Journal of Applied Pharmaceutical Science

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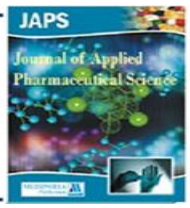

ISSN: 2231-3354
Received on: 10-12-2011
Revised on: 14:12:2011
Accepted on: 18-12-2011

The effects of penicillin-streptomycin on liver amino-transferases, alkaline phosphatase and total serum protein in rabbits (*Orcytolagus coniculus*)

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ABSTRACT

The effects of penicillin-streptomycin on some liver enzymes and total serum protein were investigated using thirty adult rabbits (*Orcytolagus coniculus*) weighing 1.8 – 2.5kg. They were divided into six groups (Groups A – F) of five animals each. Groups A – C received high, moderate and low doses of penicillin-streptomycin, respectively; Group D received penicillin at 10mg/kg twice daily, Group E received streptomycin at 50mg/kg once daily while Group F received normal saline throughout the period of drug administration. All treatments were administered intramuscularly and lasted for ten consecutive days. Serum samples were taken before drug administration (0 hour) to establish baseline parameters and then at 24 and 168 hours post administration of the last dose of the drugs, that is, 11^{th} and 17^{th} days post commencement of treatment. Aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatse (ALP) and total serum protein were determined in all the serum samples using appropriate methods. The results showed significant increase in AST and ALT when compared with baseline parameters ($p < 0.05$). In contrast, there was significant decrease in total serum protein ($p < 0.05$). However, no significant difference was observed in ALP activities before and after drug administration. In conclusion, penicillin-streptomycin could interfere with liver functions by induction of acute hepatitis especially when given in high dosages.

Keywords: Penicillin-streptomycin, Liver enzymes, Effects.

INTRODUCTION

Bacterial infections are one of the leading infectious diseases confronting public health and antibacterial therapy remains relevant in treatment and control of such infections especially in developing countries. The increase in the rate of resistance of microorganisms to monotherapy is increasingly alarming and has necessitated use of combination therapy in treatment of some infectious diseases (Brunton et al., 2008). For instance, penicillin G or amoxicillin were the mainstay therapy for pneumococcal infections, however, bacterial resistance to penicillins, cephalosporins and non-beta lactam antibiotics have escalated in the last two decades (Jones and Pfaller, 1998; Barry, 1999). Therefore, complete cure may not be achieved with penicillins or cephalopsporins monotherapies when use against *Streptococcus pnuemoniae* (Bradley and Connor, 1991; Friedland, 1994). This and many other cases have necessitated the combination of two or more different antibiotics to obtain improved effects in the treatment of infections and many have proved efficacious. In an experiment, penicillin-streptomycin gave total cure in mice infected with *Streptococcus pneumoniae* (Udeani and Kalu, 2003). Penicillin and streptomycin are narrow spectrum drugs with effects usually on Gram positive and negative organisms, respectively (Rossi, 2004) which make their combination a common practice in other to achieve broader spectrum.

However, drug interaction may result from combination of two or more safe drugs (Bishop, 2006) resulting in potential increase in toxic effects or reduced efficacy (Hansten, 1998; Brunton et al., 2008). Liver being the largest organ in the body and functionally responsible for detoxification of drugs and xenobiotics (Ganong, 2006), usually suffers most of these potential toxic effects. These could cause altered liver function usually manifesting as altered activities of liver enzymes such as liver alkaline phosphatase, transaminases and sometimes altered total serum protein (Bass, 2003). Acute liver injury has been described as the leading cause of drug withdrawal based on safety grounds (Bakke et al., 1995). To this background, we evaluated the effects of penicillin-streptomycin on liver transaminases, alkaline phosphatase and total serum protein in rabbits.

MATERIALS AND METHODS

Experimental animals

Thirty adult rabbits (*Orcytolagus coniculus*) weighing 1.8 – 2.5kg procured from Dagwom Farm of National Veterinary Research Institute (NVRI), Nigeria were used with approval from Animal Ethics Committee, NVRI, Nigeria. The rabbits were kept in rabbit pens in NITR at room temperature, fed with standard feeds (ECWA Feeds, Jos, Nigeria) and provided with access to clean drinking water *ad libitum*. They were conditioned for three weeks within which prophylactic treatment against coccidial infection and worms was given using amprolium (Petlife, UK) and piperazine (Pfizer, UK), respectively during the first week of conditioning.

Treatment drugs

Penicillin and streptomycin were procured from a standard pharmaceutical shop in Jos. The drugs were both manufactured by Shijiazhuang Pharmaceutical Factory, China and registered by National Food and Drug Administration and Control (NAFDAC). They were diluted just before administration as prescribed by the manufacturer and kept in refrigerator throughout the period of administration.

Treatment groups

The rabbits were divided into six treatment Groups A – F with five rabbits in each treatment group. The drugs were administered according to body weight as presented in Table 1 for ten (10) consecutive days while Group F was administered normal saline for same period of administration. All drugs were administered intramuscularly. Before each administration, the drug was brought to room temperature by keeping on table for some time.

Serum sampling

Blood samples were collected from ear vein of all the experimental animals into plain bottle just before administration of drugs; this period was tagged 0 hour. Then, another set of samples were collected at 24 and 168 hours post administration of the last dose on 10^{th} day of drug administration. The blood samples were allowed to clot at room temperature and the serum gently removed, dispensed into new set of plain bottles and immediately analyzed.

Table 1. Drug treatments.

Treatment group	Drug	Dose (mg/kg)
Group A	Penicillin-streptomycin	Penicillin at 20mg/kg twelve hourly plus Streptomycin at 100mg/kg, for 10 days
Group B	Penicillin-streptomycin	Penicillin at 10mg/kg twelve hourly plus Streptomycin at 50mg/kg, for 10 days
Group C	Penicillin-streptomycin	Penicillin at 5mg/kg twelve hourly plus Streptomycin at 25mg/kg, for 10 days
Group D	Penicillin	10mg/kg twelve hourly for 10 days
Group E	Streptomycin	50mg/kg daily for 10 days
Group F	Normal saline	Daily for 10 days

Enzyme assays

The enzymes were assayed using Reitman and Frankel method as previously described by Cornelius et al. (1959) for serum alanine amino transferase (ALT) and aspartate amino transferase (AST) and Biuret method as previously described by Cole (1980) for total serum protein. The activity of alkaline phosphatase (ALP) was determined as described by King and Armstrong (1974).

Data analysis

Data were presented in tables as mean $\pm$ standard deviation and analyzed using analysis of variance, ANOVA (SPSS, 2006). Significant difference was inferred at $p \leq 0.05$.

RESULTS

Effects on ALT activity

Table 2 shows the effects of penicillin-streptomycin on the ALT activities. There was significant difference in ALT activities in animals in Groups A, B and C when baseline activities were compared with activities on 24 and 168 hours post administration ($p < 0.05$).

Table 2. Effects of penicillin-streptomycin on serum alanine animotransaminase (ALT) activity in rabbits.

Treatment groups	Activities (Units/L)			p value
	0 hr	24 hr	168 hr	
Group A	4.5 $\pm$ 0.7	30.0 $\pm$ 1.4	27.7 $\pm$ 0.7	**0.0002**
Group B	6.0 $\pm$ 1.4	25.0 $\pm$ 2.1	21.5 $\pm$ 0.7	**0.00012**
Group C	5.5 $\pm$ 2.1	17.5 $\pm$ 0.7	14.0 $\pm$ 1.4	**0.017**
Group D	13.5 $\pm$ 4.9	17.0 $\pm$ 1.4	16.5 $\pm$ 0.7	0.19
Group E	10.5 $\pm$ 2.1	17.5 $\pm$ 0.7	16.0 $\pm$ 0.4	0.127
Group F	8.5 $\pm$ 2.1	10.0 $\pm$ 0.6	10.0 $\pm$ 0.2	0.57

0 hr : Before drugs/normal saline administration.
24 hr : 24 hours post administration of last dose on 10^{th} day.
168 hr : 168 hours post administration of last dose on 10^{th} day.

Effects on AST activity

There were significantly elevated AST activities in all the treatment groups when 24 and 168 hours post commencement of drug administration samples were compared with baseline samples (0 hour) ($p < 0.05$) (Table 3).

Table 3. Effects of penicillin-streptomycin on serum aspartate animotransaminase (AST) activity in rabbits.

Treatment groups	Activities (Units/L)			p value
	0 hr	24 hr	168 hr	
Group A	6.0 ± 1.4	57.5 ± 3.5	53.7 ± 2.8	**0.00001**
Group B	8.0 ± 1.4	39.0 ± 2.8	47.5 ± 0.7	**0.0008**
Group C	6.5 ± 0.7	37.5 ± 0.7	37.0 ± 1.4	**0.00047**
Group D	8.5 ± 3.5	44.0 ± 4.2	33.5 ± 2.8	**0.00013**
Group E	11.0 ± 2.8	44.5 ± 4.2	33.0 ± 1.4	**0.0005**
Group F	9.0 ± 2.8	11.0 ± 2.8	10.0 ± 1.4	**0.837**

0 hr : Before drugs/normal saline administration.
24 hr : 24 hours post administration of last dose on 10th day.
168 hr : 168 hours post administration of last dose on 10th day.

Table 4. Effects of penicillin-streptomycin on serum alkaline phosphatase (ALP) activity in rabbits.

Treatment groups	Activities (Units/L)			p value
	0 hr	24 hr	168 hr	
Group A	51.0 ± 14.1	61.0 ± 9.4	66.0 ± 7.1	0.732
Group B	56.0 ± 21.2	60.8 ± 28.9	51.0 ± 14.1	0.29
Group C	31.0 ± 7.1	56.0 ± 0.7	31.0 ± 1.4	0.07
Group D	56.0 ± 7.9	56.7 ± 7.4	56.5 ± 7.1	0.92
Group E	61.0 ± 14.1	65.5 ± 7.8	66.0 ± 7.1	0.7
Group F	46.0 ± 7.1	56.0 ± 7.1	56.0 ± 7.4	0.57

0 hr : Before drugs/normal saline administration.
24 hr : 24 hours post administration of last dose on 10th day.
168 hr : 168 hours post administration of last dose on 10th day.

Table 5. Effects of penicillin-streptomycin on serum total protein in rabbits.

Treatment groups	Activities (Units/L)			p value
	0 hr	24 hr	168 hr	
Group A	71.5 ± 5.0	51.0 ± 1.4	56.0 ± 1.4	**0.03**
Group B	69.0 ± 1.4	56.0 ± 1.4	61.0 ± 1.4	**0.045**
Group C	68.0 ± 11.3	61.0 ± 1.4	66.5 ± 2.1	**0.041**
Group D	75.0 ± 4.9	61.0 ± 1.4	61.5 ± 2.1	**0.01**
Group E	68.5 ± 16.3	59.5 ± 3.5	63.5 ± 1.4	**0.034**
Group F	68.5 ± 2.1	70.5 ± 3.5	70.5 ± 3.5	0.17

0 hr : Before drugs/normal saline administration.
24 hr : 24 hours post administration of last dose on 10th day.
168 hr : 168 hours post administration of last dose on 10th day.

Effects on ALP activity

ALT activities at baseline were similar in all the treatment groups to the activities 24 and 168 hours post drug administration ($p > 0.05$) (Table 4).

Effects on total serum protein

Table 5 shows the effects of penicillin-streptomycin on total serum protein in rabbits treated with penicillin, streptomycin and penicillin-streptomycin combination. Total serum protein was significantly lowered in all treatment groups 24 and 168 hours post drug administration ($p < 0.05$).

DISCUSSION

Liver, a vital organ in vertebrates, performs wide range of functions such as detoxification, plasma protein synthesis and metabolism of drugs among other functions (Ganong, 2006). During these activities, liver could be exposed to toxicity from these agents which may manifest clinically or only as abnormal liver enzymes activities (Lee, 2003). The abnormal liver activities are usually assessed by evaluating the activities of liver enzymes such as amino transferases, alkaline phosphatase or total serum protein (Pratt and Kaplan, 2000).

In our study, we investigated the effects of penicillin-streptomycin on liver by measuring the activities of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP) and total serum protein, post treatment. The increased activities of ALT post-treatment observed in animals in Groups A – C is similar to the report by Lee (2003). These changes are suggestive of liver injury induced by the drugs. ALT is usually released into blood in increasing amounts when the liver cell membrane is damaged and specifically used as indicator of liver injury (Pratt and Kaplan, 2000). The fact that such changes were not observed in animals in Groups D and E could be attributed to synergistic effects by the two drugs. Combination of two or more drugs with little or no toxic effects on organ(s) could greatly increase organ toxicity (Hansten, 1998). The elevated activities of AST in all drug treated groups could be attributed to non-specificity of the enzyme in evaluating liver functions (Pratt and Kaplan, 2000). It could be opined that other tissue damage might have contributed to these values, probably skeletal muscle damage resulting from frequent intramuscular injection. Significant amount of AST is also found in skeletal muscle and could be released following tissue damage (Pratt and Kaplan, 2000). Elevated ALP activities have been previously attributed to cholestasis (Pratt and Kaplan, 1999), hence, in the absence of cholestasis, hepatocellular damage causes little release of ALP (Whitby et al., 1984). No significant change in ALP activities observed in our study is an indication that the possible mechanism of liver damage induced by the drugs may not be biliary blockade. On the other hand, total plasma protein was significantly decreased in all drug-treated groups. Liver synthesizes plasma protein (Ganong, 2006) and lower protein level has been reported in compromised liver functions (Bass, 2003). Thus, the penicillin and streptomycin as well as their combination could have altered the synthetic function of the liver.

CONCLUSION

The study showed that penicillin and streptomycin when used singly or in combination could induce liver injury similar to acute liver injury. Thus, caution should be exercised when these drugs are used clinically especially in patients with underlying liver diseases and those on other hepatotoxic drug(s).

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