THE PLASMA KINETICS OF SULBACTAM-AMPICILLIN ADMINISTERED TO CALVES BY THE INTRAMUSCULAR AND SUBCUTANEOUS ROUTES

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SUMMARY

Sulbactam-ampicillin combines ampicillin, a broad spectrum beta-lactam antibiotic, with sulbactam, an irreversible beta-lactamase inhibitor. The sulbactam component prevents the degradation of ampicillin by several major classes of bacterial beta-lactamases and restores the activity of ampicillin against most strains of bacteria in which resistance is mediated by beta-lactamase production.

A crossover study was conducted in Friesian calves of 98–119 kg bodyweight in which the plasma kinetics of sulbactam-ampicillin adminstered by the intramuscular and subcutaneous routes were defined, and the plasma kinetics of ampicillin derived from sulbactam-ampicillin and a commercially available formulation of ampicillin trihydrate were compared. Subsequent to both intramuscular and subcutaneous administration of sulbactam-ampicillin, peak plasma concentrations of sulbactam and ampicillin were recorded two hours post-injection. Higher peak plasma concentrations of both sulbactam and ampicillin were achieved by the subcutaneous route of administration and, for ampicillin, the difference between the two routes was statistically significant ($p<0{\cdot}01$). However, there was no significant difference in bioavailability (as measured by area under the curve) between the two routes of administration for either component. In addition, there were no significant differences between the peak plasma concentrations or areas under the curves for ampicillin derived from intramuscular administration of sulbactam-ampicillin, and ampicillin alone, indicating that combination with sulbactam does not alter the plasma kinetics of ampicillin.

INTRODUCTION

The beta-lactam antibiotics—ie, the penicillins and their semisynthetic derivatives—are extensively used in the treatment of bacterial diseases in cattle. The development of bacterial resistance, however, has reduced their value as therapeutic agents. In both Europe and North America, resistance to antibiotics is already a significant problem among bacteria which cause economically important diseases of cattle. (Jackson, 1981; Chang & Carter, 1976; Fairbrother *et al.*, 1979).

Resistance to beta-lactam antibiotics in both Gram-positive and Gram-negative bacteria is mainly due to bacterial production of beta-lactamases which degrade beta-lactam antibiotics and thus markedly reduce their activity. A solution to this problem is the combination of a broad spectrum beta-lactam antibiotic with a beta lactamase inhibitor. Amoxycillin potentiated by clavulanic acid is the only such combination available to date (Marshall *et al.*, 1982), but has a major disadvantage in cattle in that it is restricted to the oral route of administration and thus should only be used in pre-ruminant calves.

Sulbactam, a simple derivative of the penam nucleus, is an inhibitor of several major classes of beta-lactamases and has been developed for use as a parenteral formulation in combination with ampicillin. Sulbactam has no significant antibacterial activity against animal pathogens *per se*, but the combination of sulbactam with ampicillin is highly effective against ampicillin-resistant strains of bacteria both *in vitro* (Girard *et al.*, 1987) and *in vivo* (Farrington *et al.*, 1987).

Sulbactam-ampicillin has been developed as a pre-constituted suspension containing 60 mg sulbactam activity (as the benzathine dihydrate) and 120 mg ampicillin activity (as the trihydrate) per ml in an oil vehicle. As the product is designed for administration to cattle by either the intramuscular or subcutaneous routes, a cross-over study was instigated to investigate two aspects of the plasma kinetics. First, whether there are differences in the plasma kinetics of either component when the suspension is administered by the intramuscular or subcutaneous routes and second, whether the presence of sulbactam alters the plasma kinetics of ampicillin.

MATERIALS AND METHODS

Animals

Fifteen castrated male Friesian calves weighing between 98 and 119 kg were housed in experimental groups in three adjacent pens and fed twice daily on a conventional ration of calf pellets (BOCM 190 calf-rearing pellets) with hay and water *ad libitum*.

Experimental Design

The 15 calves were individually identified, weighed, and randomized into three groups on the basis of bodyweight. The design of the cross-over study is shown in Table I.

On day 0, 10 ml of whole blood were taken from the jugular vein of each calf into an evacuated glass tube containing lithium heparin (Vacutainer, Becton and Dickinson). Immediately thereafter, calves in group A were administered a single intramuscular injection of sulbactam-ampicillin at a dose rate of 6·6 mg ampicillin activity plus 3·3 mg sulbactam activity per kg bodyweight into the left cervical musculature. Calves in group B were administered a single subcutaneous injection of sulbactam-ampicillin at the same dose rate into the subcutaneous tissue overlaying the left deltoid muscle. Calves in group C were administered a single intramuscular injection of ampicillin trihydrate (Penbritin Injectable Suspension, Beecham Animal Health) at a dose rate of 6·6 mg ampicillin activity per kg bodyweight into the right cervical musculature. Further blood samples were taken from each calf at 0·5, 1, 2, 3, 4, 6, 8, 12 and 24 h subsequent to administration of sulbactam-ampicillin or ampicillin. Within 30 min of collection the blood was centrifuged at 4°C, the plasma harvested, divided into two equal aliquots and held frozen at −20°C. This treatment and sampling procedure was repeated commencing on days 4 and 8, with the formulation and route of administration being rotated through the three groups of animals as shown in Table I.

Table I
Design of cross-over study

Animal Group	*Number of Animals*	*Mean Bodyweight (kg)±SD on Day −1*	*Treatment*		
			Day 0	*Day 4*	*Day 8*
A	5	111·1±7·2	T1	T2	T3
B	5	110·8±7·7	T2	T3	T1
C	5	111·1±7·8	T3	T1	T2

T1—Sulbactam-ampicillin: single injection at a dose rate of 6·6 mg ampicillin activity/3·3 mg sulbactam activity per kg bodyweight by the intramuscular route.
T2—Sulbactam-ampicillin: single injection at a dose rate of 6·6 mg ampicillin activity/3·3 mg sulbactam activity per kg bodyweight by the subcutaneous route.
T3—Ampicillin-trihydrate: single injection at a dose rate of 6·6 mg ampicillin activity per kg bodyweight by the intramuscular route.

Assays of Sulbactam and Ampicillin

Plasma concentrations of sulbactam and ampicillin were measured using a large plate agar diffusion technique (Sutherland & Robinson, 1978). The assay used two different bacteria for the assay of sulbactam and ampicillin. *Pasteurella haemolytica* strain 59B010 is insensitive to high concentrations of either sulbactam or ampicillin alone. However, as the resistance of this strain to ampicillin is beta-lactamase mediated, the culture responds to ampicillin with sulbactam, synergistically. Thus one aliquot of plasma was assayed for ampicillin using *Sarcina lutea* ATCC 9341 (susceptible to ampicillin but not to sulbactam) and the other for sulbactam in the presence of ampicillin using *P. haemolytica* strain 59B010. The assay procedure used Muller-Hinton agar, and compared sample concentration against a standard curve in the range 0·125—12·0 mcg/ml.

Statistical Analyses

The peak concentration (Cmax) and the area under the curve (AUC; calculated using the trapezium rule) were analysed on the log scale to achieve normality and homogeneity of variances. Any values less than the lower limit of assay sensitivity (0·125 mcg/ml) were taken as zero. As most of the 12 h post-injection values were below this limit, only observations made at or before this time point were considered in the analyses. The ANOVA tables which formed the basis of statistical testing, allowed for differences between calves, times and treatments (Cochran & Cox, 1957), and were constucted by fitting a sequence of linear models to the data. Confidence limits for differences between the means were calculated on the log scale and back-transformed. Hence, they are the confidence limits for the ratio of two geometric means.

RESULTS

The mean plasma concentrations of sulbactam and ampicillin, following intramuscular and subcutaneous administration of sulbactam-ampicillin, are recorded in Figures 1 and 2, respectively. In individual calves peak plasma concentrations of sulbactam and ampicillin occurred between one and four hours after administration of sulbactam-ampicillin

by either route. The mean peak plasma concentration of ampicillin achieved by the subcutaneous route was 49% greater than by the intramuscular route (Table II), and this difference was statistically significant (p=0·0059). The mean peak plasma concentration of sulbactam achieved by the subcutaneous route was also greater, by 36%, but the difference was not statistically significant (p=0·0502). There were no significant differences between the two routes of administration of sulbactam-ampicillin in the AUCs for either sulbactam or ampicillin (Table III).

The mean plasma concentrations of ampicillin following intramuscular administration of sulbactam-ampicillin and ampicillin alone are compared in Figure 3. There were no significant differences between sulbactam-ampicillin and ampicillin alone in either mean peak plasma ampicillin concentrations (Table II) or AUCs for ampicillin (Table III).

DISCUSSION

The results of this cross-over study demonstrate that the bioavailabilities of sulbactam and ampicillin, as measured by AUCs, are similar when on oil suspension of sulbactam-ampicillin is administered by either the intramuscular or subcutaneous routes. Various factors, including the site and route of administration and the surface area of the injected bolus, can markedly affect bioavailability of a drug (Marshall & Palmer, 1980; Bogan, 1983). In recent years, investigations of these factors indicate that in animals, as in humans, the assumption that intramuscular administration of a drug results in greater bioavailability than subcutaneous administration is not necessarily correct. The results of this study, in respect of sulbactam-ampicillin, are consistent with those obtained for ampicillin and amoxycillin by Marshall & Palmer (1980) which demonstrate that there is no significant difference in bioavailability between subcutaneous administration over the neck or ribs and intramuscular administration into the rump. Similarly, the higher peak plasma concentrations of sulbactam and ampicillin obtained with subcutaneous, as compared to intramuscular, administration of sulbactam-ampicillin, parallels the findings of Marshall & Palmer (1980) in which higher peak serum concentrations were observed when ampicillin was injected subcutaneously over the ribs compared with into the gluteus medius muscle.

Comparison of the plasma kinetics of ampicillin when derived from sulbactam-ampicillin, and ampicillin alone, confirms that the combination of sulbactam with ampicillin does not significantly affect the plasma kinetics of the ampicillin component. Thus, the well-established therapeutic properties of ampicillin are maintained in this combination. The presence of sulbactam, an irreversible inhibitor of beta-lactamases, enables the bacteriocidal activity of ampicillin to be expressed against strains of bacteria which would otherwise be resistant due to beta-lactamase production. The efficacy of sulbactam-ampicillin in the treatment of field cases of disease caused by bacteria with resistance to beta-lactam antibiotics has been confirmed in trials conducted in both housed calves and feedlot cattle with respiratory disease (Grimshaw *et al.*, 1987a; Risk & Bentley, 1987; Bentley & Cummins, 1987), and in calves with neonatal diarrhoea (Grimshaw *et al.*, 1987b).

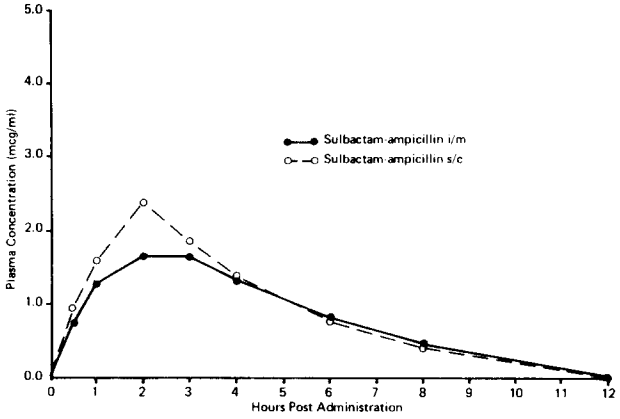

Fig. 1. Mean plasma concentrations of sulbactam subsequent to administration of sulbactam-ampicillin by the intramuscular and subcutaneous routes.

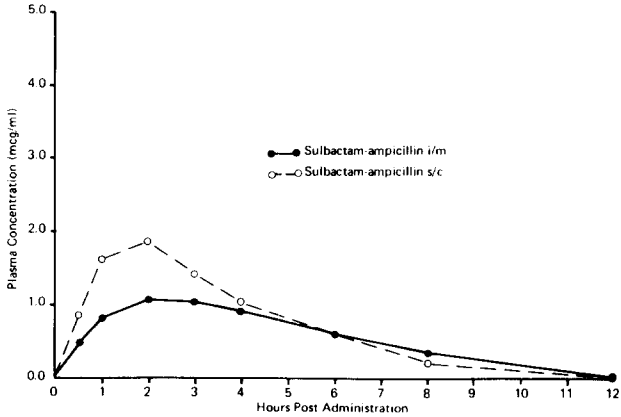

Fig. 2. Mean plasma concentrations of ampicillin subsequent to administration of sulbactam-ampicillin by the intramuscular and subcutaneous routes.

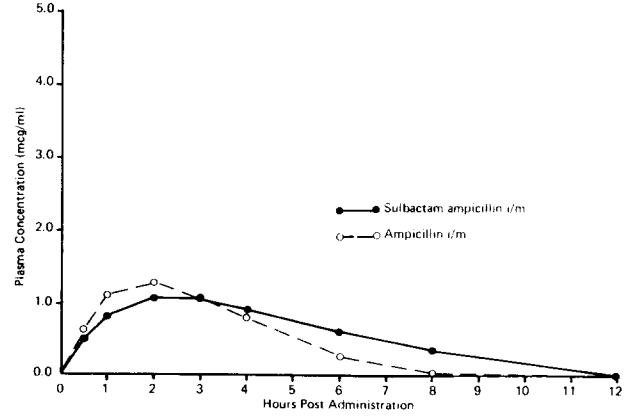

Fig. 3. Mean plasma concentrations of ampicillin subsequent to administration of sulbactam-ampicillin and ampicillin alone by the intramuscular route.

Table II

Comparison of the mean peak plasma concentrations of sulbactam and ampicillin achieved by intramuscular (im) and subcutaneous (sc) administration of sulbactam-ampicillin and intramuscular administration of ampicillin alone

Variable Analysed	*Treatment Group*	*Mean Peak Plasma Concentration (Geometric mean) (mcg/ml)*	*Percentage Difference from T1*	*95% Confidence Limits**		*p-Value*
				Lower	*Upper*	
Peak plasma concentration of Sulbactam	T1 Sulbactam-ampicillin im	2·01	NA	NA	NA	NA
	T2 Sulbactam-ampicillin sc	2·73	36%	−1%	86%	0·0502
Peak plasma concentration of Ampicillin	T1 Sulbactam-ampicillin im	1·28	NA	NA	NA	NA
	T2 Sulbactam-ampicillin sc	1·91	49%	14%	97%	0·0059
	T3 Ampicillin im	1·35	6%	−20%	39%	0·6829

*A value between the upper and lower confidence limits for the difference in means would not be rejected as the 'true' difference in means by a test which took that value in the null hypothesis. Thus, if the range does *not* include zero, the difference is statistically significant at 5% or less (cf. the final column).

Table III

Comparison of the areas under the curves (AUCs) for sulbactam and ampicillin achieved by intramuscular (im) and subcutaneous (sc) administration of sulbactam-ampicillin and intramuscular administration of ampicillin alone

Variable Analysed	*Treatment Group*	*AUC (Geometric mean) (mcg h/ml)*	*Percentage Difference from T1*	*95% confidence Limits**		
				Lower	*Upper*	*p-Value*
AUC for Sulbactam	T1 Sulbactam-ampicillin im	10·36	NA	NA	NA	NA
	T2 Sulbactam-ampicillin sc	11·58	12%	−6%	34%	0·1942
AUC for Ampicillin	T1 Sulbactam-ampicillin im	7·49	NA	NA	NA	NA
	T2 Sulbactam-ampicillin sc	8·97	20%	−4%	49%	0·1055
	T3 Ampicillin im	6·05	−24%	−54%	1%	0·0569

*See Table II.

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