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Research Article

Effects of amoxicillin repeated administration on the hemogram and biogram of sheep

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ABSTRACT

Background: The object of the present study was to investigate the possible alterations in hematological and biochemical parameters of sheep that may occur following intramuscular injection of amoxicillin.
Methods: Amoxicillin was injected to 10 sheep at a dosage regimen of 7 mg/kg of body weight for 5 successive days. Two types of blood samples (with and without ethylenediaminetetraacetic acid as an anticoagulant) were collected from the jugular vein before and after the antibiotic course.
Results: Amoxicillin significantly ($p < 0.001$) increased total leukocyte count and ($p < 0.05$) absolute eosinophilic count when compared with those of the control samples. Aspartate aminotransferase, alkaline phosphatase and cholesterol, were significantly ($p < 0.05$) higher than the corresponding control values. In addition, amoxicillin significantly ($p < 0.05$) increased blood urea nitrogen and creatinine but decreased phosphorus level when compared with those of prior-administration samples.
Conclusions: These data may suggest that although the side changes caused by amoxicillin are minor in sheep, yet the liver and kidney functions should be monitored during its usage in therapy and it should be used with care for treatment of sheep with renal and/or hepatic impairments; its dosage regimen should be adjusted to avoid its hepatotoxic and nephrotoxic effects.

Keywords: Amoxicillin, Biochemical parameters, Blood, Hepatotoxicity, Nephrotoxicity, Sheep

INTRODUCTION

Since the discovery of antibiotics, various investigations have been carried out to reveal their pharmacodynamic profile and side effects. Although antibiotics are generally considered safe and well tolerated, they have been associated with a wide range of adverse effects.[1] Side effects are many, various, and can be very serious depending on the antibiotics used and the microbial organisms targeted. Pharmacodynamic effects of different antibiotics on blood constituents, serum enzymes and electrolytes were extensively studied in the laboratory and domesticated animals.[2,3]

Amoxicillin is a bactericidal aminopenicillin, it is eliminated primarily through renal mechanism, principally by tubular secretion and glomerular filtration.[4,5] It is a broad-spectrum antibiotic useful in the treatment of clinical cases with serious infections caused by Gram-positive and Gram-negative bacteria, especially those resistant to penicillin-G and ampicillin.[6] Due to its favorable pharmacokinetics and antimicrobial efficacy, amoxicillin is widely used for pre-operative antibiotic prophylaxis,[7] also, it is widely used in veterinary medicine.[5,8] Although penicillins are not considered hepatotoxic, elevated liver enzymes have been reported by.[8]

Comparative investigations on changes in biochemical and hematological parameters after amoxicillin and other antibiotics are few in ruminants.[3,9] Since antibiotics are used for treatment of infections that may also cause changes

in some hematological and biochemical parameters, the veterinarian must take into consideration the changes caused by these drugs to avoid misdiagnosis as well.

Therefore, this study was designed to investigate the possible hematological and biochemical changes in healthy Libyan sheep after parenteral administration of amoxicillin in the therapeutic dose for 5 successive days.

METHODS

The drug

The drug used was amoxicillin sodium (Moxynil® 500 mg). It was obtained in the form of vials, each vial contains 500 mg powder of amoxicillin sodium, produced by Nile company for Pharmaceutical and Chemical industries, Cairo, Egypt.

Experimental animals

A total of 10 Libyan, apparently healthy, fat-tailed sheep aged from 10 to 12 months, belong to the faculty of veterinary medicine, Tripoli University were used in this study. Animals were kept in a semi-closed system under natural daylight and temperature and fed on standard ration of hay and concentrate ad libitum, and had free access to water. Animals were free from ectoparasites, endoparasites and blood parasites.

Experimental design

Animals were weighed individually at the beginning of the experiment; blood samples were taken from each animal and considered as control.

On the next day, animals were injected with the therapeutic dose (7 mg/kg body weight) of amoxicillin sodium, intramuscularly, twice a day, for 5 successive days.

At the end of the experiment, 2 h after the last injection, blood samples were taken from all animals for hematological and biochemical investigations.

Two blood samples were collected from the jugular vein of each sheep, one is introduced into a plain glass tube to obtain serum during the other into a glass tube containing ethylenediaminetetraacetic acid for hematological examination.

Haematological examination

Total red blood cell count, hemoglobin content, packed cell volume, and total white blood cell count were determined manually according to.[10] Blood smears were prepared, fixed and stained with May-Grunwald-Giemsa stain for differential leucocytic count; 200 leukocytes were differentiated in the blood smear of each animal.

Biochemical examination

Kidney function tests

Blood urea nitrogen (BUN) was estimated using urea kits (Bio Meriux®) according to.[11] Serum creatinine (SCr) was determined using creatinine kit (Bio Meriux®) according to.[12] Serum calcium, phosphorous, sodium and potassium were measured using flame photometer according to respectively.[13-15]

Liver function tests

The levels of alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined using transaminase kits (Bio Meriux®) according to.[16] Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels were estimated according to respectively.[17,18] Total bilirubin (TB) was measured with a colorometric method using Boehringer Mannheim GmBH® kits, according to.[19]

Statistical analysis

Data were expressed as mean±standard error (n=10) and results were statistically analyzed using student's t-test after.[20]

RESULTS

Blood picture of experimental animals at zero time (control) and after treatment is shown in Table 1. Blood parameters of animals injected with amoxicillin showed significant (p<0.001) increase in total leukocyte count and significant (p<0.05) increase in absolute eosinophil count when compared with the control.

Results of serum biochemistry applied to evaluate liver and kidney function after 5 days of treatment with amoxicillin are shown in Tables 2-4; when compared with the control; animals injected with amoxicillin exhibited significant (p<0.05) increase in AST, ALP and cholesterol.

Significant (p<0.05) increases in BUN, SCr and a decrease in phosphorus were also recorded.

DISCUSSION

Amoxicillin is a bactericidal β-lactam antibiotic with extended antibacterial spectrum used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Our study was focused on its possible side effects on hemogram and biogram of Libyan sheep. Eosinophilia observed post-amoxicillin treatment is mostly due to dose- independent hypersensitivity that can be associated with aminopenicillin treatments.[5,8,21,22]

Table 1: Haematological changes in sheep injected with amoxicillin (7 mg/kg of body weight) intramuscularly, twice daily for 5 successive days (data are expressed as mean±SE; n=10).

| | RBCs×10^6 (Cu mm) | Hb (g/dl) | PCV (%) | WBCs×10^3 (Cu mm) | Differential leuckocytic count | | | | |
					Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
Control	12.7±0.42	12.25±0.37	37.8±6.0	7.5±0.14	41.2±2.54	3.9±0.57	0.2±0.13	51.4±2.56	3.3±0.40
Amoxicillin	12.0±0.35	11.95±0.32	37.6±0.8	8.65±0.16**	41.0±2.05	5.6±0.51*	0.2±0.14	49.7±1.96	3.5±0.32

*Significance p<0.05, **Significance p<0.01. RBCs: Red blood cells, Hb: Hemoglobin, PCV: Packed cell volume, WBCs: White blood cells, SE: Standard error

Table 2: Response of blood urea nitrogen and serum creatinine levels of sheep injected with amoxicillin (7 mg/kg. of body weight) intramuscularly, twice daily for 5 successive days (data are expressed as mean±SE; n=10).

	Control	Amoxicillin
Blood urea nitrogen (mg/100 ml)	24.6±3.22	33.6±2.79*
Serum creatinine (mg/100 ml)	0.58±0.08	0.74±0.05*

*Significance p<0.05, SE: Standard error

Table 3: Response of sheep serum enzyme levels to amoxicillin (7 mg/kg. of body weight), injected intramuscularly, twice daily for 5 successive days (mean±SE; n=10).

	Control	Amoxicillin
ALT (U/L)	12.4±1.15	12.4±1.04
AST (U/L)	58.3±4.07	69.2±3.91*
GGT (U/L)	38.5±2.36	35.4±1.95
ALP (U/L)	293.3±31.41	395.6±31.16*
Cholesterol (mg/dl)	55.7±2.58	61.8±2.24*
Total bilirubin (mg/dl)	0.37±0.05	0.34±0.025

*Significance p<0.05, ALT: Alanine amino transferase, AST: Aspartate amino transferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, SE: Standard error

Table 4: Response of sheep serum electrolyte levels to amoxicillin (7 mg/kg. of body weight), injected intramuscularly, twice daily for 5 successive days (mean±SE; n=10).

	Control	Amoxicillin
Sodium (mg/100 ml)	150.3±0.65	150.8±0.39
Potassium (mg/100 ml)	4.56±0.7	4.58±0.06
Calcium (mg/100 ml)	10.18±1.9	9.79±0.27
Phosphorus (mg/100 ml)	8.6±0.40	7.48±0.48*

*Significance p<0.05, SE: Standard error

A significant increase in the level of AST and cholesterol in the amoxicillin-treated group may indicate hepatic impairment, as AST is a hepatic leakage enzyme that increases in association with the liver and muscular diseases.[23] This increase, although statistically significant, is not considered conclusive, as the level of AST remained within the lower normal range for ovines.[24] ALT, another hepatic leakage enzyme, did not show significant change, because its activity is not high in ruminants' hepatocytes.[23,24] In addition to that, a significant increase in ALP monitored in the treated group was not associated with an increase in bilirubin. It is obvious that ALP is a cholestatic marker and its synthesis and release increases in association with impaired bile flow,[23,24] and the TB in the treated group was within normal range, accordingly, this increase in ALP may be considered non-specific and attributed to its wide and fluctuating reference intervals in ruminants.[23] Supporting to this, GGT the specific cholestatic enzyme in large animals showed no significant changes in amoxicillin-treated group.

The penicillins in general are not considered hepatotoxic, but transient elevation in liver enzymes associated with their usage has been recorded.[5,25]

Amoxicillin-treated group showed a significant increase in SCr and BUN in comparison with the pre-treatment values, this increase in non-protein nitrogenous substances indicate a decrease in GFR, this finding agreed with the finding of many other workers that attribute these changes to nephrotoxicity. In this experiment, the increase in SCr is not considered significant because it remained within normal reference range for this animal species,[24] which means that the kidneys in these animals are still capable of creatinine excretion. Unlike BUN which was above the normal value of that animal species and significantly higher than the control. In ruminants increase in SCr is more specific than BUN to detect a decrease in GFR, since in these unique animals ammonia derived from urea is utilized to form amino acids,[26,27] so such increase in BUN may be in part due to increasing in protein catabolism rather than nephrotoxicosis.[28] In addition to that, SCr is freely filtered from the glomeruli and it is not reabsorbed by renal tubules, unlike urea that will passively diffuses from the tubules back to the body, and the amount of urea absorbed is inversely related to the rate of urine flow through the tubules,[26,27] this may further explain the more significant increase in BUN than SCr in the present study. In normal conditions, amoxicillin is excreted through the kidneys, 90% of the drug is secreted by the proximal tubules, while the remaining 10% is excreted by glomerular filtration.[29] Rarely, amoxicillin may induce acute renal failure, especially when it is given in very large doses, with low diuresis, fasting, acid urine pH, or

given drugs like carbamazepine, through the formation of crystaluria that leads to renal failure.[7,29,30]

The kidneys are involved in the homeostasis of calcium, phosphorous, sodium and potassium.[24] In renal tubular diseases hypernatremia is expected due to increasing in fractional excretion of sodium.[24,27] However, the level of sodium in the present study was within normal reference range. Hypocalcaemia and hyperphosphataemia[24] were detected in animals before and after treatment (Table 4), so it cannot be attributed to renal malfunction,[24,27] taking into consideration that the level of phosphorous in the amoxicillin-treated group was significantly lower than the control, the change in this electrolyte may not match with abnormal renal function.

Through conducting this study, it could be concluded that amoxicillin given in the therapeutic dose twice daily to healthy Libyan sheep caused only minor inconclusive changes in the hematological and biochemical profile of these animals, so it may be safely used in treating different susceptible bacterial infections in sheep provided that the kidney and liver are functioning normally.

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