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ARTICLE

Plasma Disposition of Ampicillin following Thiopentone Sodium Anaesthesia in Rabbits *(Oryctolagus cuniculi)*

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SUMMARY

Various anaesthetic agents have been used to relieve pain during surgical procedures and are normally followed by antibiotic cover to guard against secondary bacterial infection. This study was aimed at determining the effect of thiopentone anaesthesia on plasma disposition of ampicillin sodium in rabbits. The study involved the use of 10 rabbits divided into two groups of 5 rabbits each. In one group, ampicillin sodium alone at a dose rate of 10 mg kg^{-1} was administered intravenously via the left jugular vein. The second group was administered the same dose rate of ampicillin immediately after recovery from thiopentone sodium (20 mg kg^{-1} body weight) anaesthesia. Blood samples were collected from the contralateral vein at 0.25, 0.5, 1, 3, 6, 12 and 24 hours into sterile ethylene diamine tetraacetic acid (EDTA) vacutainer tubes post ampicillin sodium administration for plasma ampicillin level determination. Measurable plasma levels of ampicillin were obtained for twenty-four hours using spectrophotometry. The group administered ampicillin alone had maximum plasma ampicillin concentration of 0.089±0.003 mg/ml at 15 minutes, which declined steadily with time to 0.012±0.001 mg/ml at 24 hours. Those administered ampicillin following anaesthesia showed a biphasic increase and decrease in plasma ampicillin concentrations, at 15 minutes (0.083±0.004) to 3 hours (2.50±0.06), and at 6 hours (1.43±0.05) to 12 hours (3.33±0.003). This result suggests that thiopentone did alter the plasma disposition of ampicillin in rabbits

KEYWORDS: Anaesthesia, antibiotic, drug interaction, intravenous administration

INTRODUCTION

Ampicillin is a semi-synthetic beta-lactam antibiotic found to be effective against a wide variety of Gram-positive and Gram-negative bacteria (Turk *et al.,* 2009; Savadogo *et al.,* 2010; Shakak and Saeed, 2010) which is often used in the treatment of bacterial infections in humans and animals. Ampicillin prevents bacterial cell wall synthesis by inhibiting the action of the enzyme transpeptidase, with subsequent loss of stability of the cell leading to cell death (Laurence *et al.,* 1999). Its advantage over the ordinary penicillin is its ability to penetrate the cell wall of Gram-negative bacteria due to the presence of an amino group. The antibiotic is readily available, cheap, and has a wide margin of safety which makes it a drug of choice for the treatment of various bacterial infections (Vaden and Riviere, 2001). Thiopentone sodium on the other hand, is a rapid-onset short-acting barbiturate general anaesthetic belonging to a class of drugs that act on the gamma-aminobutyric acid (GABA) receptor in the brain and spinal cord (Downie *et al.,* 2000; Yang *et al.,* 2006). This anaesthetic agent has been shown to cause immobility and depresses the response to noxious stimuli, and has an elimination half-life of about 3.7-12 hours (Bertone and Horspool, 2004; Mondol and Rahim, 2007; Norman *et al.,* 2010). Longer elimination half-life has been observed during pregnancy or when there is decreased hepatic blood flow (Chestnut *et al.,* 2004). Drug interactions occur when two or more drugs are co-administered and this could lead to changes in the activity of any of the drug on the body or alterations to what the body does to the drugs from absorption to elimination (Flynn, 2003; Lees *et al.,* 2004).

The interplay between a drug's affinity for plasma proteins, defined by the dissociation constant *Kd*, and the maximum plasma protein binding capacity, defined by B_{max}, contribute to the amount of free drug available in the system. Ultimately, free drug concentration is determined by a variety of physiological mechanisms including systemic clearance as well as the binding properties defined above

(Wilkinson, 2001). Alterations in *Kd* and B_{max}, or both could cause an increase or decrease in free drug concentration (Buur *et al.,* 2009). During the period especially after a surgical procedure, broad-spectrum antibiotics such as ampicillin are normally administered to take care of any secondary bacterial infection; this could lead to drug interaction which may affect the pharmacokinetics of the antibiotics. This study is therefore aimed at investigating the effect of thiopentone sodium anaesthesia on plasma disposition of ampicillin in rabbits with a view to establishing whether thiopentone increases or decreases the plasma half-life of ampicillin. This will be helpful in antibiotic therapies especially following surgical procedures that may involve anaesthesia.

MATERIALS AND METHODS

Area of Study

The study was conducted in Maiduguri, Borno State, Nigeria. The state lies between latitude $11^0$05'N and 11^o40;N and longitude $13^0$05'E and 13^o25'E (Mbaya *et al.,* 2008). The period of the study was from February 2008-April 2008.

Drugs

Biochem GmbH, Vienna, Austria, supplied ampicillin sodium B.P. while thiopentone sodium (5%) was supplied by Elvis Pharmaceuticals, Shijlazhuang, China.

Rabbits

Ten clinically healthy New Zealand White rabbits of mixed sexes weighing between 2.5 and 3.5 kg were used. The rabbits were purchased from the Maiduguri Monday market. They were treated with amprolium as a coccidiostat at a dose rate 1.25 gL^{-1} of drinking water for two weeks before the start of the experiment. The rabbits were kept in wire gauze cages in the laboratory for the period of the study. During acclimatization and subsequent treatment periods, they were fed antibacterial free commercial feeds supplemented with lettuce and cucumber. Water was provided *ad libitum*. All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals in Biomedical Research as outlined by the Canadian Council of Animal Care (2009).

Experimental design

The ten rabbits were divided into two groups of five rabbits each. The first group was administered a single dose of ampicillin sodium (10 mg kg^{-1} body weight) intravenously through the left jugular vein using a 23 gauge needle. Blood samples were obtained from the contralateral vein before and at 0.25, 0.5, 1, 3, 6, 12, and 24 hours after administration to determine the drug concentration in the plasma. About 2 ml of blood was collected at each sampling time and placed in vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA), and centrifuged at 4000 rpm for 10 minutes to obtain plasma which was stored at -20 ^{0}C until used. The second group of rabbits were administered thiopentone sodium (20 mg kg^{-1} body weight) through the left jugular vein. Immediately after recovery from anaesthesia, the rabbits were administered ampicillin sodium (10 mg kg^{-1} body weight) via the same route and blood samples collected from the contralateral vein at the same time intervals as described above.

Analytical procedure

The spectrophotometric method described by Smith *et al.* (1967) for the determination of ampicillin in pharmaceutical preparations was used. This is based on the copper facilitated formation of stable acid degradation products as modified by Angelucci and Baldieri (1971) for the assay of ampicillin in biological fluids. Briefly, 1 ml of plasma was mixed with 4 ml of absolute alcohol in a centrifuge tube and mixed thoroughly before centrifuging at 4000 rpm for 10 minutes. 2 ml of clear supernatant was mixed with 8 ml of citrate buffer containing copper. An aliquot of 5 ml was taken in a separate test tube and incubated at 75 ^{0}C for 30 minutes with constant agitation. After this, the sample was cooled and finally read at 320 nm in a spectrophotometer (Al-Ghannam, 2008). The residual non-incubated fraction of the buffered supernatant was used as blank. The actual concentration of ampicillin was estimated by consulting a previously constructed standard curve using different concentrations of ampicillin.

Statistical analysis

The chi-square (χ^2) was used to adjudge the difference between the plasma concentration of ampicillin administered alone and after thiopentone anaesthesia.

RESULTS

The mean (±SEM) plasma concentration of ampicillin administered alone and following thiopentone sodium anaesthesia in rabbits is shown in table I. The result showed that ampicillin plasma concentration of 0.089±0.003 mg/ml was recorded at 15 minutes post treatment for the group administered ampicillin alone which decreased steadily with time to 0.075±0.001, 0.078±0.002, 0.063±0.005, 0.058±0.002, 0.025±0.002, and 0.012±0.001 at 0.5, 1, 3, 6, 12 and 24 hours post treatment respectively (Fig.1). The group administered ampicillin post recovery from thiopentone anaesthesia recorded plasma concentration of 0.083±0.004 mg/ml at 15 minutes post ampicillin administration. The concentration increased with time and at 3 hours, the concentration was 2.5±0.006 mg/ml, which afterward decreased to 1.43±0.005 mg/ml at 6 hours post treatment. The concentrations further increased to 3.33±0.003 before dropping to 2.5±0.001 mg/ml at 12 and 24 hours respectively post ampicillin administration after anaesthesia (Table I, Fig.2).

Table I. Plasma concentration of ampicillin sodium in rabbits, when administered alone and following thiopentone anaesthesia at different time intervals.

Time intervals (hours)	Mean (±SEM) concentration (mg/ml)	
	Ampicillin alone	Ampicillin following anaesthesia
0.25	0.089±0.003	0.083±0.004
0.5	0.075±0.001[a]	0.167±0.005[a]
1	0.078±0.002[b]	1.786±0.003[b]
3	0.063±0.005[c]	2.500±0.006[c]
6	0.058±0.002[d]	1.430±0.050[d]
12	0.025±0.002[e]	3.333±0.030[e]
24	0.012±0.001[f]	2.500±0.010[f]

Same superscripts on the same row denotes significant difference at P<0.05

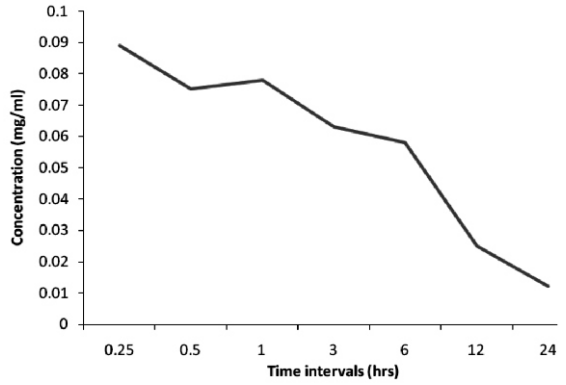

Fig. I. Plasma concentration of ampicillin administered alone to rabbits at a rate of 10 mg kg^{-1}.

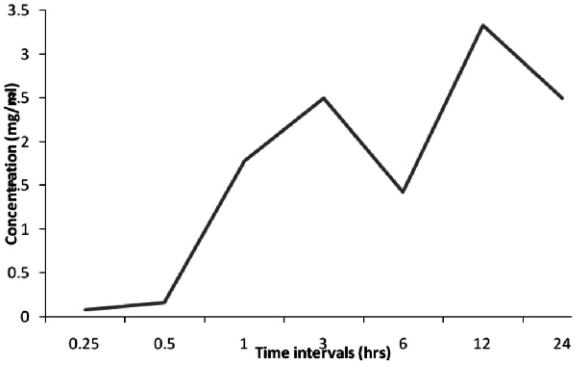

Fig. II. Plasma concentration of ampicillin administered to rabbits following thiopentone anaesthesia at a rate of 10 mg kg^{-1}.

DISCUSSION

The gradual decrease in plasma concentration of ampicillin in the first group have been supported by previous findings when administered intravenously in young calves (Long *et al.,* 1983), buffaloes, (Khanikor *et al.,* 1986) and rabbits (Olling *et al.,* 1995). Supportive findings were also obtained in kittens (Goldstein *et al.,* 1995), sheep (Montesissa *et al.,* 1994), goats (Elsheikh *et al.,* 1998) and in humans (Whyatt *et al.,*1974) on the pharmocokinetics of ampicillin following intravenous and oral administrations whereas Nawaz and Khan (1991) reported a longer elimination time of ampicillin among the local (Pakistani) sheep and goats. Variations between different animal species in elimination parameters might be related to differences in renal clearance and urinary excretion of ampicillin (Elsheikh *et al.,* 1997).

The increase in plasma concentration of ampicillin with time following thiopentone sodium anaesthesia could be as a result of hypotensive effect of thiopentone (Wood and Wood, 1990). Distribution kinetics of antibiotics would not necessarily follow normal patterns in haemodynamically depressed animal (Lavy *et al.,* 1995). Thiopentone has been shown to cause peripheral venodilation (Eckstein *et al,* 1961) and decreased renal blood flow (Onarheim and Tysseboth, 1980). These findings have supported the results of this study because ampicillin concentration was higher in the plasma of rabbits following thiopentone anaesthesia. Ampicillin is known to be excreted via urine and bile. For drugs like ampicillin with high hepatic and renal clearance ratio, clearance for such drugs are mainly determined by the liver and renal blood flow (Benowitz, 1984). It is also

possible that both thiopentone and ampicillin compete for the same transport channels in which case movement of ampicillin from blood vessels to tissues and organs of excretion will be reduced. The progressive increase in the saturation of hepatic metabolizing enzymes by thiopentone may also be responsible for increase in plasma concentration of ampicillin, as these enzymes will not be available for metabolising ampicillin (Wood and Wood, 1990). When vascular collapse and poor peripheral circulation are encountered such as in cases of hypovolaemia or septicaemic shock, substances administered into an animal are not absorbed rapidly enough and therefore may be inadequate to support large volume resuscitation and specific therapy (Orlowski, 1984). It is concluded that administration of thiopentone anaesthesia indeed altered the disposition of ampicillin in rabbits.

CONCLUSION

The results of this study did indicate that thiopentone anaesthesia alters the disposition of ampicillin when administered intravenously in rabbits. The outcome of this work may be helpful especially when considerations are made about antibiotic therapy following surgical procedures that may involve anaesthesia in order to allow practitioners to make appropriate dosage adjustment to avoid therapeutic complications.

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