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NEPHROSONOGRAPHIC EXAMINATIONS IN DOGS WITH GENTAMICIN INDUCED ACUTE RENAL FAILURE

MACANOVIĆ MIRJANA*, JANKOVIĆ-ZAGORČIĆ ANICA*, LIŠANIN LJ**, DODIĆ M*** and SPASIĆ V**

*Faculty of Veterinary Medicine, Bul. JNA 18, 11 000 Belgrade ** Military Medical Academy, Crnotravska 17, 11 000 Belgrade *** CMD, Božidara Ad'je 12, 11 000 Beograd

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In this study we investigated the feasibility of ultrasonography in the evaluation of renal parenchyma structural changes in dogs with acute renal failure (ARF) induced by high doses of gentamicin (40 mg/kg body mass, at 12 hr intervals during 7 days). Acute renal failure was induced in all experimental animals after three days and the disease had a progressive tendency till the end of the experiment. Morphometric analyses of the kidneys did not reveal changes in the size of the organ as compared to the values observed at the beginning of the study. Initial changes in the echostructure of the renal parenchyma were observed in 92% of the animals starting from day 3 of the experiment. They were manifested as enhanced echogenicity of the cortex and a less prominent corticomedullar junction and these findings correlated with the onset of ARF. During the experiment, changes in the renal structure intensifyied and on day 9 marked hyperechoity of the cortex and complete loss of the corticomedullar junction were observed in the majority of experimental animals. Our results confirmed the correlation between the changes in renal structure estimated by nephrosonography and the progress of ARF.

Key words: acute renal failure, dog, gentamicin, ultrasonography

INTRODUCTION

Acute renal failure (ARF) is a clinical syndrome that can evolve as a consequence of numerous renal parenchyma diseases including inflammation, dystrophic changes in the tubules and glomerules and acute tubular necrosis. The majority of causes of ARF have common pathogenic factors i.e. serious circulation disturbance and an influence of nephrotoxic substances (Grauer and Lane 1995).

Due to their characteristic anatomical properties and specific physiological functions, the kidneys are organs especially susceptible to various toxic substances. As a result of the extensive blood flow through the kidneys (20% of the total blood flow), large quantities of potentially toxic substances can reach these organs via the circulation. The renal cortex is especially sensitive to toxic substances because it possesses a very large capillary endothelial surface and because nearly 90% of the total renal blood flow moves toward this area.

It is well known that some toxic substances e.g. ethylene glycol, picric acid and sodium arsenate cause massive dystrophic as well as necrotic alterations in the epithelial cells of proximal renal tubules with consecutive ARF development (Tsukamoto *et al.* 1983). Similar histopathological alterations were induced by snake venom (Puig *et al.* 1995, Chen *et al.* 1997). Also, application of some therapeutic substances in high doses like oxytetracycline (Stevenson, 1980), kanamycine (Okada *et al.* 1991) and gentamicin (Spangler, 1980, Riviere *et al.* 1983, Milosavljević, 1988, Nakakuki, 1997) carry a potential risk for damage of the renal parenchyma, often resulting in ARF. Gentamicin and other aminoglycoside antibiotics as well as some cationic drugs can cause alterations of the glomerular membrane reducing the glomerular filtration rate. Following reabsorption of water and other primary urine constituents, the cells of the proximal tubules are exposed to toxic substances in high concentrations. Consequently, malabsorption of numerous substances from primary urine occurs and therefore these substances are found in the final urine (Riviere *et al.* 1983, Grauer and Lane, 1995, Nakakuki *et al.* 1996).

Ultrasonographical examinations of the kidneys following induced nephrotoxicity were performed by Rosenfild *et al.* (1985). They stated that ultrasonograms of rat kidneys, following tubular necrosis induced by K - dichromate (15 mg/kg bm), were characterized by enhanced echogenicity of the cortex. On the contrary, tubular necrosis induced by ischaemia did not change echogenicity of either medulla or cortex.

Adams *et al.* (1989) investigated changes in nephrosonograms of dogs following nephrosis induced by oral application of 95% ethylene-glycol (10 ml/kg bm). During the forth hour after administration of toxic substance, echogenicity of the renal cortex was significantly increased and was more pronounced than echogenicity of the liver. During this experiment the authors also documented hyperechogenicity of the renal medulla reaching maximal values 5 hrs after toxin ingestion.

Changes in nephrosonograms of a pregnant cat and four fetuses were also described following accidental intoxication with ethylene glycol (Adams *et al.* 1991). A hyperechoic appearance of both renal medulla and cortex was recorded in the mother while in all fetuses only the cortex had enhanced echogenicity.

Echonosonographic demonstration of pathological changes in the renal morphology and structure induced by nephrotoxic substances and evaluation of their character, size, distribution and intensity is very simple. Ultrasonography is, namely, a quick, simple and harmless method of examination that can often be repeated enabling subsequent follow up of the disease evolution. Moreover, the possibility of precisely locating the pathological process has great value for tissue sampling in ultrasound guided biopsy.

MATERIAL AND METHODS

The experiments were carried out on 12 male, mongrel dogs, aged about 12 months and of 7 - 20 kg body mass. Acute renal failure was induced by SC application of 40 mg/kg of gentamicin (Gentavan 5 %, "Zdravlje", Leskovac lic. Vana, Austria) every 12 hrs (80 mg/kg/24hrs) during a seven day period. This represented a 20 times higher dose than therapeutically (2 mg/kg/12hrs) recommended.

Nephrosonography was performed on days 0, 3, 5, 7 and 9 of the experiment using "ALOKA 500" in B mode and a 5 MHz convex transducer. Prior to examination, hair was removed in the epimesogastric region and contact gel was applied. The kidneys were examined in both saggital and transversal scans and

their width, length and surface area were measured. Also, the diameter of the renal parenchyma and diameter of the pyelocalix were then estimated. At the end, changes in the echostructure of the renal parenchyma were analyzed.

Blood sera and urine samples were collected on days 0, 3, 5, 7 and 9 of the experiment and creatinine concentration was estimated by kinetic-colorimetric assay without deproteinization. Urine samples were collected with closed catheter collection systems. Every 24 hrs the plastic bags were emptied and the amount of urine was measured.

The intensity of glomerular filtration and degree of renal function loss were estimated on days 0, 3, 5, 7 and 9 by endogenous creatinine clearance (Ccr):

Ccr= (VU x Ucr) / (t x Scr x BW)

Ccr - creatinine clearance (ml/min/kg)

VU - volume of urine (ml)

Ucr - urine creatinine concentration (mg/dl)

t - time (min)

Scr - serum creatinine concentration (mg/dl)

bm - body mass (kg)

Statistical analyses were performed by calculating mean values and standard deviations. The significance of the obtained differences was estimated by Student's t - test.

RESULTS

Induction of ARF was documented by changes in the endogenous creatinine clearance and data are presented in table 1.

Table 1: Endogenous creatinine clearance in dogs with gentamicin induced ARF (X ± SD)

	day 0	day 3	day 5	day 7	day 9
Ccr	3.22 ± 0.85	1.23± 0.38	0.80± 0.39	0.17± 0.07	0.04± 0.01

Physiological range: Ccr - 2.4 - 5.0 ml/kg/min

Creatinine clearance values were below the physiological range as early as on day 3 and decreased till the end of the experiment. Differences between mean values were statistically significant between all groups (data not shown here).

The results of the kidney morphometric analyses (length, width, surface area, renal parenchyma and pyelocalix diameter) are presented in table 2.

Table 2: Morphology of the kidneys in dogs with gentamicin induced ARF

	day 0	day 3	day 5	day 7	day 9
Length (cm)	6.2±1.50	6.3±1.52	6,2±1.43	6.4±1.52	6.3±1.47
Width (cm)	3.1±0.78	3.1±0.72	3.1±0.69	3.1±0.78	3.1±0.69
Surface area (cm^2)	12.4±2.92	12.8±2.95	12.7±2.54	13.1±2.97	13.3±3.14
Renal parenchyma diameter (cm)	1.2±0.31	1.2±0.27	1.2±0.28	1.1±0.30	1.1±0.27
Pyelocalix diameter (cm)	1.1±0.31	1.1±0.29	1.1±0.27	1.2±0.33	1.1±0.29

Analyses of the presented data did not reveal the presence of statistically significant differences in the parameters measured during the experiment in comparison to day 0.

On the dogs nephrosonograms on day 0 all segments were clearly visible. The renal capsule that surrounds the kidneys was represented on ultrasonograms as a clear, very thin hyperechoic line. The structure of the renal cortex was homogenous and slightly hypoechoic in comparison to liver tissue without focal alterations. However, the renal medulla was hypoechoic in all experimental animals. The corticomedullar junction was clearly visible in all animals while the central part of the organ (pyelon) was represented as a hyperechoic area (Figure 1.)

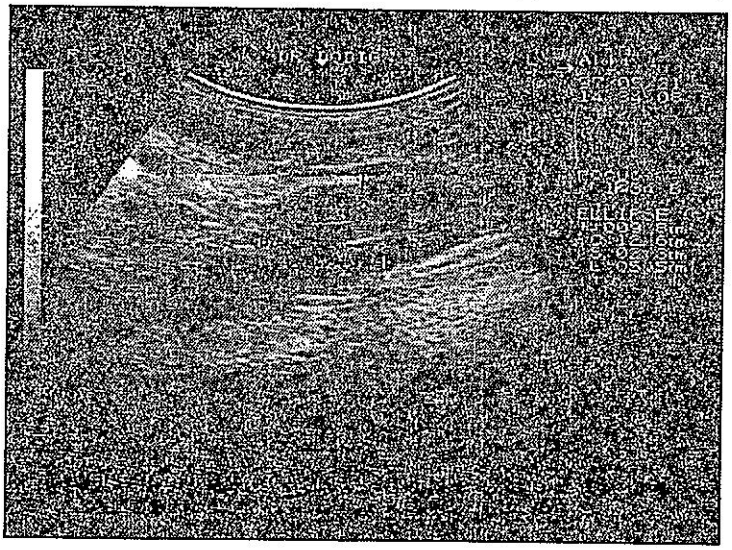

Figure 1. Nephrosonogram of a dog before ARF induction - hypoechoic cortex and clearly visible corticomedullar junction.

The results of the renal sonographic examinations during the experiment (days 3, 5, 7 and 9) were compared with findings observed on day 0 and differences in the echoic intensity and corticomedullar junction differentiation were judged on a 1-3 scale.

After three days of applying toxic gentamicin doses were not able to demonstrate structural renal changes only in one dog and this echosonogram had the same characteristics as in all animals on day 0 (Figure 1).

Initial changes in the renal parenchyma echostructure were characterized by a hyperechoic cortex (grade 1) and weaker corticomedullar junction differentiation (grade 1). The same changes were demonstrated in 9 dogs on day 3 while on day 5. all animals exibited similar patterns (Figure 2). On day 7, only three animals had kidneys with initial changes while at the end of the experiment (9 day) initial changes were not present any more.

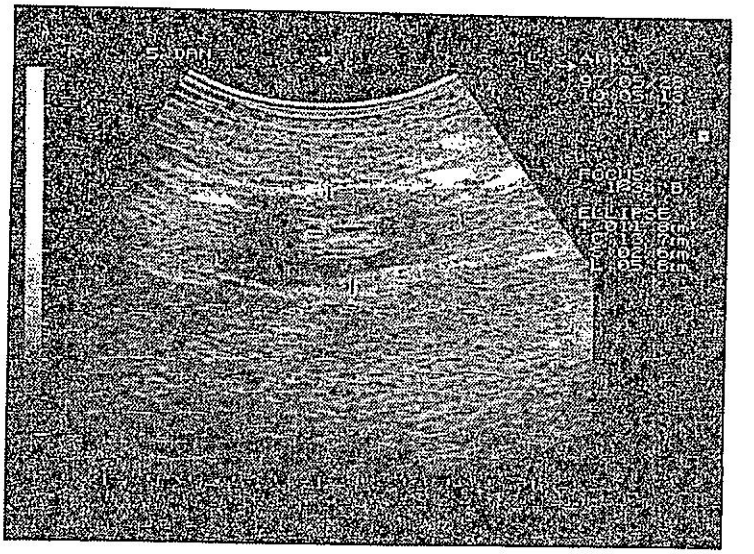

Figure 2. Nephrosonogram of a dog after gentamicin induced ARF - hyperechoic cortex (grade 1) and weaker differentiation of the corticomedullar junction (grade 1).

More pronounced changes characterized by greater echogenicity of the renal cortex (grade 2) and indistinct corticomedullar junction differentiation were demonstrable in two dogs as early as on day 3 of the experiment, while on day 5 they were not present. On the day 7, changes judged as grade 2 were demonstrated in 5 dogs and on day 9 only in one experimental animal.(Figure 3).

Massive changes in the renal parenchyma were visible as a greatly hyperechoic cortex (grade 3) while the corticomedullar junction could not be observed. This intensity of changes was present in four dogs on day 7, and in 11 animals on day 9 (Figure 4).

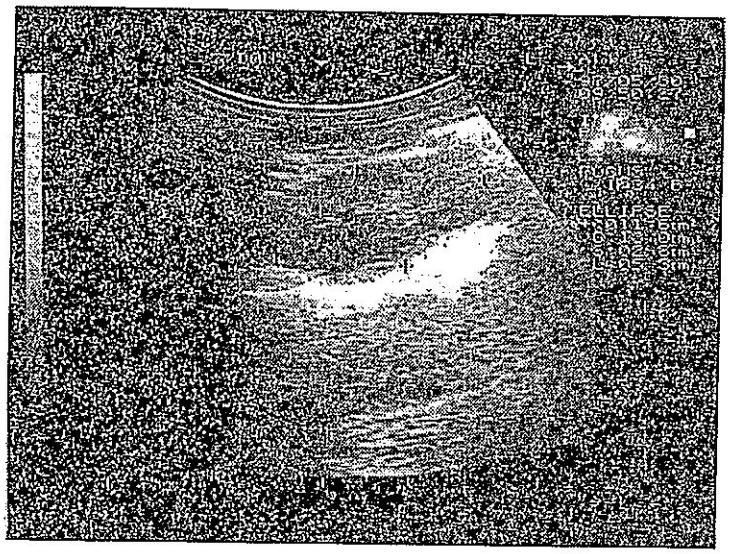

Figure 3. Nephrosonogram of a dog after gentamicin induced ARF - hyperechoic cortex (grade 2) and indistinct differentiation of the corticomedullar junction (grade 2).

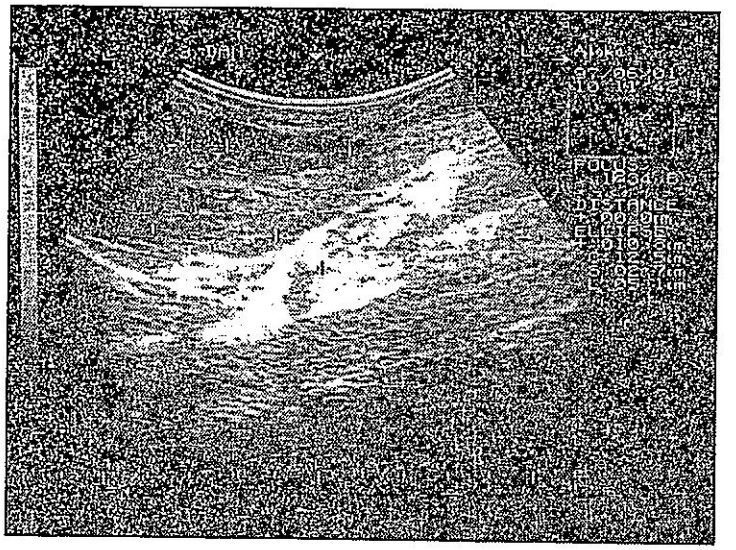

Figure 4. Nephrosonogram of a dog after gentamicin induced ARF - greatly hyperechoic cortex (grade 3) and corticomedullar junction loss (grade 3).

DISCUSSION

Sonography is a rapid, simple and harmless diagnostic procedure providing important information about the anatomy, topography, size and morphology of kidneys and other organs. The main advantage of sonography in ARF, among other diagnostic procedures in routine practice, is the possibility to discover the presence, character, distribution and localization of lesions in the renal structure.

However, these data are not always specific for particular types of ARF. Angeli and Macarini (1987) performed histopathological examinations of the kidneys in 70 patients with diffuse hyperechoic changes in the renal parenchyma. Diffuse hyperechogenicity of the renal cortex with a distinct corticomedullar junction was detected in cases of chronic glomerulonephritis, nephroangiosclerosis and amyloidosis, while diffuse hyperechogenicity with an uncertain corticomedullar junction was demonstrated in uric interstitial nephropathy. Page *et al.* (1994) performed a sonographic study of 55 patients with suspected renal parenchyma disease. The authors demonstrated enhanced hyperechogenicity in numerous disorders i.e. glomerular sclerosis, interstitial infiltration, interstitial fibrosis, glomerulonephritis and tubulo-interstitial nephritis. They also underlined the hyperechoic findings in patients with marked tubular atrophy. Since gentamicin applied in high doses leads to dystrophic and necrotic alterations, mainly in the epithelial cells of the proximal tubules (Macanović *et al.* 1999), our demonstration of hyperechoic changes in the renal cortex is in agreement with the results of Page *et al.* (1994).

Numerous authors have performed sonographic examinations of the kidneys following experimentally induced nephrotoxicity. Rosenfield *et al.* (1985) induced acute tubular necrosis in rats after single SC application of potassium bichromate (15 mg/kg bm) and demonstrated enhanced echogenicity of the cortex 4 days later. On the contrary, the same authors did not observe changes in echo properties when acute tubular necrosis was induced by experimental ischaemia. On the basis of these results, the authors suggested that nephrosonography can help in estimating the etiology of renal diseases. In 1989, Adams *et al.* observed hyperechoic changes in the renal cortex of dogs following PO application of 95% ethylene glycol (10ml/kg bm) 4 days after intoxication. Besides cortical hyperechogenicity, the renal medulla was also hyperechoic while the corticomedullar junction was slightly hypoechoic. The same findings were observed in the kidneys of a pregnant cat and four fetuses after poisoning with ethylene glycol (Adams *et al.* 1991). In our experiment enhanced echogenicity of the renal cortex was demonstrated in 11 dogs from day 3 of the experiment and had a tendency of intensifying in the majority of the animals. However, we were not able to demonstrate changes in the echoic properties of the renal medulla, while the corticomedullar junction was progressively less visible and therefore more difficult to differentiate. Hanquinet *et al.* (1995) also demonstrated enhanced echogenicity of the renal cortex in three children with developed Fanconis syndrome following ifosfamide application but in these patients the corticomedullar junction was clearly visible.

According to Huntington *et al.* (1991) ultrasonography is one of the diagnostic procedures of choice whenever acute renal failure is suspected. These authors stated that the nephrosonogram may have normal appearance at the beginning of the pathological process and that observed changes are not specific

for the particular disease. Therefore they concluded that histopathological evaluation of biopsy samples is the best method for exact diagnosis.

In our study the decrease of creatinine clearance below physiological values (day 3) correlated with the development of the initial echosonographic changes in the majority of the experimental animals. These initial changes were manifested by diffuse hyperechogenicity in 11 animals and we judged them as grade 1. At the same time, the corticomedullar junction was less clearly visible (grade 1). In the group of 12 dogs only one animal exhibited no any changes in echogenicity of the cortex while in two dogs the cortex was even more hyperechoic (grade 2) with indistinct corticomedullar junction (grade 2). On day 5 of the experiment the intensity of sonographic changes in the cortex was constant (grade 1) and the corticomedullar junction was less clearly visible (grade 1). On day 7 of the experiment 5 dogs had a hyperechoic cortex (grade 2) with indistinct corticomedullar junction. Marked hyperechogenicity of the cortex (grade 3) and absence of the corticomedullar junction (grade 3) was documented in 4 dogs. In three dogs sonographic changes were at the initial level (grade 1). Diversity in our findings could be explained by the different individual reactivity of animals to factors inducing ARF. On day 9, two days after the last gentamicin injection, 11 dogs had maximal hyperechogenicity of the cortex (grade 3) while the corticomedullar junction was absent (grade 3). These findings correlated with changes in renal function as judged by the dramatic drop in creatinine clearance value, indicating serious ARF.

Despite the fact that during *in vivo* experiments, like this one, all individuals do not react to different harmful agents in the same way, it is our opinion that nephrosonography can help in the detection of acute renal parenchyma changes, resulting in ARF.

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ULTRAZVUČNA ISPITIVANJA PROMENA U PARENHIMU BUBREGA PASA SA AKUTNOM RENALNOM INSUFICIJENCIJOM IZAZVANOM GENTAMICINOM

MACANOVIĆ MIRJANA, JANKOVIĆ - ZAGORČIĆ ANICA, LIŠANIN LJ, DODIĆ M i SPASIĆ V

SADRŽAJ

U ovom radu su vršena ispitivanja mogućnosti ultrazvučne dijagnostike promena u parenhimu bubrega pasa sa akutnom renalnom insuficijencijom eksperimentalno izazvanom s.c. aplikacijom visokih doza gentamicina (40 mg/kg), dva puta dnevno, tokom sedam dana. Postojanje akutne bubrežne slabosti je dokazano kod svih oglednih životinja već 3. dana eksperimenta sa tendencijom progresije bolesti do kraja ogleda. Analizom rezultata morfometrijskih ispitivanja bubrega nisu utvrđene promene u veličini ovih organa tokom eksperimenta u odnosu na vrednosti izmerene 0. dana. Prisustvo inicijalnih promena u ehostrukturi bubrežnog parenhima u smislu povećanja ehogenosti korteksa i slabije diferencijacije kortiko-medularnog prelaza, kod većine pasa (11) ustanovljeno je 3. dana ogleda, što se podudara sa razvojem ARI. Tokom eksperimenta promene u bubrežnoj strukturi su se intenzivirale, tako da je 9. dana kod većine pasa ustanovljena izrazita hiperehogenost korteksa sa potpunim gubitkom kortiko-medularne granice. Ovi naši nalazi potvrđuju da postoji korelacija između rezultata dobijenih ehosonografskim ispitivanjima bubrega pasa i razvoja ARI.