Diagnostic Urography of Renal Disorders in Rats

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Abstrakt

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Simple intravenous administration of a contrast medium for excretion urography was tested in 12 clinically healthy female rats (*Rattus norvegicus*, Wistar), aged from 3 to 8 months, whose weight ranged from 270 to 320 g. Excretion urography was performed in 6 rats. The rats were sedated with a combination of ketamine (40 $mg \cdot kg^{-1}$ i.m.) and diazepam (5 $mg \cdot kg^{-1}$ i.m.). Intravenous administration to *vena cephalica antebrachii* was used in three rats, and intravenous administration to *vena saphena lateralis* in nine rats. Meglumine (800 mg $I_2 \cdot kg^{-1}$) was used as the contrast medium in two rats while iopamidol (800 mg $I_2 \cdot kg^{-1}$) was used in the remaining animals. Administration of iopamidol to *v. saphena lateralis* proved to be the best choice. Excretion urography was performed at the intervals of 15 sec, 1 min, 5, 10, and 20 min. The rats were also sampled for blood, their urine was subjected to basic tests, and a renal biopsy was taken and histological examination of the kidneys was done. Details of the ureters could be observed between 5 and 10 min after administration (a.a.). Presence of the contrast medium in the urinary bladder could be observed between 1 and 10 min a.a. Details of the urinary bladder could be seen from 5 min a.a. Hydronephrosis was detected in two rats; contrast stagnation was observed in the renal pelvis and in cranial part of the ureter till 20 minutes. The modified excretion urography revealed renal changes in rats exhibiting no clinical signs of disease. The existence of these changes was subsequently confirmed by a post-mortem examination.

Imaging methods, contrast media, rodents, hydronephrosis

There is a demand for fast and reliable methods of kidney and urinary tract examination in rats especially when kept as companion animals (Konrád and Bondy 1985; Konrád 1989; Johnson-Delaney 1998; Redrobe 2001). Since kidneys and the urinary tract of small mammals in plain radiographs are hard to discern, their visualization can be facilitated with the help of other methods. Kidneys of rats can be visualized by means of a compression method using a prism from a non-contrast material (Wildnerová 2003). Using contrast media is an optimal method (Redrobe 2001). One traditional method of examining kidneys and the urinary tract in veterinary practice is excretion urography (intravenous urography) based on renal-selective concentration and excretion of an intravenously administered contrast medium (Osborne et al. 1972; Kučera 1999). Contraindications to excretion urography include iodine allergies, oliguria, and dehydration. These general techniques can be used to examining small mammals too; potential limitations reflect the patient's size and temper. Some techniques used for radiographic imaging of the urinary tract modified for use in small laboratory rodents have already been described (Hubmann 1980; Isenbügel 1985; Anderson 1994). They have, nevertheless, only rarely been used in clinical practice (Redrobe 2001). The reasons were technical sophistication and, in the classic intracardial administration of the contrast medium, also fear of injuring the patient. The aim of this study

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was to test an easy form of intravenous administration of a contrast medium in rats and a safe excretion urographic method that could be used for intravital diagnostics of renal disorders.

Materials and Methods

The trial included 12 female rats (*Rattus* norvegicus, SPF strain by Medical Faculty, Masaryk University: WISTAR), aged from 3 to 8 months, whose weight ranged between 270 and 320 g. Prior to the inclusion into the trial and immediately after completion of the experiment, a clinical examination was carried out, including evaluation of the fur, colour of mucous membranes, palpation of submandibular and poplitear lymph nodes, auscultation of the cardiovascular and respiratory systems, examination of the oral cavity, and abdominal cavity palpation. The animals were clinically healthy individuals. Before administration of the contrast medium, the rats were fasted for 4 hours; their intake of water was not restricted.

Table 1
Administration of a contrast medium to rats

Rats	Contrast medium used [a]	Leg	Administration site
1	Meglumine	Right front	v. cephalica
2	Meglumine	Left front	v. cephalica
3	Iopamidol	Right front	v. cephalica
4–12	Iopamidol	Left hind	v. saphena lateralis

[a] Contrast media administered at the dose of 800 mg I_2/kg

Preparation of the animals and contrast medium administration

To facilitate handling the animals when applying the contrast medium and to get good-quality radiographs, the animals were sedated with a ketamine/diazepam combination (ketamine 40 mg·kg^{-1} i.m., Narcamon 5%, Spofa, diazepam 5 mg·kg^{-1} i.m., Apaurin, Krka; Knotková and Knotek 2000). The site of administration of the contrast medium on a front or hind leg was shaved and treated with chlorhexidine (Nolvasan Spray, Fort Dodge). Contrast medium was applied using insulin syringes (Omnican, Braun). The contrast medium was injected in the *vena cephalica antebrachii* in 3 rats (No. 1–3), and in the *vena saphena lateralis* in 9 rats (No. 4–12) (Table 1). Meglumine was used in 2 rats (No. 1–2) (800 mg I_2·kg^{-1}, Telebrix N 350 injectable., Byk Gulden), iolpamidol was administered to the remaining animals (800 mg I_2·kg^{-1}, Iopamiro, Bracco).

Table 2
X-ray exposure values used in excretion urography in rats

Rat	Weight (g)	Projection	kV	mAs	s
4	300	LL, VD	54	6.4	0.06
5	270	LL, VD	61	6.4	0.06
6	300	LL, VD	57	6.4	0.06
7	300	LL, VD	57	6.4	0.06
8	310	LL, VD	56	6.4	0.06
9	320	LL, VD	56	6.4	0.06

Excretion urography

Excretion urography was performed in 6 rats (No. 4–9). Exposure times were set as follows: at 15 seconds (immediately after administration), at 1 min, at 5, 10, and 20 min. Ventro-dorsal (VD) projection was used during the exposures at 15 sec and 1 min; and VD and latero-lateral (LL) projections during the exposures at 5, 10 and 20 min. In VD projection compression of the abdomen with a non-contrast prism was applied, to achieve a better visualization of the kidneys by bringing them closer to the X-ray table. The X-ray system used was Durolux; supplemented with an amplification foil 100 cassette of focal length 100 cm; an 18 × 24 cm film (Agfa, SP:BU-new).

Blood sample-taking and haematological and biochemical tests

One week after excretion urography, an EDTA blood sample was taken under inhalation anaesthesia with isoflurane/oxygen mixture (Isoflurane, Rhodia Cantwell 2001). The basic haematological tests were performed with an automatic system (ACT diff, Beckman Coulter); the white blood count was determined on the basis of assessment of Pappenheim-stained blood films. Plasma biochemical parameters were evaluated using automatic analysers Cobas Mira S (Roche) and Atomspec (Hilger 1550).

Urine tests

Urine was sampled from the rats during spontaneous micturition after a one-hour isolation of each animal in a clean box without bedding. The urine tests were performed with testing papers (Hexaphan, Pliva-Lachema) using a semi-quantitative method.

Table 3
CBC (Complete blood count) and biochemical tests of blood plasma in rats after excretion urography

Plasma parameter	Units	Rat					
		4	5	6	7	8	9
Haemoglobin	g/l	149	143	131	136	141	140
PCV	l/l	0.47	0.45	0.42	0.42	0.45	0.47
RBC	T/l	8.49	7.91	7.55	7.40	8.20	7.60
WBC	G/l	7.2	12.1	12.7	5.9	9.2	11.0
Juvenile metamyelocytes	G/l	0	0	0	0	0	0
Segments	G/l	1.22	**0.12** [a]	**0.38** [a]	**0.06** [a]	3.90	3.58
Lymphocytes	G/l	**5.76** [a]	**11.74** [a]	**12.07** [a]	**5.72** [a]	**5.20** [a]	**7.16** [a]
Monocytes	G/l	0.22	0	0	0.06	0	0.10
Eosinophils	G/l	0	0.24	0.25	0.06	0.10	0.15
Basophils	G/l	0	0	0	0	0	0
Total protein	g/l	71.5	63.0	58.0	63.7	-	69.1
Urea	mmol/l	8.4	6.5	6.5	6.1	5.9	6.5
Creatinin	µmol/l	59.8	41.9	41.9	40.5	46.2	52.1
ALP	µkat/l	-	**2.01**[a]	1.50	1.60	-	1.50
ALT	µkat/l	-	0.66	1.08	0.81	-	0.70
AST	µkat/l	-	1.25	1.07	1.00	-	0.92
Phosphoru	mmol/l	3.7	**1.6** [a]	**1.6** [a]	**1.5** [a]	-	2.2
Kalium	mmol/l		**3.5** [a]	**3.5** [a]	**3.4** [a]		2.9
Glucose	mmol/l	-	**10.6** [a]	**10.6** [a]	**9.3** [a]	-	6.8

[a] values set in bold are beyond the physiological range as defined by Harkness and Wagner (1977), Anderson (1994), Hillyer and Quesenberry (1997), Knotková and Knotek (2000)

Renal biopsy

A renal biopsy was carried out on rats No. 4–8 under inhalation anaesthesia, using a mask and an isoflurane/oxygen mixture The operative field on the back spanning both kidneys was shaved clean and disinfected with chlorhexidine (Nolvasan, Fort Dodge). Needle biopsy following incision of the skin in parallel with the spine above the fixed kidney was performed. To limit the risk of undesirable muscle tissue sampling, the method was supplemented with muscle tissue incision in rats No. 6–8. The kidney was fully visualized and the biopsy could be led in the renal cortex without intervening with the renal pelvis. The incision length was approx. 1.5 cm. True-Cut 20 gauge needles, 15 cm long, with a 10 mm sample groove each (Quick-Core needles, Cook William Europe) were used. To prevent pelvis penetration, the biopsy needles were inserted into the renal parenchyma at a sharp angle. The muscle suture was done using Vicryl 3-0 (Ethicon, Johnson & Johnson). The skin was closed with a single plastic-thread U suture (Orsilon, Léčiva). The obtained bioptic samples were fixed with 10% neutral buffer formalin.

Euthanasia and pathological and histological examination

Five to seven d after renal biopsy, the rats were euthanized with T 61 (Intervet) applied intramuscularly into the gluteal muscle of the hind leg. After an external examination the organs were examined *in situ* then removed (spleen, digestive tract, liver, kidneys, urinary bladder, and female reproductive tract). After removing the capsule of the kidney, the cortex, the medulla, and the pelvis were examined. Renal samples were taken and fixed with 10% buffer formalin for histological examination. After 24 h of fixation, the samples were processed routinely into paraffin and tissue slides 4-6 µm thick were stained with hematoxyline-eosine and microscopically evaluated.

Table 4
Results of urine tests in rats after excretion urography

Urine parameters	Rat			
	4	5	6	7
pH	6-7	6-7	6-7	6-7
Proteins	±	±	±	±
Glucose	Negative	Negative	+	Negative
Bodies ketone	Negative	Negative	±	Negative
Urobilinogen	Negative	±	±	Negative
Blood	Negative	Negative	Negative	Negative

Results

Contrast medium administration and excretion urography

Intravenous administration of the contrast medium was tested in 12 female laboratory rats. The sedation of the animals by intramuscular administration of ketamine/diazepam was without complications. All animals were easy to handle and intravenous administration of the contrast medium could start five minutes after the administration. Meglumine proved to be a thick liquid rather difficult to draw into the syringe and complications due to paravenous leakage of this contrast medium occurred in two rats (No. 1 and 2) during administration into *vena cephalica*. Due to these complications, iopamidol, whose administration proved easy, was used to test in the remaining animals. The *vena cephalica* proved not really suitable for administration of the medium (Rat No. 1–3). From a practical point of view, applying the contrast medium into *vena saphena lateralis* proved more easy. The administration of iopamidol into this vein in nine rats was free of complications (Rat No. 4–12).

Excretion urography was done in 6 rats only. A nephrogram, including a pyelogram was monitored in all cases. The kidneys and the renal pelvis were visualized 15 to 60 sec after the contrast medium administration. Details of the ureters were visable from 5 to 10 min. The presence of the contrast medium in the urinary bladder could be detected after 1 to 5 min. Details of the urinary bladder could be observed from min 5 on. Hydronephrosis was detected in two rats (No. 4 and 6); contrast stagnation was observed in the renal pelvis and in the cranial part of the ureter practically throughout the total observation time. How the individual sections of the urinary tract of the rats were visualized at exposition times is shown in Table 5.

Blood and urine tests

The results of the blood count and the biochemical profile of the blood of rats No. 4–9 are presented in Table 3. The number of lymphocytes was above average in rats No. 4, 7, 8, and 9; a real lymphocytosis was diagnosed in two rats (No. 5 and 6). Rats No. 5–7 showed hypocalaemia, hypophosphataemia, and hyperglycaemia. The basic urine tests revealed no serious deviations from the physiological values of selected parameters (see Table 4).

Biopsy

Rats No. 4 and 5 urinated blood immediately after the biopsy. Their status improved soon, but the animals remained apathic for quite long. Rat No. 4 had two deep radial lesions of 5 mm of diameter on the right kidney. One of them was on *margo lateralis* and the second one on the caudal pole of the kidney. An analogous lesion was found on *margo lateralis* of the left kidney. The right kidney of rat No. 5 showed no significant macroscopic change; there was only a yellow-red discolouring sized 1 mm on *facies dorsalis*. The left kidney had a scarred recession of 3 mm in diameter in the vicinity of the cranial pole on *margo lateralis*. Rats No. 6 and 7 had only traces of blood in urine immediately after the sampling, using

Table 5
Segments of kidneys and the urinary duct observed using excretion urography

Rat	Exposure				
	15 sec VD	1 min VD	5 min VD, LL	10 min VD, LL	20 min VD, LL
4	Nephrogram and initiating pyelogram	Pyelogram – RIGHT more details and more prominent dilatation, LEFT ureter visible	Pyelogram – RIGHT prominent pelvis, ureter relatively prominent; LEFT ureter well visible; onset of urinary bladder filling	Stagnation in RIGHT pelvis lasts, parenchyma discoloured, LEFT ureter visible, urinary bladder more prominent	RIGHT pelvis visible, without details of renal parenchyma now, LEFT pelvis and ureter mildly visible, urinary bladder prominent
5	Mild details of nephrogram; both pelvises dilated (right one more so)	Prominent pelvises and cranial parts of ureters (right dilated), more intensive urinary bladder filling	Dilated pelvises, all ureters visible, cranial parts dilated, left kidney discolouring, urinary bladder prominent	Prominent stagnation in RIGHT pelvis and ureter dilatation; LEFT pelvis loses in contrast, pelvis and ureter still visible	Pelvises and ureters still visible, but less prominent; urinary bladder filling again
6	Details of the nephrogram mildly visible on both sides; pyelogram	More prominent details of the parenchyma, pelvises, and ureters	More prominent details of pelvises and ureters; details of the urinary bladder	Ureters visible; very prominent urinary bladder	Urinary bladder filling dominates
7	Nephrogram and pyelogram; urinary bladder starts to fill	Parenchyma discoloured, pelvis and ureters better visible; more prominent urinary bladder	Pelvis discolouring; details of ureters; urinary bladder very prominent	Almost nothing but ureters visible, urinary bladder easy to discern	Almost nothing but ureters visible, urinary bladder easy to discern
8	Nephrogram and pyelogram visible	Details of pelvises and ureters	Pelvis discolouring; details of ureters urinary bladder	Almost nothing but ureters visible, well-discernible and urinary bladder	Urinary bladder filled up
9	Nephrogram and initiating pyelogram	Sharp details of pelvises and ureters	Pelvis discolouring; sharp details of ureters and urinary bladder	Almost nothing but ureters visible, well-discernible urinary bladder	Urinary bladder filled up

Table 6
Histology of the kidneys in rats after excretion urography

Rat	Left kidney	Right kidney
4	Fragments of fibrous tissue with adipose cells and lymphocytes (part of the capsule)	Muscle tissue area in the material; renal medullar channels with lymphocytes in the interstitium
5	Cortex with glomerules and a group of proximal tubuli: lymphocytic infiltration, extravasations, damaged epithelium in part of the tubuli	Group of channels and part of the glomerules: many lymphocytes, haemorrhages, fat droplets in some epithelial cells
6	Cortex with glomerules, lymphocytes in the interstitium and degeneration of the proximal tubuli	Renal cortex intact, sporadic lymphocytes in the interstitium
7	Renal cortex and glomerules	Renal medulla: small extravasations, otherwise intact
8	Renal cortex and glomerules: leucocytic infiltration in the interstitium, damaged epithelium in part of the tubuli, vacuolisation	Group of channels and part of the glomerules: fat droplets in some epithelial cells

[a] In rats No. 4–7 after renal biopsy, in rat No. 8 after euthanasia

urinary test strips. After 2 d no blood was found. Rat No. 6 had a subcutaneous fibrous reaction above the site of sample-taking from the right kidney. The left kidney showed a small scar at the cranial pole and an elongated scar sized 4 ± 2 mm on *margo lateralis*. The right kidney exhibited a 3 mm scar at the cranial pole. Rat No. 8 showed haemorrhages on the renal surface; the pelvis was not penetrated.

Renal changes

The right kidney of rat No. 4 had a dilated pelvis. In the histology an indistinct leucocytic infiltrate of the interstitium, some cystically dilated tubular sections, and proximal tubuli coagulation necrosis regions were seen in both kidneys. The medulla showed no changes. The right kidney of rat No. 5 showed no significant macroscopic change. Histologically the right kidney showed glomerulonephritis, interstitial nephritis mainly in the medulla, and degeneration of the proximal tubuli. The left kidney was characterized by interstitial nephritis and degeneration of the proximal tubuli. In rat No. 6, the histological testing revealed glomerulonephritis, interstitial nephritis, degeneration of the proximal tubuli, and a sporadic lymphocytic infiltrate in the interstitium in both kidneys. In rat No. 7 no pathological changes were seen. As to rat No. 8, both its kidneys suffered from degeneration of the proximal tubuli, which was more prominent in the right kidney, and sporadic lymphocytes in the interstitium.

Discussion

There are a number of different renal and urinary tract damages. One of the aims of using imaging methods and their modifications is timely diagnosis of the changes before they reach a point of threatening the patient's life.

As far as developmental renal anomalies are concerned, unilateral or bilateral, partial or total agenesis of the kidneys can be detected. Unilateral agenesis involves compensatory hypertrophy of the contralateral kidney, which is free of clinical signs.

Renal cysts, which can be developmental non-inherited, inherited, or acquired, have also been observed in rats. They can be of different sizes

and wall thickness, solitary or multiple (polycystic renal disease, Johnson-Delaney 1996; Halouzka and Krinke 2000).

According to Jelínek (1992), renal dystrophy includes mineralization, non-viral cell inclusions, urolithiasis, and hydronephrosis. Our rats showed no signs of clinical disease, but the urography revealed renal changes that could subsequently be confirmed by a post-mortem examination. Neither the clinical examination nor the blood tests indicated any excretion disorders. The deviations of several blood parameters and the biochemical profiles that were observed would not be regarded as a reason for a detailed examination of the kidneys and the urinary tract in clinical practice. A recurring finding was a renal pelvis dilatation, potentially due to hydronephrosis (Halouzka and Krinke 2000). Genetically conditioned hydronephrosis has been reported in some rat strains, where no macroscopic change in the urinary duct can be demonstrated. Acquired hydronephrosis may be due to defective urinary draining caused by concretions, inflammation, or another pathological process (Jelínek 1992). Where the condition is bilateral, it usually leads to renal failure. Urine production is maintained even in cases of urinary tract obstruction, urine accumulates and leads to atrophy of the renal parenchyma. In the rats in this study, there was one animal in which the renal pelvis dilatation was bilateral, but the status was compensated and renal failure did not occur.

Female rats over seven weeks of age often suffer from nephrocalcinosis, involving calcium phosphate deposition in the basal membrane of *pars recta* of tubuli, the loop of Henle, the cortical zone of the medulla, and at the corticomedullar junction. Intraluminar concretions are believed to be associated with atrophy and tubular cells loss. In serious cases tubular dilatation and hyperplasia occur. Isostenuria and prominent proteinuria are diagnosed from urine (Anderson 1994). These changes have not been observed in the rats of our study.

The most frequent and most important renal disease observed in old rats, whose cause has not been traced down yet, is chronic progressive nephrosis of rats (synonyms: nephropathy of old rats, glomerulonephrosis, nephrosclerosis, chronic nephrosis, chronic nephritis; Jelínek 1992). The condition has been attributed to the effect of fast growth and excessive protein intake, and primary glomerular damage associated with development of dystrophy of the tubular epithelium due to protein and iron absorption. Males are affected more often. Macroscopically the kidneys are hypertrophic in the beginning and later granulated and atrophic with small cysts (Hillyer and Quesenberry 1997; Johnson-Delaney 1998). The interstitium is infiltrated with lymphocytes and plasmacells at an advanced stage (Jelínek 1992), the result being chronic renal failure accompanied by isostenuria and proteinuria.

Struvite uroliths in the urinary bladder lumen or adhering to the mucosa have been reported in elderly rats (Johnson-Delaney 1998). Anderson (1994) reports as frequent calcium carbonate uroliths. Protein concretions have been observed in elderly male rats (Johnson-Delaney 1998).

Most of the above-mentioned diseases can be diagnosed by using our modification of excretion urography rats.

Diagnostická urografie poruch ledvin u potkanů

U 12 klinicky zdravých samic potkanů (*Rattus* norvegicus, Wistar), stáří od 3 do 8 měsíců, hmotnosti 270-320 g byla ověřována jednoduchá intravenózní aplikace kontrastní látky pro vylučovací urografii. U šesti potkanů byla provedena i vylučovací urografie. Potkani byli sedováni kombinací ketaminu (40 mg·kg^{-1} i.m.) s diazepamem (5 mg·kg^{-1} i.m.). U tří potkanů byla zvolena intravenózní aplikace do *vena cephalica antebrachii*, u devíti potkanů byla kontrastní látka aplikována do *vena saphena lateralis*. U dvou potkanů byla aplikována kon-

trastní látka meglumin (800 mg $I_2 \cdot kg^{-1}$), u ostatních zvířat byl zvolen iopamidol (800 mg $I_2 \cdot kg^{-1}$). Jako optimální se ukázala aplikace iopamidolu do v. saphena lateralis. Vylučovací urografie byla provedena v intervalech 15 sekund, 1 min, 5, 10 a 20 min. Potkanům byla rovněž odebrána krev, orientačně vyšetřena moč, provedena biopsie ledvin a histologické vyšetření ledvin. K zobrazení ledvin a pánvičky došlo při expozici 15 až 60 sekund po aplikaci kontrastní látky. Prokreslení ureterů bylo patrné od páté do desáté minuty. Přítomnost kontrastní látky v močovém měchýři bylo možno zachytit již mezi první a pátou minutou. Kvalitní zobrazení močového měchýře nastalo od páté minuty. U dvou samic byla odhalena hydronefróza, při které byla patrná stagnace kontrastu v ledvinné pánvičce a v počátečním úseku ureteru v podstatě po celou dobu expozice. Modifikovaná metoda vylučovací urografie odhalila změny na ledvinách u potkanů, kteří nevykazovali klinické projevy onemocnění. Existence těchto změn byla následně potvrzena postmortálním vyšetřením.

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