubule; DCT: distal convoluted tubule; BB: brush border in the lumen of PCT.

The histological findings are summarized in Table 2 and partly illustrated in Figs. 5–8. The main findings were: 1) proximal tubular and glomerular necrosis, 2) hemorrhage/congestion of the cortex, medulla, and glomeruli, 3) proximal tubular vacuolation, and 4) protein-filled tubules in the cortex and medulla. All findings were similar for the ventral and dorsal sample of each kidney.

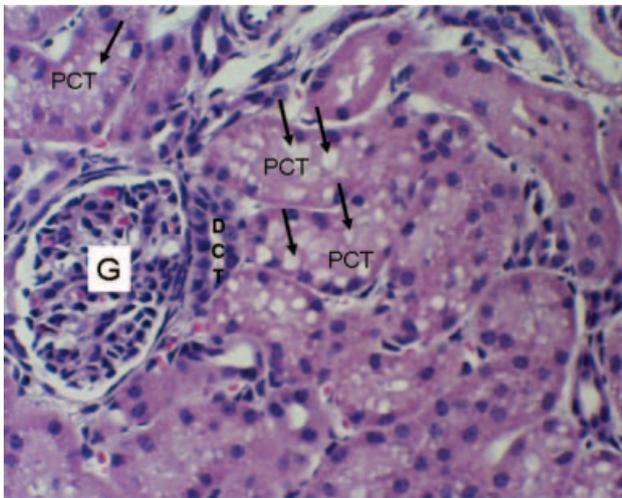


Fig. 4. Gadodiamide-treated animal. Light micrograph, histological sections of immersion-fixed kidney, cortex. H&E,  $\times 400$ . Arrows indicate vacuoles within proximal convoluted tubules (PCT; grade 3). The glomerulus (G) and distal convoluted tubule (DCT) are not affected.

### Necrosis of proximal tubules and glomeruli

Proximal tubular necrosis was significantly greater in the kidneys injected with 0.5 M gadopentetate, mannitol/io 1.96, and 1.0 M gadobutrol compared to all other groups ( $P < 0.001$ ). Iohexol 0.5 M (190 mg I/ml) induced significantly more proximal tubular necrosis than 0.5 M gadodiamide and mannitol/io 0.82 ( $P < 0.01$ ). There were no significant differences between iohexol 190, mannitol/io 0.43 ( $P = 0.061$ ), and iopromide 150 ( $P = 0.289$ ). There was no proximal tubular necrosis in the kidneys injected with iodixanol 150 and 320 or saline, and only a low incidence of minimal necrosis after treatment with iohexol 70.

Necrosis of glomeruli was only found in the groups injected with gadopentetate, mannitol/io 1.96, and gadobutrol, and was significantly greater compared to all other groups ( $P < 0.001$ ). There was no statistically significant difference between gadopentetate, gadobutrol, and mannitol/io 1.96.

### Hemorrhage and congestion

Gadopentetate, mannitol/io 1.96, and gadobutrol induced significantly more frequent and severe hemorrhages/congestions of the cortex, medulla, and glomeruli compared to all other groups ( $P \leq 0.005$ ), with the exception of changes in the medulla following injection of gadobutrol (Table 2). All other test solutions, including saline, did not cause any or induced only minor occasional hemorrhage/congestion.

### Proximal tubular vacuolation

Contrary to necrosis and hemorrhage/congestion, there was no proximal tubular vacuolation in the kidneys injected with gadopentetate, gadobutrol, or mannitol/io 1.96. All other test solutions resulted in some degree of vacuolation. There were significantly more vacuoles in the gadodiamide group compared to all other groups ( $P \leq 0.001$ ), and the least vacuolation was seen in animals treated with saline. There was no significant difference between the various I-CM.

### Protein-filled tubules

The pattern of protein-filled cortical tubules resembled the proximal tubular vacuolation (Table 2). Numerically, there were most protein-filled tubules in kidneys injected with gadodiamide, and the differences between gadodiamide and all other groups were significant ( $P < 0.01$ ) except for the comparison with gadobutrol ( $P = 0.714$ ). There was no statistically significant difference between the

Table 2. Summary of renal histomorphological findings

Grade	Necrosis								Hemorrhage/congestion								Vacuolation				Protein-filled tubules																			
	proximal tubules				glomeruli				cortex				medulla				glomeruli				proximal tubules				cortex				medulla											
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4				
CM																																								
Gadopentetate 1.96*, n=16				100	50	19	31		56	44			25	38	31	6	50	38	13																					
Mannitol/io 1.96 1.96†, n=8				100		50	38	13	75	25			13	63	25		50	50							25															
Gadobutrol 1.60*, n=8				100	13	13	50	25	50	50				25			13	13	75							13	38	25		25	25	38								
Gadodiamide 0.78*, n=24	29												8								8	75	8		4	13	54	4												
Mannitol/io 0.82 0.82†, n=8									25				13								13	50	25		13	50	25													
Iohexol 190 0.40*, n=16	38	25							6				13								13	44	13		13	50	6													
Mannitol/io 0.43 0.43†, n=8	25	13							13	13											50				50															
Iopromide 150 0.34*, n=8	50										13		13								75	13		13	75	13		13	13	13	13		13							
Iodixanol 150 0.29*, n=8												13	13								38	25	25		25	25	25													
Iodixanol 320 0.29*, n=8																					50	13			50	13														
Iohexol 70 0.29*, n=16	6																				19	25			19	25														
NaCl-1 0.29*, n=8									13				13								13				13															
NaCl-2 0.29*, n=8																																								
NaCl-3 0.29*, n=8																					13				13															

\* Osmolality (Osm/kg H<sub>2</sub>O) according to manufacturers.

† Osmolality according to measurement using a Vapor Pressure Osmometer 5500 XR (16).

The severity of changes was graded as follows: 1=minimal, 2=mild, 3=moderate, and 4=marked. n: number of animals in the group. Figures denote percent of animals.

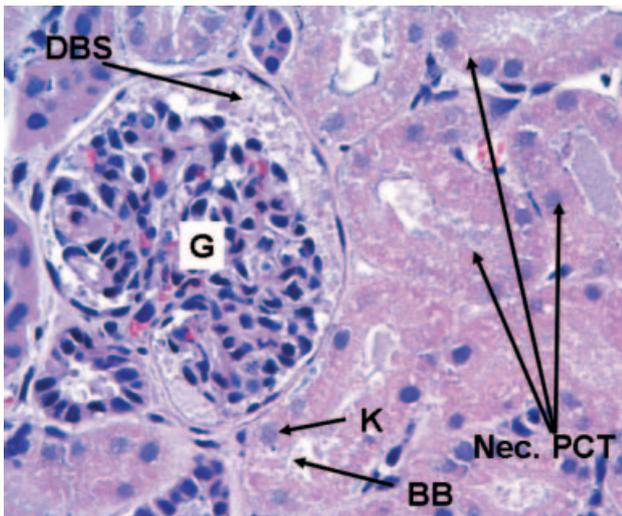


Fig. 5. Gadopentetate-treated animal. Light micrograph, histological section of immersion-fixed kidney, cortex. H&E,  $\times 400$ . There is extensive coagulative necrosis of the proximal convoluted tubules (Nec. PCT; grade 4), with karyolysis (K; ghosting) of the nuclei and loss of the brush border (BB) being noted. There is cellular debris (DBS) present in the Bowman's space of the renal corpuscle; however, the glomerulus (G) is not affected. The distal convoluted tubules (DCT) appear normal.

various I-CM. Again, saline caused the least changes.

Protein-filled tubules in the medulla were only found in kidneys injected with gadobutrol or iopromide 150 mg I/ml. The difference between the two CM was not statistically significant.

**Discussion**

The present porcine model of renal ischemia used for histomorphological analysis (14–16) was designed to provoke an acute CIN after selective

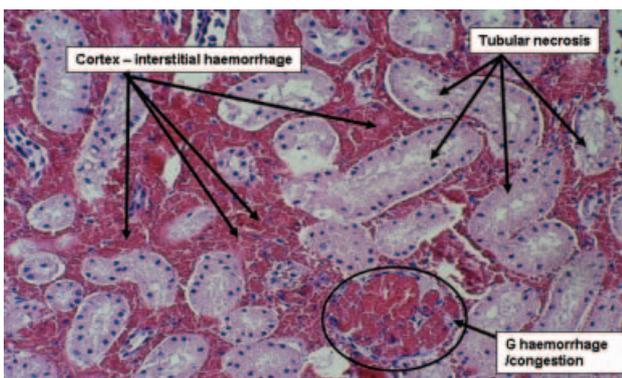


Fig. 6. Gadobutrol-treated animal. Light micrograph, histological section of immersion-fixed kidney, cortex. H&E,  $\times 200$ . Hemorrhage/congestion of the glomerulus (G, grade 3) and within the interstitium of the cortex (grade 3). There is also extensive necrosis of the proximal convoluted tubules (grade 4).

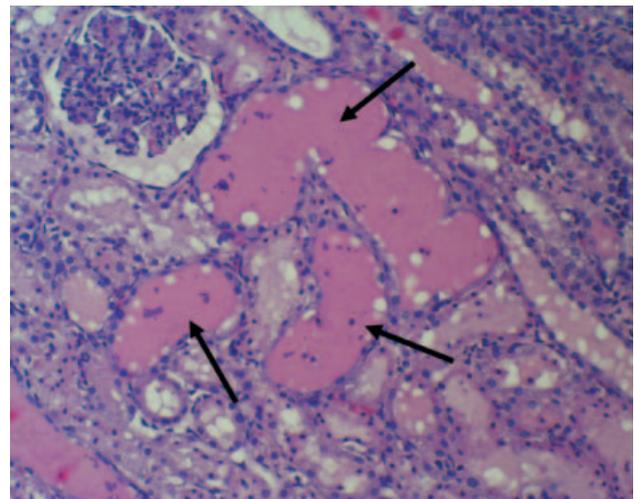


Fig. 7. Gadobutrol-treated animal. Light micrograph, histological section of immersion-fixed kidney, cortex. H&E,  $\times 400$ . The tubules are dilated and contain a brightly eosinophilic homogenous material (arrows; protein-filled tubules, grade 4).

injection of high doses of Gd- and I-CM into the renal artery. In a pilot study, we injected CM into the patent right renal artery. Since we did not notice any decline in renal function, temporary occlusion of the right renal artery was added to potentiate the effect of the CM. The model may imitate a clinical situation where patients with ischemic kidneys undergo renal arteriography/angioplasty, repeated CM injections, and periods of renal ischemia from balloon occlusions of the renal artery. To the best of our knowledge, pigs have rarely been used in investigations of CIN, although their kidneys are morphologically and physiologically considered more similar to human kidneys than most other species (13, 45, 48), possibly only with the exception of the dwarf water buffalo (49).

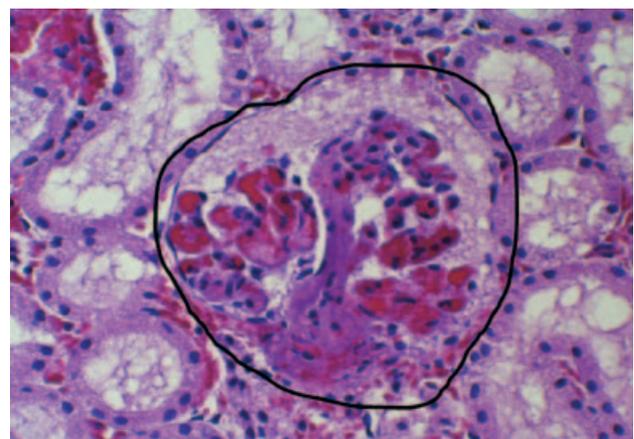


Fig. 8. Mannitol/io 1.96-treated animal. Light micrograph, histological section of immersion-fixed kidney, cortex. H&E,  $\times 400$ . The renal corpuscle (encircled) shows severe glomerular necrosis (grade 4) and cellular debris within the Bowman's space.

*Macroscopic renal findings*

Discolored and swollen kidneys were seen only in the groups injected with gadopentetate, mannitol/io 1.96, and gadobutrol. The high osmolalities of these test solutions are believed to contribute to these morphological changes. This is supported by the fact that mannitol/io 1.96 with the same high osmolality as gadopentetate caused similar changes. Comparable macroscopically alterations were observed in canine kidneys injected with highly hyperosmotic solutions of sodium (18) or diatrizoate with an osmolality approximately 7 times that of plasma (29).

*Necrosis and hemorrhage / congestion*

Tubular and glomerular necrosis as well as hemorrhagic/congestion of the cortex, medulla, and glomeruli was in general found in all kidneys injected with gadopentetate, mannitol/io 1.96, and gadobutrol. These test solutions have an osmolality 5.5 to 7 times that of plasma, and the frequency and severity of the changes they induced were significantly higher than those caused by the test solutions with lower osmolality, i.e., 1.0–2.7 times that of plasma. Gadopentetate, mannitol/io 1.96, and gadobutrol also caused severe impairment of renal function, while animals subjected to the other test solutions had much less effect on GFR, as reported separately (Table 1) (14–16). These findings indicate that osmolality was the predominant factor for inducing necrosis, hemorrhage/congestion, and severe renal impairment.

The pathophysiological mechanisms behind the necrosis and hemorrhage/congestion mainly caused by the “high-osmolal” solutions in the present model may be multifactorial and dependent on the osmotic and chemotoxic effects of the CM in combination with ischemia. The “high-osmolal” test solutions in the present study may act in a similar way as iodine HOEM by extracting water from the red blood cells due to the osmotic gradient, resulting in shrinkage and crenation of red blood cells with secondary trapping and obstruction of the microcirculation (4, 5, 18, 28). The effect of balloon occlusion may prolong the contact time between the “high-osmolal” solutions and the vascular endothelial long enough to induce endothelial injuries with secondary platelet aggregation contributing to obstruction of the microcirculation (3). HOEM in an in vitro study was also found to be directly toxic to renal proximal tubular cells (26).

*Proximal tubular necrosis*

Marked necrosis of proximal tubules was found in all kidneys injected with gadopentetate, mannitol/io

1.96, and gadobutrol. When comparing with kidneys injected with solutions iso-osmolal to plasma, no proximal tubular necrosis was noted. In agreement with the findings in the present investigation, tubular necrosis after injection of iodine HOEM has also been described in several other animal studies (3, 12, 29).

The proximal tubules are particularly sensitive to toxic influences (33). This could be due to many factors, such as the large volume of fluid entering and concentrating within proximal tubule cells, cytoplasmatic and mitochondrial enzyme activities, active transport system, and the associated high metabolic rate (6).

According to some authors, acute tubular necrosis represents a nonspecific response to a variety of renal insults (39). Acute renal failure (ARF) is generally characterized by limited alterations of the proximal tubules (33, 40), although overt tubular necrosis may also occur (27). ARF is caused by ischemic (50%) or nephrotoxic (35%) injury to the kidney, and is often multifactorial (27). In the present porcine model, the marked necrosis of proximal tubules paralleled severe impairment of renal function (14, 16), and it can be postulated that the mechanisms behind these changes probably include, as in human ARF, ischemic and toxic effects of CM.

*Vacuolation of proximal tubules*

Vacuolation of proximal tubules was found in all kidneys except those injected with the high-osmolal test solutions, i.e., gadopentetate, mannitol/io 1.96, and gadobutrol. All three test solutions significantly impaired renal function (14, 16), indicating that there is no covariation between vacuolation and major impairment of renal function. The findings in the kidneys injected with the other CM suggest that vacuolation is not dependent on osmolality. All the I-CM caused vacuolation but did not cause any major impairment of renal function (14–16). Consequently, vacuolation is unlikely to be a major factor in the pathogenesis of CIN in our model.

All kidneys injected with gadodiamide showed vacuolation of proximal tubules. The vacuolation was mostly of moderate severity and was significantly higher than in kidneys injected with all other test solutions. In a previous study, plasma iso-osmolal iodixanol produced pronounced vacuolation (36). Iodixanol 320 has a much higher viscosity (25.4 mPa·s at 20°C) than gadodiamide (2.8 mPa·s at 20°C). The results in the present study do not indicate that vacuolation was dependent on viscosity of the CM. A study in normal rats revealed

vacuoles in all kidneys exposed to I-CM but not in kidneys exposed to Gd-CM (53). No vacuoles were seen in rats with cisplatin nephropathy (54). Thus, vacuolation may vary according to the type of animal model and the pre-experimental status of the kidneys.

The mechanism behind the formation of vacuoles is not well known. For I-CM, experimental evidence indicates that the contents of these vacuoles are CM that has entered the cells by endocytosis. A high concentration of iodine has been detected from these intracellular vacuoles, which have been suggested to be secondary lysosomes (32). POWELL et al. (35) also found 1.5% of the dose of  $^{14}\text{C}$ -iodixanol injected 24 hours previously located in the cortex of rat kidneys. These authors proposed that the CM that enter the cell may interact with secondary lysosomes and interfere with protein catabolism, and suggested that vacuolation is not pathogenic in healthy subjects, but that it may be the first stage of a pathologic process, with cell destruction, in patients with impaired renal function (34). However, others have suggested that vacuolation is reversible (55) and a normal physiological phenomenon of little clinical significance (35). Interestingly, when gadopentetate in our ischemic model caused an almost complete cessation of glomerular filtration, there may only have been scant amounts of excreted CM to be absorbed by the lysosomes, explaining the lack of vacuoles. However, in a non-ischemic pilot study with gadopentetate prior to the present experiments, glomerular filtration was preserved allowing CM to be excreted into the tubules, and vacuolation was in fact observed in these kidneys (unpublished data).

#### *Protein-filled tubules*

No or minimal occasional protein filling of cortical tubules was only seen in the kidneys injected with gadopentetate, mannitol/io 1.96, and saline. Protein-filled tubules in the medulla were found exclusively in some kidneys injected with gadobutrol and iopromide. The appearance of protein-filled tubules did not appear to show a relationship with renal function in the present model, and seemed to covariate with vacuolation.

The main pathway for extraction of proteins from the circulation is by glomerular filtration (10). The amount of protein reaching the urinary space is dependent on glomerular filtration rate, plasma concentration, and the physicochemical characteristics of the protein. The degree of uptake is inversely related to the molecular size of the protein. The majority of the protein within the tubular fluid

is taken up into the proximal tubule epithelial cells by endocytosis. Absorbed proteins within endocytic vacuoles are transported to regions of the tubular cell rich in lysosomes where fusion takes place and hydrolysis to amino acids occurs. Amino acids are then returned to the circulation. Usually, the presence of increased protein in the lumen of proximal tubules indicates that there is increased glomerular permeability in the affected nephron and/or decreased uptake by the proximal tubules (20). It is well known that at least I-CM may induce proteinuria following selective nephroangiography in humans and experimental animals (24, 25). It may be speculated that protein filling of tubules may be secondary to CM-induced proteinuria combined with restrained protein uptake due to vacuolation of the proximal tubules. The scant or lack of protein-filled tubules following injections of gadopentetate and mannitol/io 1.96 may simply be due to the fact that these two agents seemed to have caused the most severe depression of glomerular filtration (14–16), thus preventing any protein reaching the tubules.

#### *Present results vs. histomorphological changes in humans*

To our knowledge, there are very few descriptions of renal CM-induced histomorphological changes in humans in the literature (2, 21, 31). GRUSKIN and MOREAU described vacuolation. Medullary necrosis was seen in some infants following cardiac studies with the HOCM iohalamate or diatrizoate at a dose of 3 ml/kg b.w. or more, apart from one patient who had received only 1.1 ml/kg b.w. (21). Interestingly, no vacuolation was found in patients who developed oligoanuric ARF, which may have prevented excretion of CM into the tubules. A renal biopsy in a recently presented case report of Gd-CM-induced CIN (2) showed acute tubular cell injury including patchy tubular cell necrosis, tubular cell degeneration, and marked proliferation of tubular cells, together with mild interstitial edema and interstitial inflammation.

In conclusion, in ischemic porcine kidneys, the histomorphological changes caused by Gd-CM are similar to those caused by I-CM. The vacuolation appears to be independent of the osmolality and viscosity of the CM, and does not seem to be an indicator of renal impairment. The “high-osmolal” Gd-CM are more nephrotoxic than “low- and iso-osmolal” I-CM when compared in equal volumes of concentrations, resulting in equal X-ray attenuation.

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