Original Article

Gentamicin-Induced Nephrotoxicity in Adult Sheep

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ABSTRACT

Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life-threatening gram-negative infections. Its use is, however, restricted because of several problems such as development of ototoxicity and nephrotoxicity. The GM-nephrotoxicity involves renal free radical generation, reduction in antioxidant defense mechanisms, acute tubular necrosis and glomerular congestion, resulting in diminished glomerular filtration rate and renal dysfunction. The objective of this study was to monitor the nephrotoxic effects of intravenous injection of gentamicin in adult male sheep, and to identify the earliest signs of toxicity and the extent of clinical and biochemical changes. To this aim 12 clinically healthy, yearling male Iranian fat-tailed sheep injected with gentamicin sulfate at a dose rate of 80 mg/kg per day. Regularly blood samples were collected weekly before and after induction of nephrotoxicosis, throughout the study. GM-induced nephrotoxicity was characterized by an increase in serum creatinine and urea, electrolyte imbalances and incidences of albuminuria and renal dysfunction. Elevation in respiratory and heart rates were significantly increased one week after treatment (p<0.05).There were noticeable increased in thirst and water consumption, lethargy and loss of appetite in treated sheep. There were significant correlations between serum creatinine with potassium (P=0.004, r=0.759), sodium (P=0.017, r=0.501) and urea (P=0.021, r=0.617)levels.Additionally, there were significant negative correlations between serum total protein and albumin with creatinine (P=0.023, r=-0.484) and urea (P=0.036, r=-0.381). At necropsy kidneys were pale and swollen and wet on the cut surface and edema, especially of perirenal tissues and ureters were apparent. These findings werecorroborated the previous reports in other species.
Key words: Experimental Chronic Renal Failure, Nephrectomy, Sheep, Uremia

INTRODUCTION

Aminoglycosides are employed in the treatment of bacterial infections. Gentamicin (GM), an aminoglycoside class of bactericidal antibiotic, was first isolated from *Micromonosporapurpurea*, gram-positive bacteria widely present in water and soil, is effective against gram-negative bacterial infections and is particularly valuable in severe sepsis[3,16]. Gentamicin inhibits bacterial protein synthesis by binding to 30S ribosomal subunit and preventing the formation of initiation complex with mRNA and also inducing the event of misreading of the mRNA message, leading to the production of defective proteins that afford bactericidal action [3]. Gentamicin is not intended to be used orally since it is not absorbed to any appreciable extent from the small intestine. It is administered intravenously, intramuscularly or topically to treat bacterial infections. It is completely eliminated as unchanged form by glomerular filtration via urine [21].However, nephrotoxicity is a major side effect of gentamicin that restricts its clinical application [7,15], so that 30% of patients treated with GM for more than 7 d show some signs of nephrotoxicity[18].Renal toxicity of gentamicin is linked to its selective accumulation in the renal cortex especiallyin proximal tubule cells[12,16]. Morphologic lesions of proximal tubules have been documented in optic microscopy [16]. Renal injury progresses slowly and its various stages can be closely followed [7].Various experimental models including rat [4,8,13,17] , dog [20], lamb[7] and horse [12] are employed to induce nephropathy in order to identify the potential pharmacological targets for nephropathy. It has also been administered to ewes to produce aminoglycoside nephrotoxicosis (AGNT) as a model of acute renal failure [10, 11]. The objective of this study was to monitor the nephrotoxic effects of gentamicin in adult male sheep, and to identify the earliest signs of toxicity and the extent of clinical and biochemical changes.

MATERIALS AND METHODS

Experimental animals

Twelve clinically healthy, yearling male Iranian fat-tailed sheep and weighing 45-50 kg were used. All animals were kept indoor in group box during the whole experimental period, under similar conditions and manual feed including alfalfa, barley, treated wheat straw (treated with 5% urea, 2.5% molasses and 2% salt) with free access to waterfor several weeks before the trial. After deworming of sheep with Albendazol 5% (Dieverm®, 15 mg/kg, PO, Damloran Razakpharma, Lorestan, Iran) and Ivermectin 1% (Intermectin®, Interchemie, Holland, 200 µg/kg,SC) animal were prepared for experiment. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care, and Ethics Committee of Faculty of Veterinary Medicine, Islamic Azad University approved the protocol.

Induction of Nephrosis

Gentamicin-induced in animals by intravenous injection of gentamicin sulphate (Gentacin 5%, Nasr,Fariman, Iran) at a dose rate of 80 mg/kg per day.Gentamicin was given daily until the plasma creatinine level of each sheep increased to a minimum of 132 µmol/litre and was then discontinued, as described by Garry et al.[10].

Sampling and laboratory procedures

Regularly blood samples were collected weekly before and after induction of nephrotoxicosis, throughout the study.For biochemical analysis, approximately 5 mL blood samples were collected from jugular vein into plain serum tubes. Sera were analyzed for urea, creatinine, total protein and albumin by an automatic analyzer (Alcyon™ 300, Abbott Lab., Illinois, USA), using commercial kits (Pars Azmoon Co. INC., Karadj, Iran). Also serum potassium and sodium value were quantified by Flame photometric method.

Statistical analysis

All statistical analyzed by Statistical Package for Social Sciences for Windows, version 17.0 (SPSS Inc.).Data normality was tested by Kolmogorov-Smirnov test. Analysis of variance (ANOVA) test was used for comparison of measured factors in various times. All values were expressed as mean and standard deviation (SD), and *P*<0.05 was considered as statistically significant.

RESULT

There were noticeable increased in thirst and water consumption in treated sheep. Lethargy and loss of appetite were recorded after fifth day. Approximately 8 – 10 days after gentamicin injection, food consumption was reduced to one-half to one-third of the previous rate. Respiratory and heart rateswere significantly increased one week after treatment (p<0.05). There is no significant difference in rectal temperature, until animals become recumbent. After sternal and lateral recumbency, body temperature significantly decreased (p<0.05 and p<0.01, respectively). Bottle jaw was seen in one sheep at days 30 due to hypoproteinemia.

The changes in blood constituents are shown in Table 1 and Figure 1. All sheep became uremic. Ten days after treatment began, significant and progressive increase in creatinine levels were occurred (p<0.05). Changes in the mean value of serum urea, potassium, total protein and albumin were not significant at this time. There were significant correlations between serum creatinine with potassium (P=0.004, r=0.759), sodium (P=0.017, r=0.501) and urea (P=0.021, r=0.617)levels. Additionally, there were significant negative correlations between serum total protein and albumin with creatinine (P=0.023, r=-0.484) and urea (P=0.036, r=-0.381). There was no significant correlation between serum sodium levels and any of other biochemical factors.

Animals died on 27 – 33 days after treatment began, due to renal failure. Toxic nephrosis signs were macroscopically apparent at necropsy. So that kidneys were pale and swollen and wet on the cut surface and edema, especially of perirenal tissues and ureters were apparent (Figure 2).There were hemorrhagic and gray necrotic streaks radiate out through the medulla and extend to the cortex (Figure 3).

Table 1: serum biochemical parameters in sheep with experimental renal failure (Mean±SD)

| Time (Days)	0	5	10	15	20	25	30	35	40
Urea(mg/dL)	50.3 ± 4	53.2 ± 6	57.0 ± 8	55.1 ± 9	79.6 ± 7	102.3 ± 4	134.3 ± 8	150.3 ± 4	202.6 ± 7
Creatinin(mg/dL)	0.23 ± 0.05	0.25 ± 0.04	1.69 ± 0.9	3.19 ± 1.2	4.89 ± 2.0	6.61 ± 2.3	7.75 ± 2.2	8.02 ± 1.7	8.14 ± 1.8
Sodium(mmol/dL)	141 ± 6	142 ± 11	140 ± 9	138 ± 18	138 ± 16	137 ± 21	136 ± 13	138 ± 12	139 ± 9
Potassium(mmol/dL)	4.07 ± 0.7	4.14 ± 0.6	4.23 ± 0.8	4.42 ± 0.7	5.65 ± 0.9	6.47 ± 0.5	7.12 ± 1.0	7.65 ± 1.1	8.89 ± 1.6
Protein(g/dL)	66.1 ± 5.3	68.22 ± 7.9	67.48 ± 8.1	66.26 ± 10.2	64.15 ± 10.6	62.81 ± 12.8	61.07 ± 11.3	60.18 ± 13.4	58.68 ± 15.0
Albumin(g/dL)	23.23 ± 4.0	24.84 ± 3.8	23.64 ± 4.7	24.49 ± 4.2	25.20 ± 3.3	24.66 ± 3.6	21.43 ± 4.4	21.73 ± 7.2	19.10 ± 8.4
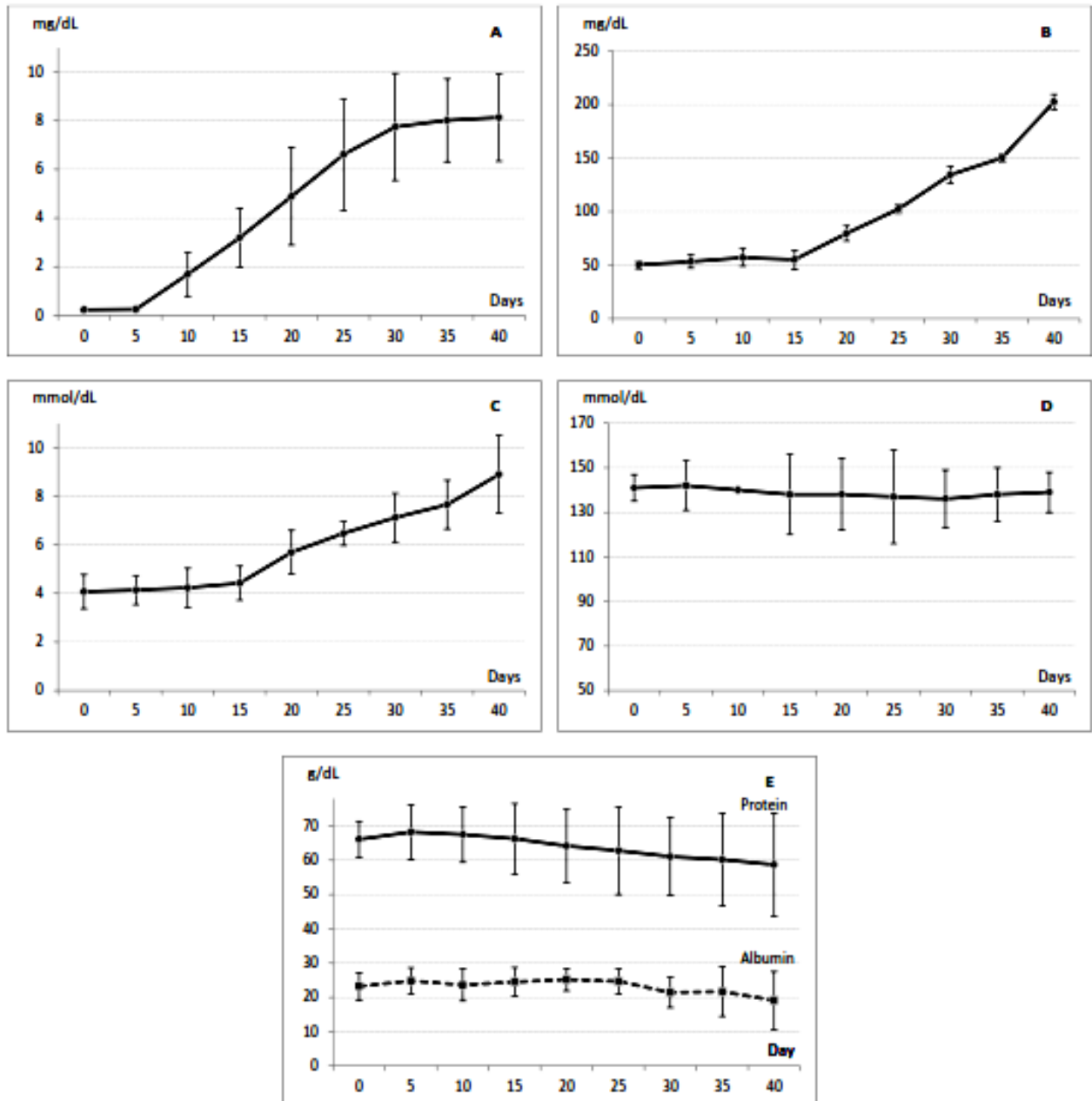
Figure 1: Serum levels of creatinine (A), urea(B), potassium(C), sodium(D), total protein and albumin(E) in sheep with nephrotoxicosis
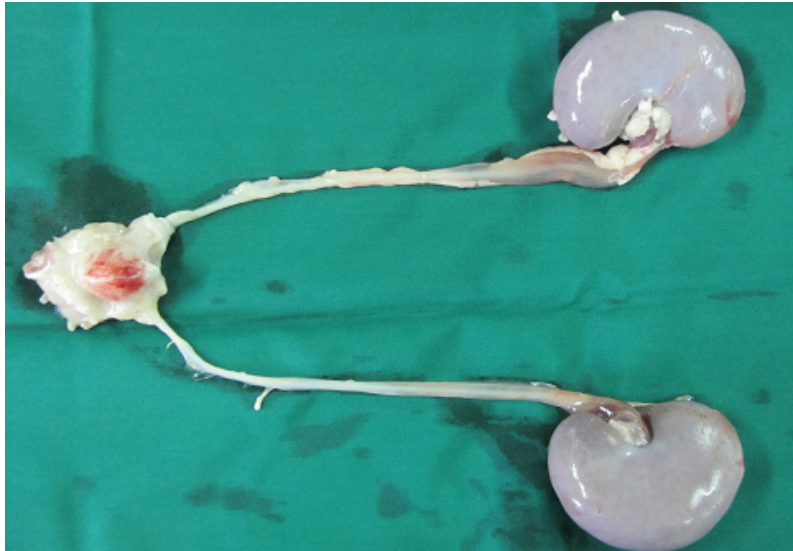
Figure 2: Pale and swollen kidneys, and edematous ureters; hemorrhagic points in bladder
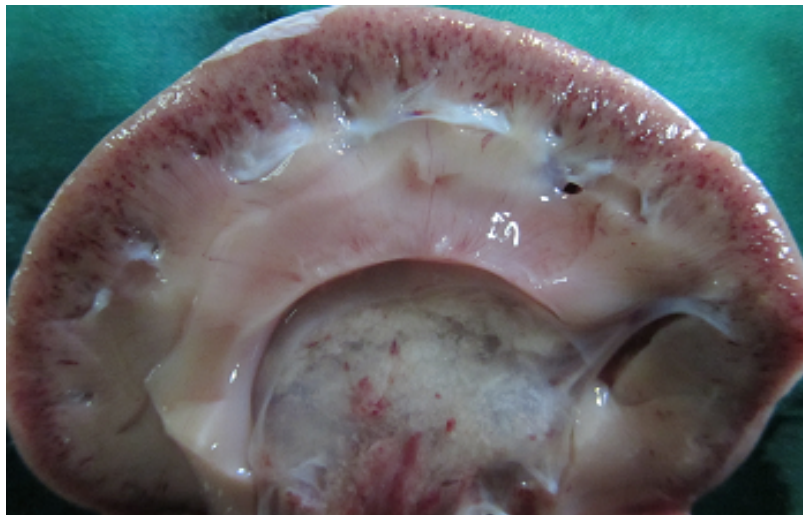

Figure 3: wetness of cut surface of kidney and swollen kidneys, and hemorrhagic points and gray necrotic streaks were apparent.

DISCUSSION

Results of this study corroborated the previous reports in other species in which gentamicin at dose of 80 mg/kg body weight significantly produced nephrotoxicity[1,5,12]. It has been reported that GM-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule[18].The specificity of gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum)[2].The exact mechanism of GM-induced nephrotoxicity is unknown. However, studies showed that primary retention of gentamicin in proximal tubular cells following production of oxygen-associated metabolites and free radicals precede gentamicin-induced nephrotoxicity [14,16,18,22].At the ultrastructural level, the earliest lesions observed concern the lysosomes, which show an accumulation of myeloid bodies[16].

This study showed that after intravenous administration of gentamicin (80 mg/kg BW) caused a significant increase in serum BUN and decreased creatinine clearance. Fukuda et al. [9]91) explicitly showed the electrolyte abnormalities upon treatment of rats with gentamicin.Such immediate formation of disturbance further supports the notion in which inactivation of Na/K ATPase is a very early event during interaction of gentamicin with proximal tubular cells.It also indicates that simultaneous inhibition of very different membrane protein species is not necessarily a prerequisite for the initial depression of Na/K ATPase and afterwards, multifactorial

cell death processes [9].Elevation of fractional excretion rates of Na, K, Cl, and P to many folds above baseline values on days 7 and 8 after gentamicin treatment has been reported [10], indicating decreased tubular reabsorption or increased tubular secretion. Nephropathy developed after administration of gentamicin (80 mg/kg, i.p.) for eight days in rats with proteinuria, increased serum creatinine and BUN, increased urinary loss of sodium and potassium, and glomerulosclerosis[13]. Remarkable observations offered in (1992) a well-define theory, in which hypocalcemia has been recognized of subsequent intracellular events between either inhibition of basolateral calcium ATPase, Na/K ATPase or blockage of intraluminal calcium channels and competition of gentamicin with calcium for binding brush border[6]. In addition to conjugated hypo-phosphatemia in gentamicin-treated rats, it was known to eliminate the problems of consequent nephrocalcinosis with the hypocalcemia [19].

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