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IN VITRO TISSUE PENETRATION STUDY OF SULBACTOMAX : A NOVEL FIXED DOSE COMBINATION OF CEFTRIAXONE AND SULBACTAM IN RATS

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ABSTRACT

The role of EDTA in tissue penetration was studied in present investigation. The present study has been designed to determine the most appropriate concentration of EDTA in FDC of ceftriaxone and sulbactam (Sulbactomax; 500 mg) in various tissues with three different concentration of EDTA. Total 18 rats were divided into three groups. Group I (n=6) treated with sulbactomax drug + EDTA (0.37mg/ml); group II (n=6) treated with sulbactomax + EDTA (3.7mg/ml) and group III (n=6) treated with sulbactomax + EDTA (37.0 mg/ml). The drug was analyzed by HPLC in various tissues. Our results showed that the concentration of ceftriaxone was statistically significantly (p<0.001) increased in kidney, lung and stomach tissues. Similarly, concentration of sulbactam was also found to be increased in kidney, liver and stomach tissues of group II as compared to the group I and III. The penetration of both drug was increased in group II of various tissues due to EDTA in comparison to ceftriaxone alone (without EDTA). EDTA competes with microorganism for any of the trace metal ions that are essential to the maintenance of their life cycle or it disturbs the integrity of cell membrane. Our finding concluded that sulbactomax combined with EDTA (3.7 mg/ml) has better tissue penetration in vitro and hence improves efficacy that might be effective against a variety of infections.

Key Words: *Fixed dose combination, Sulbactomax, EDTA, tissue penetration*

Introduction

Over the past 20 years, the increase in the prevalence of β-lactamase-producing strains of gram-positive and gram-negative bacteria has restricted the usefulness of β -lactam antibiotics. Sulbactomax is fixed dose combination of ceftriaxone and sulbactam antibiotic. Sulbactam is a molecule which is given in combination with the beta-lactam antibiotics to inhibit beta-lactamases, an enzyme produced by bacteria that destroys the antibiotics. Ceftriaxone is a third-generation cephalosporin antibiotic that exhibits a long half life[1]. Like other third-generation cephalosporins, Ceftriaxone has broad spectrum activity against Gram positive and Gram negative bacteria. It is commonly used in intensive care units, there are few data reported on the pharmacokinetics of ceftriaxone in critically ill patients. Ceftriaxone is partially eliminated by the kidneys about 60-70% of the total dose[2]. Sulbactam is able to inhibit the most common forms of beta-lactamases but is not able to interact with the amp C cephalosporinase. When Sulbactam combined with ceftriaxone or other β-lactams in a physical mixture, it restores their original activity both *in vitro* and *in vivo* [3]. Because

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of its greater stability in solution, Sulbactam may have an advantage over clavulanic acid as a companion drug to various β-lactam [4]. It is used in combination with ceftriaxone and other beta-lactam antibiotics to enhance the spectra [5]. Sulbactam is a beta-lactam agent that acts as an irreversible inhibitor of beta-lactamase activity by combining with the enzyme and rendering it inactive. In the present study, authors have tried to determine the *in vitro* tissue penetration of sulbactomax (a fixed dose combination of ceftriaxone and Sulbactam) drug in various tissues of healthy wistar rats.

Materials and Methods

Chemicals

Tetrabutyl ammonium hydroxide; (TBAH, AE 8AF58232), acetonitrile (SG8F80852) were purchased from Merck grade. Ceftriaxone and Sulbactam standard were purchased from Zhejianj Yonging and Harbin pharmaceutical company Ltd, China. Standard solution of ceftriaxone (25.0 mg) and Sulbactam (12.5mg) were prepared in 100 ml of distilled water. Sulbactomax (fixed dose combination of ceftriaxone and Sulbactam) drug was obtained from Venus Remedies Ltd. The ratio of sulbactomax drug was 2: 1 respectively.

Animals and groups

Eighteen wistar rats were (weighing 225 to 250 g) used in the experiment. They were housed at controlled temperature and humidity in an alternating 12-hr light and dark cycle with free access to food and water. The study was approved by the institutional animal ethical committee. The rats were divided into three groups of six rats each as given below.

Group I: Normal saline + sulbactomax + EDTA (0.37mg/ml; 0.001M) treated group

Group II: Normal saline+ sulbactomax+ EDTA (3.7mg/ml; 0.01M) treated group

Group III: Normal saline+ sulbactomax+ EDTA (37.0 mg/ml; 0.1M) treated group

In present study concentration of sulbactomax drug (Fixed dose combination of Ceftriaxone + Sulbactam) was used 500mg/ml in each group. Drug was dissolved in normal saline and three different concentration of EDTA(0.1M to 0.001M). All the rats were sacrificed under light ether and tissues (liver, lung, kidney, stomach, heart and intestine) were removed. These tissues were washed three times with normal saline (0.9% NaCl). The various tissue samples from the individual animals were examined for their antibiotic concentration separately.

Sample preparation

Each groups of different tissues were weighed (100 to 125mg) separately and kept into different concentration of EDTA, drug+ normal saline and incubated at 37 °C for 45 min. After 45 minutes tissues were removed and homogenized with 3.0 ml of chilled distilled water in an Ultra-Turrax homogenizer (Janke & Kunkel KG, FRG). The homogenates were immediately centrifuged at 8500 rpm for 15 minutes and supernatant were separated and kept in other tubes. One ml of acetonitrile were added into supernatant and mixed properly and left it for 10 minutes to precipitate the plasma proteins. Each samples were again centrifuged at 5000 rpm for 20 min at 0-4 °C and supernatant were aspirated out carefully for analysis of drug concentration.

Chromatographic eluent

A buffer solution consisted of 50 ml of tetrabutyl ammonium hydroxide (TBAH) in 1000 ml of distilled water and adjusted to pH 7.0 with

orthophosphoric acid. The solvent used for the mobile phase was a mixture of buffer–acetonitrile (70: 30). The mobile phase was passed through membrane filter (Millipore corp.), 0.45 µm pore size and deaerated under reduced pressure .

Ceftriaxone and Sulbactam drug analysis

For the analysis of Ceftriaxone and Sulbactam concentration in various tissues, 500 µl supernatant was mixed with 200 µl of mobile phase and shaken vigorously. The chromatographic separation was performed by high performance liquid chromatography (Agilent,1200 series, CA, USA) with a mobile phase containing buffer and acetonitrile. The column C-18 hypersil ODS (5 µ, 4.6 x 250mm) was used for the analysis of antibiotics. The flow rate and column temperature were maintained at 1.5 ml/min at 25°C respectively. After an equilibration of column with mobile phase for 2 hour, 20µl of sample was injected and detection of ceftriaxone and Sulbactam antibiotics was performed at 220 nm UV wavelength. Under these chromatographic conditions, the retention time of ceftriaxone and Sulbactam were found to be 6.9 and 3.5 minutes (Figure 1).

Statistical analysis

The resulting data was analyzed statistically. All values are expressed in mean ± SD. One-way analysis of variance (ANOVA) with student-Newman-Keuls comparison test was used to determine statistical difference between group I, group II and III. P values <0.05 were considered statistically significant.

Results and Discussion

Ceftriaxone and sulbactam were well tolerated when given either as a single agent or in combination. In present study, ceftriaxone and Sulbactam drug concentration were measured in various tissue organs at different concentration of EDTA (0.37mg/ml, 3.7 mg/ml and 37.0 mg/ml). In group II and group III ceftriaxone drug was distributed maximally significant ($p<0.001^{***}$) as compared with group I . The mean level of ceftriaxone drug was found higher in kidney tissue of group II (4.12 mg/ml) than group III (2.75 mg/ml). The mean levels of ceftriaxone drug in kidney tissue was found highly significant in group II and III in comparison to group I (0.37 mg/ml EDTA). Ceftriaxone drug concentration was found higher in lung (3.67 mg/ml) in comparison to group III (1.99 mg/ml). The mean level of ceftriaxone concentration was found very low in all various tissues of group I . The concentration of ceftriaxone drug was found higher in kidney, lung, stomach and heart tissue in group II .In case of group III, the concentration of ceftriaxone was found higher only in heart tissue as well as lower concentration was observed in lung, and kidney tissue. There was no significant change observed in liver and intistine tissues (Table 1). Similarly the concentration of Sulbactam was found higher and highly significant ($p<0.001^{***}$) in group II as compared to group I &III. When group I was compared with group III, there was no altered significant change observed in the level of Sulbactam drug in all tissues. When group I was compared to group II , the level of Sulbactam drug was found higher in kidney (2.00 mg/ml), liver (1.25mg/ml), stomach (1.66mg/ml) and intistine (1.19mg/ml) tissues and lesser in lung (1.14 mg/ml) and heart (1.16 mg/ml) tissues of group II respectively (Table 2). The percentage changes of ceftriaxone and Sulbactam drugs were presented in various tissues at two different concentration of EDTA (3.7mg/ml, 37.0 mg/ml) and drug+normal saline in Figure 2 and Figure 3. The levels of ceftriaxone and Sulbactam were not significantly increased in different tissues in group I after administartion of EDTA (0.37mg/ml)and

drug+normal saline in comparison to group II and group III. In choosing a β-lactam to accompany a β-lactamase inhibitor, it is necessary to ensure that the *in vitro* activity and the pharmacological properties are such that synergistic interaction occur *in vivo*. Sulbactomax is a fixed dose combination of ceftriaxone and Sulbactam antibiotic. There are several studies have been done on tissue distribution of combination of two drugs [6.] Ceftriaxone/sulbactam is unlikely to cover gram-negatives and gram-positive frequently implicated in post-neurosurgery procedures. Ceftriaxone is commonly used in intensive care units, there are few data on the pharmacokinetics of ceftriaxone in critically ill patients [7]. Ceftriaxone is excreted largely unchanged and is dealt with in approximately equal proportions by the liver (in bile) and kidneys, where it is eliminated by glomerular filtration. Ceftriaxone compound have the greater binding ratio to human serum proteins, and also have a distinctly longer half-life in humans 6-9 hr [8]. In the present study the concentration of Ceftraixone drug was observed higher in kidney, lung and stomach tissues of group II in comparison to group I and III. Higher concentration of ceftriaxone drug was found in lung tissue it means that the drug play a significant therapeutic role in management of pneumonia infection. Similar result was obtained by Bergeron *et al* in pneumonia infected mice model [9]. There are several reports suggested that sustained high tissue concentrations after administration of cephalosporin has been demonstrated in small animals, particularly in the lungs, liver and kidneys [10]. Numerous studies with ceftriaxone, a correlation between protein binding and pharmacokinetic behavior can also be observed in humans. Sulbactam belong to penicillanic acid sulfone which is a competitive and non competitive β-lactamase inhibitor [11-12]. It is co- administered with cefoperazone/ ceftriaxone to expand the antimicrobial spectrum to include β-lactam-resistant microorganism. Co-administration of Sulbactam with tested beta lactam antibiotics including ceftriaxone, cefoperazone did not significantly alter the pharmacokinetics of either drug. *In Vitro* the combination of cephalosporin and Sulbactam shows a marked degree of synergy against some microorganisms which is resistant to cephalosporins. For this reason, simultaneous administration of ceftriaxone-Sulbactam may improve the therapeutic role under certain clinical conditions. In this penetration study of sulbactomax, the renal clearance of Sulbactam was found approximately 50% lower than ceftriaxone. The concentration of Sulbactam drug was found higher in kidney, liver and stomach tissues of group II as compared to group I and III. The higher concentration of Sulbactam were found in kidney, liver and stomach tissues and lesser concentration were found in lung, heart and intestine tissues. Similarly this result was reported by English *et al* in mice, rats and dogs [13]. Higher concentration of both drugs were observed in kidney, liver, lung , stomach tissues of group II in comparison to group I and III. The level of ceftriaxone +Sulbactam was highly increased in different tisssues of group II due to EDTA.

EDTA (ethylene diamaine tretra acetate) is potent chelating agent which suppress hydroxyl radical formation by fenton reaction with the increase of its concentration. Besides chelating property, EDTA has a antimicrobial property which plays a significant role in growth inhibitory effect against several microbes [14]. The mechanism of EDTA is likely that it competes with microorganism for any of the trace metal ions which are essential to the maintenance of their life cycle [15], or it disturbs the integrity of cell membrane [16]. It has a high affinity for the potentially catalytic form of iron, and removes it from the body [17].

EDTA can prevent the peptide degradation by the metalloprotease [18]. EDTA penetrates the cell membrane and open the Ca^{+2} Channel and enhanced the concentration of drug in the body. This study concluded that combination of sulbactomax drug with 3.7 mg/ml (0.01M) of EDTA concentraion is most effective which provide a wide distrbution to the various tissues and play a theraputic role against bacterial infections produced by β-lactam-resistant as well as β-lactam-susceptible microorganisms.

Table 1: Status of ceftriaxone plus Sulbactam drug in three different concentration of EDTA in various tissues.

S.No.	Tissue	Ceftriaxone (mg/ml) EDTA			Sulbactam (mg/ml) EDTA		
		0.37mg/ml G (I)	3.7mg/ml G (II)	37.0 mg/ml G (III)	0.37mg/ml G (I)	3.7mg/ml G (II)	37.0mg/ml G (III)
1	Kidney	0.53 ± 0.40	4.12 ± 0.54 [a]	2.75 ± 0.05[a]	1.14 ± 0.11	2.00 ±0.35[a]	1.17 ±0.05[d]
2	Lung	0.16 ± 0.04	3.67 ± 1.23 [a]	1.99 ± 0.65[b]	1.02 ± 0.21	1.14±0.22[d]	1.05 ±0.14[d]
3	Liver	0.38 ± 0.27	2.03 ± 0.74[a]	2.03 ± 0.42[a]	1.09 ± 0.08	1.25 ±0.26[d]	1.16 ±0.17[d]
4	Heart	0.28± 0.12	2.55± 0.45[a]	3.13 ± 0.37[a]	0.91 ± 0.09	1.16 ±0.27[d]	1.14 ±0.22[d]
5	Intestine	0.35 ± 0.11	2.44 ± 0.60[a]	2.48 ± 0.53[a]	0.88 ± 0.12	1.19 ±0.23[c]	1.15 ±0.14[c]
6	Stomach	0.17± 0.04	3.02 ± 1.41[a]	2.88± 1.13[a]	1.29 ± 0.16	1.66 ±0.38[d]	1.24 ±0.36[d]

Data are expressed in Mean ± SD . Where G is group. a = p<0.001*** (highly significant), b = p<0.01** (significant), c = p <0.05* (less significant), d = p>0.05 (not significant)

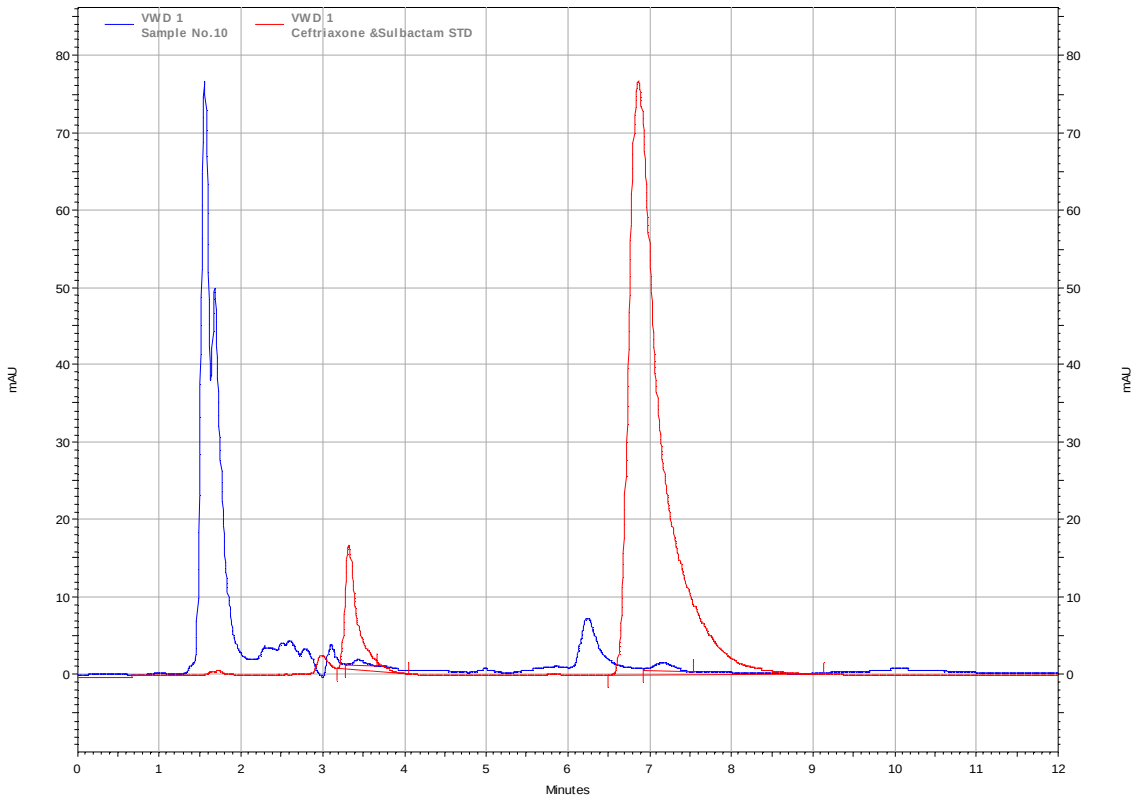

Fig. 1: Chromatogram of ceftriaxone and Sulbactam in tissue: Peak A (Red) shows the Sulbactam (RT about = 3.5min) and peak B (Red) shows Ceftriaxone (RTabout =6.9 min) antibiotic (Standard) and Similar C (Blue) and D (Blue) peaks was found in sample of tissues.

Percentage distribution of ceftriaxone drug combined with different concentration of EDTA in various tissues

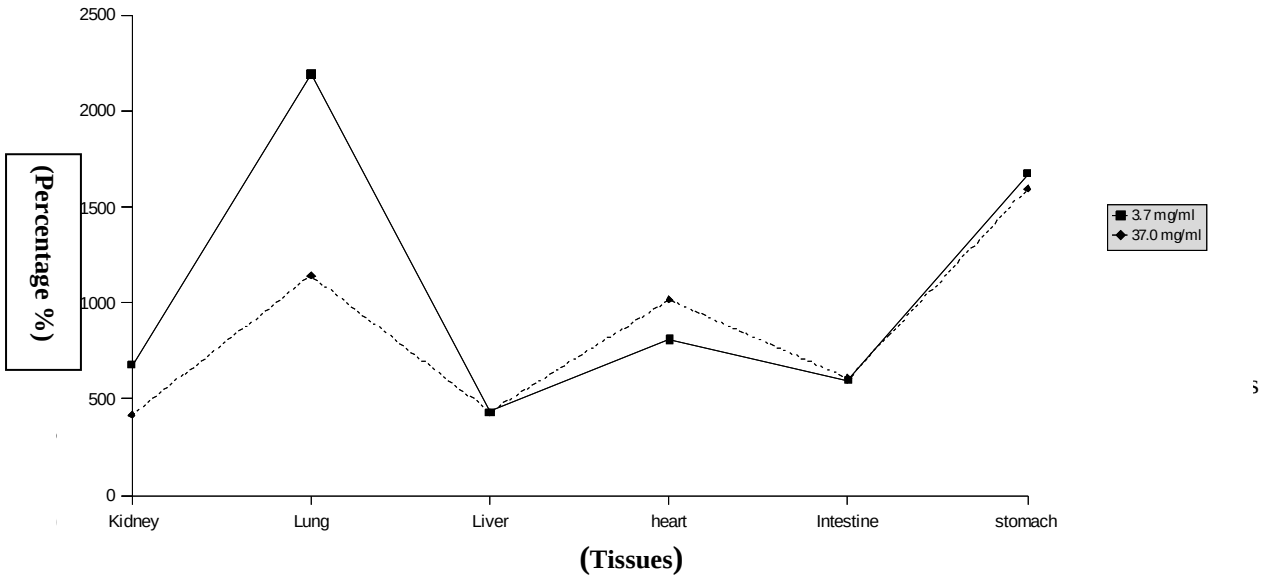

Fig.2: Value are expressed are Mean. All values are parenthesis % changes between3.7mg/ml, 37.0mg/ml vs 0.37 mg/ml concentration of EDTA.

Percentage distribution of ceftriaxone drug combined with different concentration of EDTA in various tissues

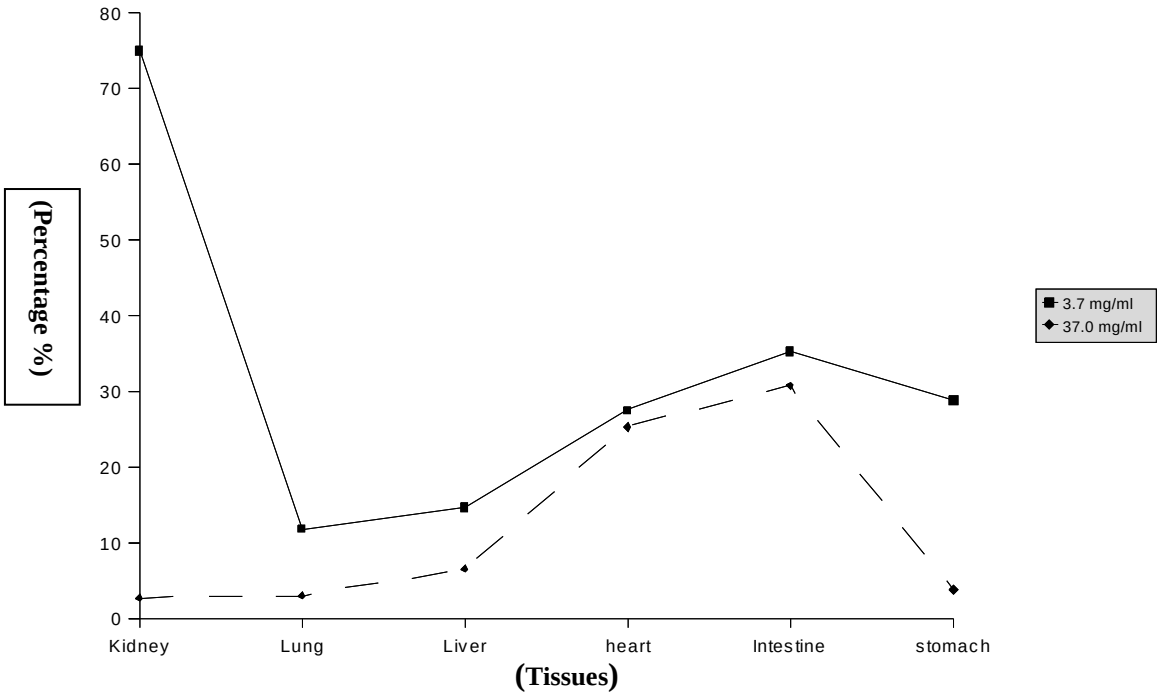

Fig.3: Value are expressed are Mean. All values are parenthesis % changes between3.7mg/ml, 37.0mg/ml vs 0.37mg/ml concentration of EDTA.

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