Experimental gentamicin-induced nephrotoxicity in the sheep

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Abstract

The aim of the study was to investigate 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. Twenty clinically healthy yearling male Iranian fat-tailed sheep were injected with gentamicin sulfate at a daily dose of 80 mg/kg for 9-10 d when nephrotoxicosis was induced. Blood samples were collected weekly before and after induction of nephrotoxicosis. Gentamicin-induced nephrotoxicity was characterised by increased creatinine and urea levels in serum, electrolyte imbalances, occurrence of albuminuria, and renal dysfunction. Significant elevation in respiratory and heart rates were observed one week after treatment (P < 0.05). There was a noticeable increase in water consumption, lethargy, and loss of appetite in treated sheep. There were significant correlations between serum creatinine and potassium (P = 0.004, r = 0.759), sodium (P = 0.017, r = 0.501), and urea (P = 0.021, r = 0.617) levels. Additionally, significant negative correlations between serum total protein and albumin and creatinine (P = 0.023, r = -0.484) and urea (P = 0.036, r = -0.381) were found. At necropsy, the kidneys were pale, swollen, and wet on the cut surface, especially perirenal tissues and ureters were oedematous. These findings confirmed the previous reports in other species.

Keywords: sheep, gentamicin, experimental nephrotoxicity.

Introduction

Aminoglycosides such as gentamicin (GM) are the most commonly used antibiotics worldwide. Gentamicin was first isolated from Micromonospora purpurea, gram-positive bacteria widely present in water and soil (3). Because of its effectiveness and the low rate of bacterial resistance, GM is often considered as a drug of choice to treat life-threatening infections such as sepsis (15, 20). It acts by irreversibly binding the 30S subunit of the bacterial ribosome, causing misreading of t-RNA, leaving the bacterium unable to synthesise proteins vital to its growth (3). Like all aminoglycosides, when gentamicin is administered orally, it is not systemically active because GM is not absorbed to any appreciable extent from the small intestine. Therefore, it is administered intravenously, intramuscularly, or locally. It is a low-protein binding drug, filtered through the kidney glomeruli without being metabolised in the body (16). Nephrotoxicity is the main limitation of its therapeutic use (7, 14, 19). Renal toxicity of GM is linked to its selective accumulation in epithelial cells of the renal cortex, especially in proximal tubule cells (15, 22), which may range from a mere loss of the brush border in epithelial cells to an overt tubular necrosis (15). Kidney damage progresses slowly and the various stages can be followed closely (7). Nephrotoxic effects of GM had been studied in various experimental models including rats (4, 8, 12, 17), dogs (21), lambs (7), and horses (22). A model of acute renal failure due to aminoglycoside nephrotoxicosis (AGNT) was induced in ewes (10, 11). The aim of this paper was to study 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.

Material and Methods

Animals. Twelve clinically healthy yearling male Iranian fat-tailed sheep, weighing 45-50 kg, were used. All animals were kept indoor in a group box under similar conditions, and manual feed
included alfalfa, barley, and wheat straw treated with 5% urea, 2.5% molasses, and 2% salt, with free access to water for several weeks before the trial. The animals were dewormed with 5% Albendazol (Dieverm®, Damloran Razak Pharma, Iran, 15 mg/kg, p.o.) and 1% Ivermectin (Intermectin®, Interchemie, the Netherland, 200 µg/kg, s.c.). The experimental protocol was approved by the Ethics Committee of Faculty of Veterinary Medicine, Islamic Azad University.

Induction of nephrosis. Nephrotoxicity was induced by daily intravenous injection of gentamicin sulphate (5% Gentacin, Nasr, Fariman, Iran) at a daily dose of 80 mg/kg. GM was given until the plasma creatinine concentration of each sheep elevated to a minimum of 132 μmol/L (≥1.5 mg/dL), and then the treatment was discontinued, as described by Garry et al. (10). Nine to ten days were required to reach this point in all sheep.

Sampling and laboratory procedures. Blood samples were collected from the jugular vein into plain tubes before and after GM-induced nephrotoxicity. Sera were analysed for urea, creatinine, total protein, and albumin by an automatic analyser (Alcyon™ 300, Abbott Lab., USA), using commercial kits (Pars Azmoon Co. INC., Iran). Serum concentrations of potassium and sodium were also determined by Flame photometric method.

Statistical analysis. Data was analysed statistically using the Statistical Package for Social Sciences statistical (SPSS) package, version 17. Normality of data was tested by Kolmgorov-Smirnov test and analysis of variance (ANOVA) was used for comparison of measured parameters in various times. Results were presented as mean ± and standard deviation (SD), and P < 0.05 was considered as statistically significant.

Results

There was a noticeable increase in water consumption in treated sheep. Lethargy and loss of appetite were recorded after the fifth day. Approximately 9-10 d after gentamicin injection, food consumption was reduced from one-half to one-third of the previous rate. Respiratory and heart rates were significantly increased one week after treatment (P < 0.05). There was no significant difference in rectal temperature, until animals became recumbent. After sternal and lateral recumbency, body temperature significantly decreased (P < 0.05 and P < 0.01 respectively). Bottle jaw was observed in one sheep on day 30 due to hypoproteinaemia.

The changes in blood constituents are shown in Fig. 1. All sheep became uremic. Ten days after onset of the treatment, a significant and progressive increase in creatinine levels 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 and 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 and creatinine (P = 0.023, r = -0.484) and urea (P = 0.036, r = -0.381). There was no significant correlation between serum concentration of sodium and any of other biochemical factors.

The animals died on 27-33 d after treatment began, due to renal failure. Toxic nephrosis signs were macroscopically apparent at necropsy. The kidneys were pale, swollen, and wet on the cut surface, especially perirenal tissues and ureters were oedematous (Fig. 2). There were haemorrhagic and gray necrotic streaks radiating out through the medulla and extending to the cortex (Fig. 3).

Fig. 2. Pale and swollen kidneys, oedematous ureters; and haemorrhagic points in bladder
Fig. 1. Serum levels of creatinin (A), urea (B), potassium (C), sodium (D), total protein, and albumin (E) in sheep with nephrototoxicosis (Mean ± SD)

Fig. 3. Swollen and wet on cut surface kidneys, haemorrhagic points and gray necrotic streaks are apparent
Discussion

Results of the study corroborated the findings of previous investigations in which significant nephrotoxic effect of GM, administered at a dose of 80 mg/kg b.w., was observed in other animal species (1, 5, 22). Tubular necrosis, mainly in the proximal tubules, following GM-induced nephrotoxicity has been reported (18). The exact mechanism of GM-induced nephrotoxicity is unknown. Tubular cytotoxicity is the consequence of many interconnected actions, triggered by drug accumulation in epithelial tubular cells (19). Most of the intravenously administered GM is excreted with urine, whereas a relatively sizable portion is selectively accumulated in the renal cortex, where the concentration of the GM amounts is 50 to 100 times greater than in serum (2). Subsequently, GM remains with a long-half life in the renal proximal tubular cells, leading to renal damage such as structural changes and functional impairments of the plasma membrane, mitochondria, and lysosomes (16).

More recently, it has been reported that megalin, a giant endocytic receptor abundantly expressed at the apical membrane of renal proximal tubules, plays an important role in binding and endocytosis of aminoglycosides in the proximal tubular cells. In megalin-deficient mice, lack of this uptake pathway results in tubular resorption deficiency and low molecular weight proteinuria (20). Other effects of gentamicin such as phospholipidosis, oxidative stress, extracellular calcium sensing receptor stimulation, and energetic catastrophe have been also connected with cell death. It has been shown that primary retention of gentamicin in proximal tubular cells following production of oxygen-associated metabolites and free radicals precedes gentamicin-induced nephrotoxicity (13, 15, 18, 23). Besides, indirect effects of gentamicin, such as reduced renal blood flow and inflammation, may also contribute or amplify its cytotoxicity (19).

This study showed that after intravenous administration of gentamicin (80 mg/kg b.w.) a significant increase in serum blood urea nitrogen and decreased creatinine clearance occurred. Fukuda et al. (9) explicitly showed the electrolyte abnormalities upon treatment of rats with gentamicin. Such immediate formation of the 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 8 d in rats with proteinuria, increased serum creatinine and blood urea nitrogen, urinary loss of sodium and potassium, and glomerulosclerosis (12). Remarkable observations provided a well-defined theory, in which hypocalcemia has been recognised as 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).

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References