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SERUM AND URINE LABORATORY ANALYSES IN DOGS WITH GENTAMICIN INDUCED ACUTE RENAL FAILURE

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The use of aminoglucoside antibiotics can be a potential risk for renal parenchyma damage and consequently acute renal failure (ARF). ARF is a syndrome that develops from progressive nephron damage resulting in the loss of renal function. Numerous experimental models have been used to study acute renal failure mainly describing histo-pathological changes in the structure of this organ. Our investigations were conducted in order to evaluate the functional capacity of kidneys in dogs with ARF induced by application of gentamicin in high doses of 80 mg/kg/24^{h}, during 7 days. For that purpose, physico-chemical properties of the urine, concentrations of relevant parameters in the sera and urine and endogenous creatinine clearance were estimated. Our results indicate that gentamicin, in doses 20 times higher than therapeutic ones, causes progressive ARF starting from the 3rd day of application.

Key words: dog, gentamicin, acute renal failure

INTRODUCTION

Renal failure is a syndrome that emerges from progressive nephron damage, leading to the loss of renal function. Depending on the nature, intensity and duration of the insult, renal failure can be acute or chronic. Acute renal failure (ARF) is characterized by a sudden and persistent decrease in glomerular filtration followed by azotemia. Besides pre-renal and post-renal failure this term includes acute renal failure due to various diseases of the renal parenchyma (inflammation, dystrophy in glomerules and tubules, acute tubular necrosis etc.). The majority of causes that induce this type of ARF have common pathogenic factors, mainly intense circulation disturbance and injury due to nephrotoxic substances (Grauer and Lane,1995).

It is well known that the use of some therapeutic substances in high doses bears a potential risk of renal parenchyma damage, often resulting in acute renal failure. There is also evidence that epithelial cells in renal tubules might be altered

following application of toxic doses of oxytetracycline (Stevenson, 1980), kanamycin (Okada et al.,1991) and gentamicin (Spangler, 1980, Riviere et al., 1983, Nakakuki et al., 1997). Histopathological changes in the form of massive dystrophy and necrosis of epithelial cells in proximal renal tubules were described following accidental poisoning with ethylen-glycol, picric acid or arsenate (Tsukamoto et al.,1983). It is also documented that snake poison can induce very similar changes (Puig et al., 1995, Chen et al., 1997).

Gentamicin, applied in high doses, results in dystrophy of tubulocytes (Milosavljević, 1988) followed by acute renal failure. The prefered site for gentamicin action is the proximal segment of renal tubules localized in the renal cortex where intensive water reabsorption as well as reabsorption of substances from primary urine takes place. Gentamicin can bind to the phospholipid membrane, disturbing transport mechanisms and increasing Ca^{++} influx into cells that results in serious membrane damage. Due to the fact that most intensive Na^{+} reabsorption takes place in this tubular segment, the described changes in tubulocytes result in significantly decreased reabsorption. The increase in Na^{+} concentration in the tubular lumen, near macula densa, lowers glomerular filtration and therefore water reabsorption from tubules will be increased. As a consequence, gentamicin will accumulate in tubulocytes in high concentrations. Furthermore, apart from primary nephrotoxic effects on tubular cells, gentamicin has effects on glomerules as well. Loss of the surface capillary layer and decrease in the number and size of fenestrae in capillary endothelia manifest this effect. For these reasons glomerular filtration coefficient rate is also decreased. In addition, gentamicin and other cationic drugs can interact with negative charges on the surface of endothelial cells of blood capillaries in glomeruli resulting in the increased permeability for proteins with a negative charge (Grauer and Lane, 1995). Decreased glomerular filtration rate also decreases the elimination of protein catabolism products (creatinine and urea) and their concentration in the blood increases which is known as azotemia. At the end, alterations of the tubulocytes in the proximal part of the tubular system, result in altered reabsorption of numerous substances from the primary urine so their presence can be detected in the final urine (Stevenson, 1980, Riviere et al., 1983, Tsukamoto et al.,1983, Nakakuki et al., 1997).

MATERIALS AND METHODS

This experiment was conducted on 12 male, mongrel dogs, of body weight from 7 - 20 kg at the age of 12 months. Acute renal failure was induced with SC application of gentamicin every 12 hrs (80 mg/kg/24^{h}) during a seven day period. The applied doses were 20 times higher than the therapeutic dose (4 mg/kg/24^{h}).

Blood sera samples were taken on days 0,3,5,7 and 9 of the experiment and glucose concentration (GOD-PAP method, enzymatic-colorimetric assay without deproteinization), creatinine concentration (kinetic-colorimetric assay without deproteinization), urea concentration (enzymatic-colorimetric assay) and Na^{+} and K^{+} concentrations (ion - exchange method) were estimated. All analyses were performed in fresh sera samples without freezing.

Urine samples were collected with closed catheter collection systems. Every 24 hrs plastic bags were emptied and the amount of urine was measured. In the fresh urine samples (10 ml) collected in the morning on days 1,4,6,8 and 10 representing urine collected during the previous 24 hrs, the following parameters were estimated: pH, specific gravity, glucose concentration (GOD-PAP method, enzymatic-colorimetric assay without deproteinization), protein concentration (colorimetric end-point method), creatinine concentration (kinetic-colorimetric assay without deproteinization), urea concentration (enzymatic-colorimetric assay) and Na^+ and K^+ concentrations (ion - exchange method). Microscopic evaluation of the urine sediment was performed following 24 hrs sedimentation in a conical vessel. The intensity of glomerular filtration was estimated by endogenous creatinine clearance (Ccr):

$$Ccr = (VU \times Ucr) / (t \times Scr \times BW)$$

Ccr - creatinine clearance (ml/min/kg)
VU - urine volume (ml)
Ucr - urine creatinine concentration (mg/dl)
t - time (min)
Scr - serum creatinine concentration (mg/dl)
BW - body weight (kg)

Statistical analyses were performed by calculating mean values and standard deviations. The significance of the obtained differences was estimated by Students t - test.

RESULTS

The concentrations of glucose, urea and creatinine found in the sera of dogs receiving high doses of gentamicin are presented in Table 1.

Table 1. Glucose, urea and creatinine concentration in the sera of dogs with gentamicin induced ARF ($\bar{X} \pm SD$)

	day 0	day 3	day 5	day 7	day 9
Glucose	3.91 ± 0.17	3.86 ± 0.47	4.78 ± 0.65	4.69 ± 0.41	5.28 ± 0.55
Urea	2.96 ± 0.26	4.71 ± 0.79	7.91 ± 0.66	10.23 ± 2.77	35.95 ± 8.60
Creatinine	79.9 ± 10.48	81.41 ± 6.48	93.83 ± 15.03	117.83 ±18.52	427.08±113,25

Physiological range: Glucose 3.61 - 6.55 mmol/l
Urea 1.67 - 3.33 mmol/l
Creatinine 44.2 - 132.6 μmol/l

Serum urea concentration exceeded physiological values from the 3rd day of the experiment and dogs developed uremia, while creatinine values remained normal except at the end of the experiment (day 9). Glucose concentration in the

sera of the experimental dogs was within physiological limits during the experiment. The same pattern was observed for the Na^+ concentration, while K^+ concentration was slightly decreased below physiological values from day 5 with a tendency of further decreasing (data not shown here).

Table 1. Statistical significance of differences in glucose, urea and creatinine concentrations in the sera of dogs with gentamicin induced ARF (t test)

	Day	3.	5.	7.	9.
Glucose	0.	NS	$p < 0,01$	$p < 0,001$	$p < 0,001$
	3.		$p < 0,01$	$p < 0,001$	$p < 0,001$
	5.			NS	$p < 0,001$
	7.				$p < 0,001$
Urea	0.	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$
	3.		$p < 0,001$	$p < 0,001$	$p < 0,001$
	5.			$p < 0,01$	$p < 0,001$
	7.				$p < 0,001$
Creatinine	0.	NS	$p < 0,01$	$p < 0,001$	$p < 0,001$
	3.		$p < 0,01$	$p < 0,001$	$p < 0,001$
	5.			$p < 0,001$	$p < 0,001$
	7.				$p < 0,001$

NS - non significant

Although glucose concentration was within physiological limits during the experiment, there was a tendency of increase and in most cases differences between groups were significant. Differences in urea and creatinine concentration were significant with only one exception.

Results of the physico-chemical analyses of the urine samples from the experimental dogs are presented in Table 2.

Table 2. Specific gravity (SG), pH of urine and diuresis in dogs with gentamicin induced ARF ($X \pm SD$)

	day 0	day 3	day 5	day 7	day 9
SG	1.021 ± 0.005	1.018 ± 0.005	1.016 ± 0.005	1.016 ± 0.004	1.016 ± 0.03
pH	6.42 ± 0.29	6.33 ± 0.32	6.29 ± 0.25	6.04 ± 0.33	5.96 ± 0.33
Diuresis	33.72 ± 6.73	30.58 ± 10.45	31.11 ± 13.26	33.66 ±10.77	29.5 ±10.14

Physiological range: SG 1,015 - 1,045
pH < 7
Diuresis 17 - 45 ml/kg/24^h

It is evident that neither specific gravity and pH of the urine samples nor the diuresis value in experimental dogs exceeded physiological values during the experiment.

Table 2. Statistical significance of differences in SG, pH of urine and diuresis in dogs with gentamicin induced ARF (t test)

	Day	3.	5.	7.	9.
SG	0.	NS	$p < 0,05$	NS	$p < 0,01$
	3.		NS	NS	NS
	5.			NS	NS
	7.				NS
pH	0.	NS	NS	$p < 0,05$	$p < 0,01$
	3.		NS	NS	$p < 0,05$
	5.			$p < 0,05$	$p < 0,05$
	7.				NS
Diuresis	0.	NS	NS	NS	NS
	3.		NS	NS	NS
	5.			NS	NS
	7.				NS

NS - non significant

Despite the fact that the examined physico-chemical variables were within physiological limits we were able to demonstrate significant differences between some groups except for the diuresis values.

Values for the examined biochemical parameters in urine and the protein/creatinine ratio (P/C) are presented in Table 3.

From day 3 treated dogs developed glucosuria that increased to day 7 (end of gentamicin application) and decreased on day 9. Proteinuria also developed during gentamicin treatment and exceeded physiological values on days 5, 7 and 9. We were also able to demonstrate a consistent drop in amount of protein in the urine on day 9. The amount of urea and creatinine in the urine of dogs exibited a significant drop (below the physiological range) from day 3. followed by a the continuous decrease. The ratio between protein and creatinine concentration (P/C) was elevated during the experiment and exceeded physiological values on day 5. The amounts of Na^+ and K^+ in the urine of dogs treated with gentamicin were within the physiological range but their fractionated excretion (FE) was constantly rising and above the physiological limit from day 7 (data not shown here).

Table 3. Glucose concentration and amount of protein, urea and creatinine excreted per day together with P/C ratio in the urine of dogs with gentamicin induced ARF (X ± SD)

	day 0	day 3	day 5	day 7	day 9
Glucose	0	1.18 ± 1.16	2.96 ± 0.92	3.44 ± 1.51	1.62 ± 0.83
Proteins	1.32 ± 26.36	7.34 ± 8.59	41.03 ± 32.62	43.64 ± 17.41	33.07 ± 12.10
Urea	168.02 ± 26.36	60.45 ± 21.36	50.24 ± 33.51	21.85 ± 5.03	12.73 ± 4.40
Creatinine	41.19 ± 9.25	16.77 ± 3.89	12.33 ± 6.29	3.29 ± 1.25	2.47 ± 0.94
P/C	0.03 ± 0.01	0.46 ± 0.54	3.02 ± 1.90	14.05 ± 6.14	14.51 ± 8.47

Physiological range: Glucose 0 mmol/l
Proteins 30 mg/kg/24^h
Urea - 140 230 mg/kg/24h
Creatinine 30 - 80 mg/kg/24^h
P / C >1 = significant proteinuria

Table 3. Statistical significance of differences in glucose concentration, amount of protein, urea and creatinine and protein / creatinine (P/C) ratio in the urine of dogs with gentamicin induced ARF (t test)

	Day	3.	5.	7.	9.
Glucose	0.				
	3.		NS	NS	NS
	5.			NS	p < 0,05
	7.				p < 0,001
Proteins	0.	p < 0,01	p < 0,001	p < 0,001	p < 0,001
	3.		p < 0,001	p < 0,001	p < 0,001
	5.			NS	NS
	7.				p < 0,05
Urea	0.	p < 0,001	p < 0,001	p < 0,001	p < 0,001
	3.		NS	p < 0,001	p < 0,001
	5.			p < 0,01	p < 0,01
	7.				p < 0,001
Creatinine	0.	p < 0,001	p < 0,001	p < 0,001	p < 0,001
	3.		p < 0,01	p < 0,001	p < 0,001
	5.			p < 0,001	p < 0,001
	7.				NS
P/C	0.	p < 0,05	p < 0,001	p < 0,001	p < 0,001
	3.		p < 0,001	p < 0,001	p < 0,001
	5.			p < 0,001	p < 0,001
	7.				NS

NS - non significant

Table 4. contains data for the endogenous creatinine clearance in dogs treated with high doses of gentamicin.

Table 4. Endogenous creatinine clearance in dogs with gentamicin induced ARE ($\bar{X} \pm SD$)

	day 0	day 3	day 5	day 7	day 9
Ccr	3.222 ± 0.850	1.231 ± 0.376	0.803 ± 0.393	0.173 ± 0.072	0.036 ± 0.015

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

Creatinine clearance values were within physiological limits only at the very beginning of the experiment. Starting from day 3 they decreased till the end of the experiment.

Table 4. Statistical significance of differences in creatinine clearance in dogs with gentamicin induced ARF (t test)

	Day	3.	5.	7.	9.
Creatinine clearance	0.	$p < 0,001$	$p < 0,001$	$p < 0,001$	$p < 0,001$
	3.		$p < 0,01$	$p < 0,001$	$p < 0,001$
	5.			$p < 0,001$	$p < 0,001$
	7.				$p < 0,001$

DISCUSSION

There is a voluminous scientific literature concerning pathological changes in renal parenchyma, sera and urine of experimental animals following application of nephrotoxic substances. Pathological changes in the kidneys of dogs were induced with gentamicin application in doses of 40 mg/kg/24^h for 15 days (Black, 1964) but also with 10 mg/kg/24^h for 10 days (Spangler, 1980). Morteover, Riviere et al. (1983) reported that even one dose of gentamicin (15 mg/kg body weight) can lead to pathological alterations in renal tissue in dogs. Experimental nephrotoxicosis was induced in rats with 45 mg/kg/24^h of gentamicin during 7 days (Milosavljević, 1988). These different data led us to use gentamicin in high doses in order to induce ARF efficiently. In our study the dogs received 40 mg/kg of gentamicin twice a day (80 mg/kg/24^h) during a 7 day period.

One of the most frequent findings in ARF is azotemia - elevation of the urea and creatinine concentration in blood sera. Furthermore, because ARF can be induced by prerenal, renal and postrenal factors, it is necessary to conduct laboratory examination of the urine in order to achieve correct interpretation of the results. Patients with renal azotemia that develops after gentamicin application in high doses have isostenuric urine in nearly all cases. The amount of daily urine

can vary and patients can be anuric, oliguric or even polyuric (Less et al., 1994). In our experiment azotemia appeared very early and the amount of urea was significantly increased from day 3 and creatinine from day 5. Azotemia had a tendency to increase and reached the maximum value on the last day of the experiment (9).

Specific gravity and urine pH were also estimated in order to evaluate complete renal function in dogs with gentamicin induced ARF. Mean values obtained for the specific gravity of urine remained within the physiological range during the whole experiment. According to that and since azotemia appeared early in the experimental animals we were able to conclude that gentamicin induced ARF is a consequence of pathological changes in the renal parenchyma (acute tubular necrosis). During the experiment, mean pH values were within the physiological range as well.

Our data concerning diuresis in gentamicin treated dogs do not display significant differences in daily urine production during the experiment. A very important difference between ARF induced with aminoglucoside antibiotics (gentamicin) from ARF resulting from ischaemia or other nephrotoxic substances is the absence of oliguria. Oliguria was thought to be one of the dominant ARF symptoms but Grauer and Lane (1995) stated that aminoglucoside antibiotics induce nonoliguric renal failure. In cases of intoxication with substances such as gentamicin, daily urine production is not reduced and can even be slightly elevated.

Laboratory examination of the urine samples revealed the presence of glucosuria from the third day of the experiment. Glucosuria is mostly connected with proximal renal tubule dysfunction and it is documented that aminoglucoside antibiotics induce glucosuria, proteinuria and cilindruria before azotemia occurs (Scott et al., 1986, Less et al., 1994). Estimation of proteinuria and its significance is one of the basic moments in patients suspected for ARF, bearing in mind that proteinuria should be interpreted according to the specific gravity values (Meyer and Harvey, 1998). The best way to determine the significance of proteinuria is to measure the amount of protein excreted in 24 hrs (the upper limit is 30 mg/kg/24^h) as well as the amount creatinine (the physiological range is 30 - 80 mg/kg/24^h) and to calculate protein/creatinine (P/C) ratio. If this value is bigger than 1, that means the amount of protein in urine is more than 30 mg/kg/24^h (Weber, 1988, Waller et al., 1989, Lulich and Osborne, 1990, Bernard and Lauwerys, 1991). Analysis of our results reveals a marked increase of protein content in 24 hrs isostenuric urine between days 3 and 5 indicating early nephrotoxicity. On day 5, the P/C ratio was 3.017 representing significant proteinuria. On the day 7 the P/C ratio rose further and remained high till the end of the experiment (day 9). The amount of creatinine in the urine of dogs with gentamicin induced ARF was below physiological values on day 3 and constantly decreased further. The ratio between urine creatinine and serum creatinine concentrations is another relevant parameter used to distinguish prerenal azotemia from renal failure due to renal tubular necrosis. Markedly expressed renal azotemia due to tubular necrosis, followed by oliguria or anuria can reduce this ratio to 20 : 1 or even 5 : 1. (Less et al., 1994). On day 0 in our experiment the urine creatinine / serum creatinine ratio was 139 : 1. On day 7 it was 7.37 : 1 and on the day 9 1.86 : 1. In animals with nonaltered

kidney function urea is normally excreted through the urine. Patients with ARF have elevated urea concentrations in serum and an equivalent decrease in the urine (Less et al., 1994). In our study a sudden fall in urinary urea concentration appeared 3 days after gentamicin application with a tendency of further decline till the end. Decreased creatinine clearance is a serious sign of disturbed glomerular filtration that appears in numerous renal diseases and also after application of some drugs and nephrotoxic substances (Less et al., 1994, Nakakuki et al. 1997). This is in agreement with our results and we noticed a fall in creatinine clearance on day 3 of the experiment.

Moreover, analyses of the urine sediment in dogs with induced ARF confirmed the presence of leukocytes, bacteria and erythrocytes starting from day 3 after the first gentamicin application. From day 5 till the end of the experiment polygonal epithelial cells, mucus and hyaline cylinders appeared in the urine sediment indicating pathological changes in glomerules and tubules characteristic for acute nephrosis. (Meyer and Harvey, 1998).

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LABORATORIJSKE ANALIZE KRVNOG SERUMA I URINA PASA SA AKUTNOM RENALNOM INSUFICIJENCIJOM IZAZVANOM GENTAMICINOM

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SADRŽAJ

Pod određenim uslovima upotreba aminoglukozidnih antibiotika mo'e predstavljati potencijalni rizik za nastanak oštećenja u parenhimu bubrega i posledične akutne renalne insuficijencije (ARI). ARI je sindrom koji nastaje usled progresivnog propadanja nefrona, što dovodi do gubitka sposobnosti bubrega da obavljaju svoju funkciju. Za proučavanje akutne renalne insuficijencije korišćen je veći broj različitih eksperimentalnih modela pri čemu su uglavnom opisivane histopatološke promene u ovom organu. Naša ispitivanja su imala za cilj da se utvrdi funkcionalna sposobnost bubrega pasa u toku akutne renalne insuficijencije, izazvane visokim dozama gentamicina (80 mg/kg/24^{h}), aplikovanim tokom 7 dana. U tom cilju su određivane fizičko-hemijske karakteristike mokraće, koncentracija relevantnih sastojaka u serumu i urinu kao i klirens endogenog kreatininina. Dobijeni rezultati ukazuju da se dozama gentamicina, dvadeset puta većim od terapijskih, već posle tri dana aplikacije izaziva ARI koja ima progresivan tok do kraja ogleda.