# PHARMACOKINETICS OF SULBACTAM AND AMPICILLIN IN MICE AND IN DOGS

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**ABSTRACT** Sulbactam is a new beta-lactamase inhibitor. The pharmacokinetic characteristics of sulbactam was similar to ampicillin after a single intravenous injection of 200 mg/kg in mice with a half-life of approximately 50 min. The two drugs appear to equilibrate rapidly between central and peripheral compartments. The Vc and Vd suggest that they are widely distributed in the extracellular fluid and into tissues. Co-administration of sulbactam and ampicillin in mice and in dogs showed essentially no change on the kinetics of either ampicillin or sulbactam.

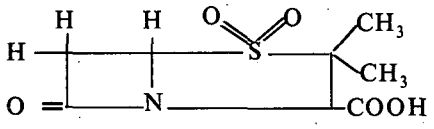
**Key words** Sulbactam; Ampicillin; Pharmacokinetics

Sulbactam (penicillanic acid sulfone, cp-45899) is a new beta-lactamase inhibitor presently under clinical investigation [1,2]. The combination of sulbactam and ampicillin in a 1 : 1 weight ratio was shown to produce potent, synergistic antibiotic activity in mice infected with some ampicillin-resistant strains. Potent antibiotic activity of co-administration of the two compounds has been demonstrated against these infections after both oral and parenteral administration [3].

The present report describes the pharmacokinetics of sulbactam and ampicillin in mice and in dogs after given separately or in combination by intravenous injection.

## MATERIALS AND METHODS

Sulbactam, {3,3-dimethyl-7-oxo-4-thio-1-azabicyclo (3,2,0) heptane-2-carboxylic acid, 4, 4-dioxide, [2s-(2α, 5α)]}, is a water-soluble, white crystalline solid with the following chemical structure:



A single intravenous injection of 200 mg/kg of sulbactam or ampicillin was given to mice. A combined dose of 200 mg/kg of sulbactam and ampicillin in a 1 : 1 weight ratio (100 mg/kg of sulbactam and 100 mg/kg of ampicillin) was given to mice and dogs. Following intravenous injection, blood samples were taken at 1, 5, 10, 20, 30, 60, 120, 150 and 180 min. The blood samples were centrifuged at room temperature after clotting and the serum was stored at -10 ℃ and assayed for drug concentrations

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within one week.

The concentrations of the two drugs in serum were determined by a thin layer chromatography (TLC) method established by our laboratory.

**Assay of sulbactam**

Serum (0.2 ml) was acidified with 0.2 ml of 0.25 mol/L hydrochloric acid and shaken with 0.5 ml of ethyl acetate for one min. The mixture was centrifuged at 10000 r/min for 10 min, and the ethyl acetate layer was separated. Portions (10 to 40 μl) of the extract was spotted on CMC – silica gel plate (20 × 20 cm or 10 × 20 cm). The plate was developed with an ethyl acetate–acetone–water–formic acid (65 :15 :13 :9, v/v) mixture to a height of 15 cm from origin. Then the plate was sprayed with 0.3 % ninhydrin alcohol solution and heated at about 110 ℃ for 20 min. All of the known metabolites of the two drugs could be separated on the TLC plate (Fig 1). The Rf value was 0.85 for sulbactam. Quantitative analysis was performed by a Shimadzu S – 910 Scanning Densitometer at 480 nm. The concentrations were calculated by an absolute calibration standard curve method. The sensitivity was 1 μg of sulbactam. The correlation coefficients of the peak area versus concentration were, in all cases, greater than 0.99. The TLC scanning result of sulbactam is shown in Fig 2.

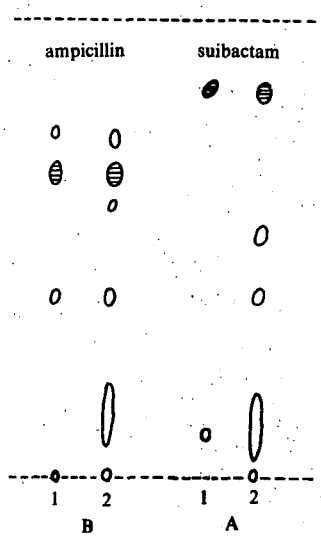


Fig 1. TLC graph of sulbactam and ampicillin
A. Sulbactam B. ampicillin 1. standard sample;
2. drug extracted from serum of dog injected drug.

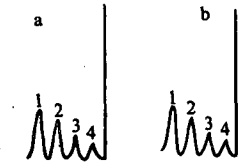


Fig 2. TLC record of different concentrations of sulbactam.
a. Standard solution; b. Extracted from dog serum containing drug.
1. 20 μg; 2. 10 μg; 3. 5μg; 4. 2.5μg.

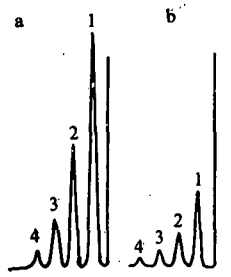


Fig 3. TLC record of different concentrations of ampicillin
a. Standard solution; b. Extracted from dog serum containing drug.
1 ~ 4 cf Fig 2.

**Assay of ampicillin**

Serum (0.2 ml) was added to 0.3 ml alcohol and acidified with 1 mol/L hydrochloric acid, then mixed and shaken for one min. The mixture was centrifuged at 10000r/min for 10 min. A portion (10 to 40 μl) of the alcohol solution was spotted on CMC – silica gel plate. The plate was developed, dyed and determined at 400 nm as described above. The Rf value was 0.65 for ampicillin and the sensitivity was 1 μg. The correletion coefficients of peak area versus concentration were, in all cases, greater than 0.99. The TLC scanning result of ampicillin is shown in Fig 3.

**Recovery and precision of quantitative assay**

The total absolute recovery for the two drugs in spiked serum was in range 70 ~ 85%. The test provided an excellent assay reproducibility. The within-day coefficient of variation for the two compounds was less than 10%.

**Pharmacokinetic parameters**

The experimental serum drug concentration versus time curves of sulbactam and ampicillin were in all cases fitted to a two-compartment pharmacokinetic model by computer BASIC program[4].

The model can be described as

$$C = A \times exp\ (-\alpha t) + B \times exp\ (-\beta t)$$

where, $\alpha$ is the slope of the distribution phase, $\beta$ is the slope of the elimination phase, A is the intercept of the distribution slope with the ordinate and B is the intercept of the back-extrapolated monoexponential elimination slope with the ordinate. The pharmacokinetic parameters were calculated from the fitted equation.

## RESULTS AND DISCUSSION

**Pharmacokinetics in mice**

The serum drug concentration-time curves after a single intravenous injection of sulbactam and ampicillin at 200 mg/kg are shown in Fig 4. The concentration changes with time for sulbactam could be expressed with the following equation:

$$C = 1186.6 \times exp\ (-0.0877 \times t) + 317.4 \times exp\ (-0.016 \times t)$$

and for ampicillin

$$C = 5249.9 \times exp\ (-0.1187 \times t) + 805.7\ exp\ (-0.012 \times t)$$

The concentrations of ampicillin were higher than those of sulbactam at all time points. The pharmacokinetic parameters of a single administration were calculated and are listed in Table 1.

**Tab 1. Pharmacokinetic parameters of sulbactam and ampicillin after intravenous injection to mice separately (each 200 mg/kg) or in combination (each 100 mg/kg)**

| Parameters | Separate injection | | Combined injection | |
|---|---|---|---|---|
| | Sulbactam | Ampicillin | Sulbactam | Ampicillin |
| $T_{1/2\alpha}$ (min) | 7.7±2.1 | 5.8±1.8 | 7.0±3.1 | 5.7±2.2 |
| $T_{1/2\beta}$ (min) | 43.1±10.5 | 57.8±1.8 | 53.0±9.8 | 60.0±13.4 |
| $K_e$ ($min^{-1}$) | 0.045±0.012 | 0.054±0.015 | 0.048±0.012 | 0.060±0.013 |
| $K_{21}$ ($min^{-1}$) | 0.032±0.007 | 0.026±0.007 | 0.032±0.011 | 0.048±0.009 |
| $K_{12}$ ($min^{-1}$) | 0.166±0.085 | 0.115±0.002 | 0.034±0.012 | 0.030±0.006 |
| Vc (ml/kg) | 132.9±12.7 | 33.0±5.6 | 105.0±13.5 | 40.0±6.7 |
| Vd (ml/kg) | 377.1±22.3 | 149.6±12 | 305.0±20.7 | 160.0±14.4 |
| CL (ml/min) | 6.0±0.9 | 1.7±0.4 | 3.9±0.8 | 1.8±0.4 |
| AUC ($\mu g$·ml·min) | 32941±1733 | 111368±15550 | 19841±1069 | 101666±8924 |

n = 9

The results show that the pharmacokinetic parameters of sulbactam are similar to those of ampicillin except AUC and CL. The drugs appear to equilibrate rapidly between the central and the peripheral compartments. The Vc, 133 ml/kg, and the Vd, 377.1 ml/kg, are over half of the total extracellular fluid and the total body fluid, and show that sulbactam is not only widely distributed into the extracellular fluid, but could also be widely distributed into the tissues.

When a combination of sulbactam and ampicillin was given to mice, the pharmacokinetic parameters were calculated from the concentration－time curves (see Table 1).

Comparison of Fig 5 with Fig 4 shows that the concentration－time curves of the two drugs are very similar after separate and combined administrations. Comparison of the pharmacokinetic parameters indicated that co－administration of sulbactam with ampicillin has essentially no effect upon the kinetics of either ampicillin or sulbactam.

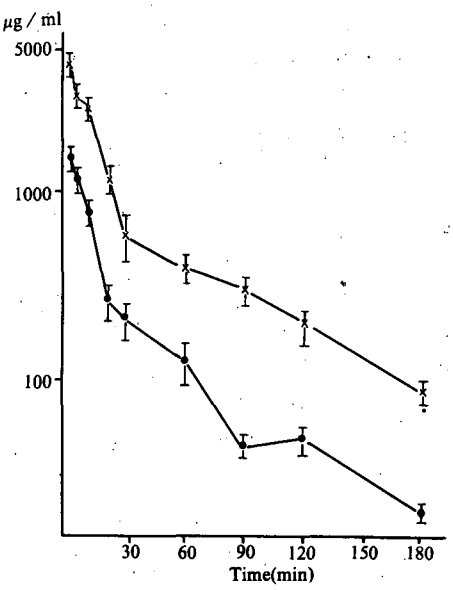


Fig 4. Serum drug concentration－time curves of sulbactam (·——·) and ampicillin (×——×) after single intravenous injection to mice (n＝9) at doses of 200 mg/kg ($\bar{x}\pm SD$).

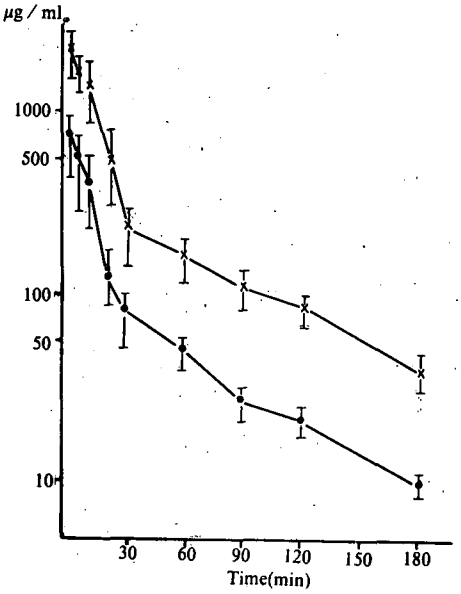


Fig 5. Serum drug concentration－time curves of sulbactam (·——·) and ampicillin (×——×) after combined intravenous injection to mice (n＝9) at doses of 100 mg/kg of each drug ($\bar{x}\pm SD$).

**Pharmacokinetics in dogs**

The serum concentration－time curves of sulbactam and ampicillin are shown in Fig 6 after a combined intravenous injection of the two drugs. The pharmacokinetic parameters are listed in Table 2. The results show that the kinetic characteristics do not differ between the two drugs.

Comparison of the pharmacokinetics in mice and in dogs showed no significant changes between the kinetic parameters after single administration and co－administration. However, higher clearance and larger volume of distribution for sulbactam compared with ampicillin was observed in mice, and higher clearance and larger volume of distribution for ampicillin compared with sulbactam was resulted in the dogs. The differences of CLs and

**Tab 2. Pharmacokinetic parameters of sulbactam and ampicilin after combined intravenous injection to dogs at a dose of 100 mg//kg of each drug**

| Parameter | Sulbactam | Ampicillin |
|---|---|---|
| $T_{1/2\alpha}$ (min) | 5.1±4.7 | 5.3±0.7 |
| $T_{1/2\beta}$ (min) | 57.5±10.2 | 48.1±11.0 |
| $K_e$ ($min^{-1}$) | 0.024±0.001 | 0.061±0.006 |
| $K_{21}$ ($min^{-1}$) | 0.109±0.076 | 0.032±0.008 |
| $K_{12}$ ($min^{-1}$) | 0.243±0.008 | 0.125±0.019 |
| $V_c$ (ml/kg) | 66.2±18.2 | 67.1±7.7 |
| $V_d$ (ml/kg) | 128.5±14.8 | 277.7±12.9 |
| CL (ml/min) | 1.5±0.3 | 4.1±0.6 |
| AUC (μg/ml·min) | 65722±1733 | 24870±4495 |

n=4

Vds for the two drugs between the two animal species are significant ($p<0.05$). The differences between the two drugs possibly resulted from animal species difference. Further study needs to be carried out to find out whether it is related to kinetic differences.

The concept of combining a beta-lactamase inhibitor such as sulbactam with a beta-lactam antibiotic such as ampicillin has a considerable appeal. The 1 :1 combination of sulbactam and ampicillin demonstrated that sulbactam significantly increases the activity of beta-lactam antibiotics in experimental and clinical studies[5,6]. In this study, ampicillin and sulbactam were given intravenously in equivalent doses. However, ampicillin showed a significantly higher concentration and larger AUC than did sulbactam. The result will be of great value in increasing the activity of beta-lactam antibiotics. The pharmacokinetic characterisitics of sulbactam appeared to be similar to those of ampicillin in single and combined administrations. This finding will be helpful to determine the clinical scheme of combination therapy.

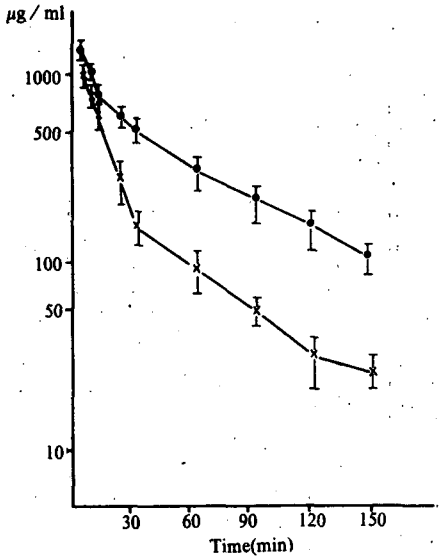


Fig 6. Serum drug concentration-time curves of sulbactam (·— ·) and ampicillin (×— ×) after combined intravenous injection to dogs (n=4) at doses of 100 mg/kg of each drug ($\bar{x}$ ±SD).

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# 青霉烷砜和氨苄青霉素在小鼠和狗的药代动力学

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**提要** 青霉烷砜是一新的β-内酰胺酶抑制剂。本文报告青霉烷砜与氨苄青霉素在小鼠和狗的药代动学,两药血清药物浓度采用TLC法测定.在小鼠分别静脉注射青霉烷砜和氨苄青霉素,前者的药代动力学特征与后者相似,半衰期约为50 min,两药均可在中央室和外周室间迅速平衡,并能广泛分布于细胞外液和组织.两药合并静脉注射给药,在两种动物上两药的药代动力学基本互不影响.

**关键词** 青霉烷砜;氨苄青霉素;药代动力学

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