Unilateral Renal Agenesis/Dysplasia Associated with Contralateral End-Stage Kidney Disease in Two Wistar Rats

F. Schorsch*, G. Pohlmeyer-Esch and D. Lasserre-Bigot
Aventis CropScience, Pathology Unit of the Sophia Antipolis Research Center, 355 Rue Dostoievski, F-06903 Sophia Antipolis, France
*Corresponding author (e-mail: frederic.schorsch@aventis.com)

KEY WORDS
Renal agenesis, renal dysplasia, chronic progressive nephropathy, Wistar rat.

SUMMARY
Unilateral abnormal renal development (agenesis, hypoplasia, dysplasia) has been described in humans and many animal species as a rare incidental finding that is usually compatible with life. This paper describes one case each of right renal agenesis and dysplasia in Wistar rats which belonged to a control group of a carcinogenicity study. Both rats passed unnoticed during earlier study phases and died spontaneously at an animal age of 60 and 83 weeks, respectively.

Compared to the remaining animals of the study, they showed a severely accelerated and aggravated chronic progressive nephropathy in the contralateral kidney, resulting in end-stage kidney disease that was considered the cause of death. The disease process was accompanied by characteristic clinicopathological changes and multi-organ lesions of secondary hyperparathyroidism. Interestingly, the right renal malformation was associated in both cases with hypoplasia of the right testis and epididymis. The mechanism underlying this rare malformation in Wistar rats is undetermined.

INTRODUCTION
Whereas renal agenesis is the complete absence of renal structures, renal hypoplasia consists of an underdevelopment in size of one or both kidneys that otherwise contain normal structures. Renal dysplasia, on the other hand, is defined as a disorganized development of renal parenchyma, and characteristically comprises the following histologic features: 1. Bone or cartilage in the parenchyma; 2. Persistent metanephric ducts surrounded by primitive mesenchyme; 3. Fetal or immature glomeruli; 4. Fetal or immature tubules; 5. Anomalous presence of interstitial fibrous tissue (JUBB et al., 1993; PICUT & LEWIS, 1987). All three malformations are recognized to be the consequence of a more or less imperfect inductive interaction between the mesonephric duct (Wolffian duct) and the metanephric blastema during embryonal development (JUBB et al., 1993). They may occur uni- or bilaterally, and may be seen alone or accompanying multi-organ malformations.

Bilateral agenesis of the kidneys invariably results in early postnatal death. Unilateral renal agenesis, hypoplasia and dysplasia, however, are compatible with life under normal circumstances, and may be found during clinical examination or at necropsy. Such malformations are rare, and case reports exist in the literature for humans, primates (KIM & KALTER, 1972; WADSWORTH & SQUIRES, 1980), dogs (VAN PELT & SACHTJEN, 1973; PICUT & LEWIS, 1987; KERLIN & VAN WINKLE, 1995; HOPPE & KARLSTAM, 2000), cats (LULICH et al., 1987), horses (ZICKER et al., 1990; JONES et al., 1994), and cattle (DUNHAM et al., 1989). In rodents, they have been found in some specific strains and have been induced in transgenic mice (KNEIDL et al., 1995; MAAS et al., 1994).

In this paper, we describe the clinico-pathologic and microscopic features of two aged male Wistar rats, control animals of a carcinogenicity study, which presented unilateral renal agenesis or dysplasia. Both animals passed unnoticed during the earlier study phases and died spontaneously at more
than one year of age. They died due to contra-lateral end-stage kidney disease with classical renal and extra-renal lesions, indicating that the strain- and age-associated chronic progressive nephropathy (CPN) was accelerated and aggravated.

MATERIALS AND METHODS

Study design

Sixty male and 60 female Wistar rats (WI (IOPS HAN), R. Janvier, Le Genest St. Isle, France), 6-7 weeks old at study start, were used in a 104-week carcinogenicity study to generate historical control data. They received no treatment throughout the study. The animals were housed in groups of 5 in stainless steel wire mesh cages in a controlled-environment barrier-system room, maintained at 22±2°C and at 55±15% relative humidity with a 12 hour light/12 hour dark cycle. Water and commercial powdered rodent diet (M20, Pietrement, Provins, France) were allowed ad libitum, except for food deprivation periods before urine collection and blood sampling.

Case history

Rat n°1 was found dead on Day 370 of the study, corresponding to an animal age of 60 weeks. Rat n°2 was found dead on Day 526 of the study, corresponding to an animal age of 83 weeks. Both rats had shown a reduced body weight gain for approximately six weeks preceding death, when compared to the group mean value. Predominant clinical findings, noticed only during a short period before death, were a thin appearance, piloerection and reduced motor activity in both rats. Furthermore, general pallor and coldness were found in rat n°1 and a soiled fur around the anogenital region was seen in rat n°2.

Clinical pathology results

According to the study design, urinalysis as well as clinical chemistry and hematology analyses were carried out at 4, 6 and 12 months on a proportion of the animals only and were therefore only available for rat n°1. The results of case-relevant parameters at 4 and 12 months are presented in Table 1.

When compared to the group mean values, predominant clinico-pathological findings at 4 months consisted of a mild polyuria and marked proteinuria, mildly elevated plasma urea and creatinine levels, mild hypercholesterolemia and hypertriglyceridemia, as well as a tendency towards decreased red blood cell parameters. At 6 months, results corresponded largely to those obtained at 4 months and are therefore not presented.

At 12 months, shortly before rat n°1 died, changes were much more prominent and were consistent with a decompensated chronic renal damage. In urinalysis, a marked polyuria and marked proteinuria were recorded. Clinical chemistry showed a severe uremia and creatininemia, a moderate hypoproteinemia as well as persistent hypercholesterolemia/hypertriglyceridemia and increased plasma activity of alkaline phosphatase. Plasma potassium and inorganic phosphorus levels were increased, plasma calcium and chloride levels were decreased. The rat showed a marked microcytic, normochromic anemia.

Methods

A complete necropsy was performed on both rats. According to routine procedures, tissues were preserved in 10% neutral buffered formalin or Davidson’s fluid (testis and epididymis), and were trimmed and processed to paraffin blocks. Sections were cut at 4 µm and stained with hematoxylin and eosin (HE). In addition, selected representative sections from each case were also stained with PAS, Congo red, Azan, Masson’s trichrome, van Kossa or Alcian blue as considered appropriate to characterize pathological changes.

RESULTS

Necropsy findings

In rat n°1, both kidneys were present, the right one being abnormally small with a diameter inferior to 0.4 cm. In rat n°2, by conventional macroscopic observation, no right kidney was found at necropsy. In both rats, the left kidney appeared obviously large with an irregular granular surface.

Associated with the findings in the urinary tract, the size of the right testis and epididymis was reduced in both rats. In rat n°2, the right seminal vesicle was also noticed to be small.

Other necropsy findings in both rats were red foci on the gastric glandular mucosa, an obviously small thymus, and a dilated, white mottled and rigid aorta. In rat n°2, the parathyroid glands appeared bilaterally enlarged and the lung had a firm consistency. A general pale appearance of all organs was observed in rat n°1.

Renal histopathology

In rat n°1, the small right kidney had an abnormal elongated shape. The central part of the organ consisted of loose mesenchyme, containing some
vascular spaces that were lined by endothelial cells, without any collecting structures. No regular medullary or renal pelvic structures could be identified (Figure 1). The mesenchyme stained positive with Masson’s trichrome and Alcian blue, disclosing collagen fibers and glycoproteins. At the periphery, enclosed within the same loose mesenchyme, there were some dilated ductular structures, lined by a tall cuboidal bi- or tri-layered and basophilic epithelium and interpreted as primitive ducts (Figure 2). Most of them contained hyaline casts. Glomeruli with condensed glomerular tufts and dilated Bowman’s spaces were observed between the tubules and enclosed in the mesenchyme (Figure 3). Atrophic tubular elements were present and some glomeruli were undergoing sclerosis. Vascularization appeared also abnormal, with numerous small arteries and venules. Altogether, the changes were interpreted as renal dysplasia.

In the contralateral kidney of both rats, the renal tissue had a similar histologic appearance.
Extra-renal histopathology

The unilateral renal malformation was associated with several ipsilateral anomalies of the genital tract. In both rats, the right testis (Figure 6) and epididymis were hypoplastic, and the left seminal vesicle and the prostate gland showed secretory inactivity. In rat n°1, the left testicle displayed a moderate diffuse tubular degeneration, and in rat n°2, the right seminal vesicle was hypoplastic.

Most other extra-renal lesions were considered to be related to the end-stage kidney disease. There was a marked diffuse bilateral hyperplasia of the parathyroid glands. A fibrous osteodystrophy was diagnosed on the routinely sectioned bone tissue of sternum and femur. Numerous organs showed metastatic calcification, especially the myocardium, skeletal muscle and gastric mucosa. Additionally, mineral deposition was observed in blood ves-

### Table 2

<table>
<thead>
<tr>
<th>Organ and lesion</th>
<th>Rat n° 1</th>
<th>Rat n° 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kidney, right</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agenesis</td>
<td>n.o.</td>
<td>present</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>present</td>
<td>n.o.</td>
</tr>
<tr>
<td><strong>Kidney, left</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td>severe</td>
<td>severe</td>
</tr>
<tr>
<td>Interstitial fibrosis</td>
<td>marked</td>
<td>marked</td>
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<tr>
<td>Atypical small tubules</td>
<td>marked</td>
<td>moderate</td>
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<tr>
<td>Dilated tubules with hyaline casts</td>
<td>moderate</td>
<td>marked</td>
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<tr>
<td>Interstitial mononuclear cell infiltration</td>
<td>mild</td>
<td>mild</td>
</tr>
<tr>
<td>Interstitial hemosiderin pigment</td>
<td>moderate</td>
<td>n.o.</td>
</tr>
<tr>
<td>Mineralization of basement membranes</td>
<td>mild</td>
<td>marked</td>
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<tr>
<td>Dilated renal pelvis</td>
<td>mild</td>
<td>mild</td>
</tr>
<tr>
<td><strong>Testis, right</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplasia</td>
<td>marked</td>
<td>severe</td>
</tr>
<tr>
<td><strong>Testis, left</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffuse tubular degeneration</td>
<td>moderate</td>
<td>n.o.</td>
</tr>
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<td>Multifocal atrophic tubules</td>
<td>n.o.</td>
<td>slight</td>
</tr>
<tr>
<td><strong>Epididymis, right</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplasia</td>
<td>marked</td>
<td>severe</td>
</tr>
<tr>
<td><strong>Epididymis, left</strong></td>
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<td></td>
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<tr>
<td>Hypospermia with intraluminal spermatic debris</td>
<td>moderate</td>
<td>n.o.</td>
</tr>
<tr>
<td><strong>Seminal vesicle, right</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoplasia</td>
<td>n.o.</td>
<td>present</td>
</tr>
<tr>
<td><strong>Seminal vesicle, left</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretory inactivity</td>
<td>moderate</td>
<td>moderate</td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secretory inactivity</td>
<td>marked</td>
<td>moderate</td>
</tr>
</tbody>
</table>

n.o.: not observed
Grade: slight, mild, moderate, marked, severe

(Table 2). Glomeruli were increased in size, with a thickened Bowman’s capsule (Figures 4 and 5). The glomerular tuft appeared hypocellular, with focal capsular adhesions. The mesangium was thickened by a hyaline, amyloid-negative substance. Many tubules were dilated and contained hyaline casts (Figure 4). Small tubules were also present, with cuboidal epithelial cells and a narrow lumen. There was a diffuse interstitial and peritubular fibrosis and an increased number of mononuclear cells in the interstitium. Mineral deposition was prominent in the basement membranes around tubules and glomeruli (Figure 5). Moreover, hemosiderin pigment was present in the interstitium, and in both rats, the renal pelvis was mildly dilated. These renal lesions were considered to represent a severe CPN with superimposed ectopic calcification.
Figure 1: Dysplastic right kidney, Wistar Rat n°1, HE, x2.5: Reduced and abnormal shape; Abnormal dilated tubules with hyaline casts and few cystic glomeruli at the periphery; Loose mesenchymal tissue in the central part; Central vascular spaces.

Figure 2: Dysplastic right kidney, Wistar Rat n°1, Alcian blue, x20: Abnormal dilated tubules lined by cuboidal bilayered epithelium enclosed in a loose mesenchyme, stained positively by Alcian blue.

Figure 3: Dysplastic right kidney, Wistar Rat n°1, HE, x20: Primitive cystic glomeruli with dilated Bowman’s spaces (arrow), enclosed within a loose mesenchyme and multilayered dilated tubules (arrowhead).

Figure 4: Left kidney, Wistar Rat n°1, HE, x10: Lesions of chronic progressive nephropathy: Glomeruli are increased in size and hypocellular; Many dilated tubules that contain hyaline casts (arrow); Some small tubules with narrow lumen (arrowhead); Chronic interstitial nephritis.

Figure 5: Left kidney, Wistar Rat n°2, Van Kossa, x20: Glomerulosclerosis, with thickened basement membrane and calcium deposit on basement membranes around tubules and glomeruli.

Figure 6: Right testis, Wistar Rat n°2, HE, x10: Hypoplastic seminiferous tubules.
sel walls, especially in the aorta. In rat n°2, the pulmonary alveolar septa were markedly mineralized. A mild diffuse hypertrophy of the left heart ventricle was found in both rats, associated with moderate progressive cardiomyopathy. All other histopathologic findings corresponded to those usually observed in rats of this strain and age and were considered unrelated to the specific disease process.

DISCUSSION

The present two cases of unilateral developmental anomalies of the urogenital tract in Wistar rats are part of a more regular, although infrequent, observation of these malformations during short- and medium-term toxicity studies with this strain. Although rare and usually noticed incidentally, there are some case reports in the literature that indicate the occurrence of unilateral renal agenesis or dysplasia in many domestic and laboratory rodent species.

It is well assumed that both observed renal abnormalities are the result of a similar defect to the developing embryological structures, including the ureteric bud (metanephric blastema) and the mesonephic (Wolffian) and Müllerian ducts. The main difference in the two cases reported here is the severity in anomaly of renal development: in rat n°2, the right kidney was completely missing (agenesis), whereas in rat n°1, the right kidney was abnormally small and disorganized (dysplasia). In rats, a similar urogenital developmental disorder is well described only in the ACI rat (FUJIKURA, 1970). In 12 to 30% of the animals, there is a unilateral renal agenesis with ipsilateral agenesis or hypoplasia of genital organs of either sex (CRAMER & GILL 3rd, 1975; SHOJI & HARATA, 1977; MARSHALL et al., 1978; VASSILACOPOULOU & BOYLAN, 1992; KNEIDL et al., 1995). As in the two Wistar rats described in this paper, the right side is more frequently involved.

Another similar syndrome is described in mice which are homozygous for the limb deformity (ld) mutation. They are characterized by unilateral or bilateral renal agenesis, and a delayed outgrowth or complete absence of the ureteric bud has been demonstrated (KLEINEBRECHT et al., 1982; MAAS et al., 1994).

Although the exact pathophysiologic mechanisms that underlie renal agenesis remain unknown, abnormal temporal and spatial expression of master genes have been proposed as a possible cause for such dysplastic development. The main gene described in the control of urogenital development is PAX2 (WINYARD et al., 1996). This gene encodes a transcription factor expressed during fetal development in the central nervous system, eye, ear and urogenital tract (ECCLES, 1998). PAX2 is required in the earliest phase of mesenchyme-to-epithelium conversion (ROTHENPIELER & DRESSLER, 1993), but then repression of PAX2 is required for normal kidney development. Persistent expression of PAX2 results in histologically abnormal and dysfunctional renal epithelium. This hypothesis has been demonstrated by deregulating the expression of the PAX2 gene in transgenic mice (DRESSLER et al., 1993). The role of the PAX2 gene has been recently associated with an increased apoptosis in the ureteric bud and could be the cause of renal anomalies found in mice and humans (PORTEOUS et al., 2000).

In males, the epididymes, vas deferens and seminal vesicles are derived from the Wolffian ducts and in females, the oviducts, uterus and upper part of the vagina develop from the Müllerian duct. As PAX2 has also been found expressed in the Wolffian and Müllerian precursor ducts of the male and female genital tract (TORRES et al., 1995), it is not surprising that renal and genital malformations may be combined.

Whatever the cause and the mode of inheritance of such renal agenesis/dysplasia in Wistar rats, it is interesting to find that the abnormal development may remain unnoticed, even under clinicopathological monitoring, for the duration of a chronic study. This explains why its eradication is difficult. All the lesions of the contralateral kidney are consistent with CPN (GRAY, 1986). The opportunity we had in this study to examine these two rats at the end of their life suggests that an abnormal or missing kidney may accelerate the progression of the disease.

Both these rats showed renal failure, associated with secondary extra-renal changes. Clinical pathology confirmed the reduced function of renal parenchyma. Renal azotemia generally occurs after approximately three-fourths of the nephrons are nonfunctional and plasma creatinine, a more accurate measurement of glomerular filtration rate is also increased (DUNCAN et al., 1994). Progressive nonregenerative anemia is also observed, probably mainly due to a reduced renal erythropoietin secretion. Consequent to glomerular damage, proteinuria, hypoproteinemia, and hypercholesterolemia are typically observed and constitute the hallmark of CPN (PERCY & BARTHOld, 1993). Potassium levels were high as well, as it is usually found in renal failure (RAGAN, 1989). Parathyroid hyperplasia was observed and has already been reported in a similar case of unilateral kidney agenesis.
(FEEBACK et al., 1986). Typical lesions of fibrous osteodystrophy in the two sections of bone examined were the consequence of this secondary hyperparathyroidism.

In conclusion, these two cases of unilateral renal agenesis and dysplasia were very similar in that they were associated with ipsilateral testis and epididymis hypoplasia and ended by a nephrotic syndrome probably induced and/or amplified by the overall reduced parenchymal tissue. Although mechanisms and genetics of this syndrome in Wistar rats have not been studied, similarities with cases in mice and humans with PAX2 mutation indicate that it could be interesting to look for a defect in this gene in rats.

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