STUDIES ON TRANSFER OF INJECTED CEFMENOXIME TO EXUDATES (BLISTER) OF WOUNDS OF BURN PATIENTS

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The transport of cefmenoxime, a cephalosporin antibiotic, into three kinds of exudates (exudate from excoriated skin, exudate from burn wounds, and burn blister fluid) from blood was studied on 37 burn patients after they were given an intravenous bolus of 50 mg cefmenoxime per kg body weight. Concentrations of cefmenoxime in serum and in the above exudates or fluid were determined by bioassay. Concentrations of cefmenoxime in serum reached 189±29.7 μg/ml (mean±SD) 15 min after administration, decreasing to 4.8±1.3 μg/ml at 8 hr. Peak concentrations of the antibiotic in exudates from burn wounds and excoriated skin and in blister fluid of burn patients were all found at 1 hr. Calculated by a two-compartment open model, the half-life of the elimination phase ($t_{1/2(\beta)}$) was 1.53 hr ; volume of distribution ($V_{d\beta}$), 0.33 l/kg ; and area under the curve (AUC), 346 μg·hr/ml. C_{max} (maximum concentration), T_{max} (time of maximum concentration), and apparent transport ratios ($F : k_1V_e/k_2V_e$) for exudates from excoriated skin, exudates from burn wounds, and burn blister fluid were 34.9, 19.7, 22.9 μg/ml ; 1.39, 1.47, 2.03 hr ; and 0.47, 0.28, 0.43, respectively.

Introduction

To control the burn wound infection, antibiotics have been administered topically[1] or systemically to burn patients. When antibiotics are administered systemically, it is important to obtain information about the efficacy of transfer of the antibiotics from the blood to the burn wounds for proper control of burn wound infections. So far, transfer of various antibiotics administered to the skin of healthy subjects or to exudates elicited from them have been studied using homogenates of skin[2,3] or exudates (blister) induced artificially by the skin window technique (dermabrasion)[4,5,6], dermal suction[7,8], or application of cantharidin ointment[9,10,11,12].

In the present study, cefmenoxime concentrations in three kinds of exudates (from excoriated skin, from burn wounds, and burn blister fluid) from the wounds of burn patients were determined over a 3-5 min period after a bolus injection of the antibiotic. The transport of cefmenoxime from serum to the exudates was studied, and the data were analysed by pharmacokinetics.

I. MATERIALS AND METHODS

Cefmenoxime, a new cephalosporin derivative[13], (7 β-[2-(2-aminothiazol-4-yl)-(Z)-2 methoxyimino-acetamido]-3-[(1-methyl-1 H-tetrazol-5-yl) thiomethyl] ceph-3-em-4-carboxylic acid), was provided by Takeda Chemical Industries Ltd., Osaka, Japan, for use in this study.

Subjects used in this pharmacological study of cefmenoxime were 37 burn patients (25 males and 12 females between 16 and 67 years of age) with a mean body weight of 52 kg. The trial was conducted on inpatients in the Burn Center who agreed to take part in the study after it was explained to them. They had no former special medical his-

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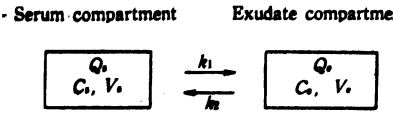
tory, and their kidney and liver functions were within the normal range. Among the 37 patients, 6 were used for the study of blood levels of cefmenoxime, 15 for the level in exudates from excoriated skin, 8 for that in exudates from wounds and 8 for the blister fluid level. The dose of cefmenoxime used in the burn patients was set at 50 mg per kg and delivered by bolus injection, because the authors observed clear clinical effects with this dose. After the injection, blood samples were taken at 15, 30, 60 min, and thereafter hourly until 8 hr had passed. Samples of the sera were obtained by the usual centrifugation method. Exudate samples were obtained at 30 min and thereafter hourly through the eighth hour.

Burn blister fluid (0.5-1 ml) was aspirated by syringe from blisters formed on the surfaces of second degree burn wounds of patients. The exudate fluids (30-50 μl) on excoriated skin and on the burn wounds were collected by the aid of filter paper disks (WHATMAN 13 mm AA disk). The sample laden disks were weighed and stored at −20°C until the determination of the drug concentration was made (within 10 days of collection).

The assays were performed by an agar plate diffusion technique with *Escherichia coli* NIHJ JC-2 supplied from National Hygienic Institute, Tokyo, and plate medium (No. 2, pH 6.4-6.6, Daigo Nutritive Chemicals Ltd., Osaka). Standard curves for determining serum and exudate antibiotic levels were prepared by using known concentrations (1.25-40 μg/ml) of cefmenoxime in pooled human serum. No inhibition of bacterial growth was observed in plates which contained sera or exudates from burn patients who had not been given cefmenoxime. To diffuse the sample on plate medium sufficiently, the plates were stored at 4°C for 1 hr prior to incubation. Incubation was carried out for 18 hr at 37°C before the diameters of inhibition zones were measured.

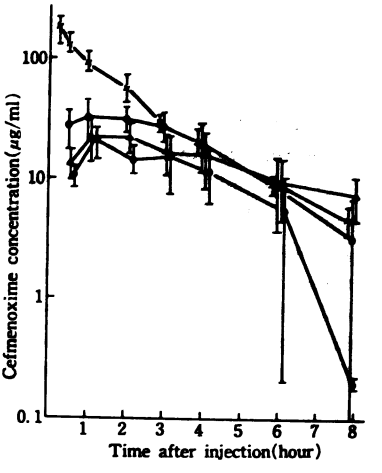
All data about the drug level in sera of the 6 patients were pharmacokinetically analyzed by IBM 3081 type computer using a two-compartment open model program. While the data for the exudates were analyzed using a modified two-compartment open model method as follows: In a two-compartment open system, there occurs the following relationship as shown in Fig. 1. Q_s, C_s, and V_s represent amount, concentration, and distribution

Fig. 1 Two compartment model for analysis of distribution of cefmenoxime in serum and various exudates



Q_s, Q_e : amount of drugs in serum or exudate(blister)
C_s, C_e : concentration in serum or exudate(blister)
V_s, V_e : distribution volume of serum or exudate(blister)
k_1, k_2 : transport rate constant

Fig. 2 Cefmenoxime concentrations in serum (×), exudates from excoriated skin (○) and burn wounds (●), and burn blister fluid (△) after bolus injection (50 mg/kg) of the antibiotic to 37 burn patients.



volume of drug in compartment 1 (serum), while Q_e, C_e, V_e are those in compartment 2 (exudate), respectively. k_1 and k_2 represent the rate constants of the equilibrium, and F (apparent transport ratio) is $k_1 \cdot V_s / k_2 \cdot V_e$. F is expressed as the transport ratio from serum to exudate. When $V_s = V_e$ is obtained (usually $V_s \gg V_e$), F is the veritable transport ratio. The transfer rate of the drug from serum into the exudate can be expressed by equation (I):

$$\frac{dQ_e}{dt} = k_1 Q_s - k_2 Q_e \qquad (I)$$

The rate of increase in the concentration of the drug in the exudate can be also expressed by equation (II):

$$\frac{dC_e}{dt} = \frac{k_1 V_s}{V_e} C_s - k_2 C_e = k_2 (F \cdot C_s - C_e) \qquad (II)$$

And the decrease in drug concentration in the serum can be determined by the well known equa-

Table 1 Cefmenoxime concentrations in serum, exudates from excoriated skin and burn wounds, and burn blister fluid after bolus injection (50 mg/kg) of the drug to 37 burn patients.

hrs	Serum (n=6)	Exudate from excoriated skin (n=15)	Exudate from burn wound (n=8)	Burn blister fluid (n=8)
0.25	189. ±29.7			
0.5	133. ±20.3	27.4±10.5	10.8±2.6	13.9±4.1
1	96.0±18.0	32.4±12.7	21.3±6.4	22.8±8.8
2	59.5±15.3	31.2± 7.7	14.8±3.7	22.7±6.0
3	50.0± 4.7	28.7± 8.1	15.4±7.7	17.3±6.5
4	20.9± 4.8	20.4± 9.0	11.7±5.7	16.2±8.0
6	9.7± 2.6	9.0± 6.6	5.2±5.0	9.7±5.3
8	4.8± 1.3	3.2± 3.9	0.2±0.05	7.7±2.9

tion (Ⅲ).

$$C_e = A_1 e^{-\alpha t} + B_1 e^{-\beta t} \quad (Ⅲ)$$

A_1 and B_1 are the extraporated intercepts of the distribution and elimination curves.

Then, when the C_s term of equation(Ⅱ) is substituted by equation(Ⅲ), the differential equation is solved to obtain equation(Ⅳ) as follows:

$$C_e = F \cdot k_2 [A_2 e^{-\alpha t} + B_2 e^{-\beta t} - (A_2 + B_2) e^{-k_2 t}] \quad (Ⅳ)$$

$$A_2 = A_1/(k_2 - \alpha) \quad (Ⅴ)$$

$$B_2 = B_1/(k_2 - \beta) \quad (Ⅵ)$$

The values of A_2 and B_2 are obtained from equations(Ⅴ) and (Ⅵ), respectively, where the values of α and β are calculated from the slopes of the distribution and elimination curves, respectively, of the drug in serum (Fig. 2).

The appropriate values for F and k_2 were calculated by an IBM 3081 type computer using equation (Ⅳ) with the values of A_2, B_2, α, and β. The F value is theoretically the same as AUC_e/AUC_s.

$V_{d\beta}$ and AUC values were calculated from the following equations.

$V_{d\beta} = k_e V_s/\beta$, $AUC = Dose/k_e V_s$ where k_e represents the elimination rate constant.

II. RESULTS

Changes in cefmenoxime concentrations in serum and various exudates of burn patients after a bolus injection of cefmenoxime. The changes in cefmenoxime concentrations in serum after a bolus injection of the drug (50 mg/kg) are shown in Table 1 and Fig. 2. Cefmenoxime concentrations in serum reached 189±29.7 μg/ml (mean±SD) at 15 min and then decreased to 4.8± 1.3 μg/ml by 8 hr. Split-thickness skin autografts made with a knife are preferred for replacing the skin lost from burn surfaces in the 2nd week after the burn episode and thus provide a suitable source of excoriated skin. The exudate from the donor site was collected hourly with a sterile filter paper disk. The peak concentration in the above exudate from excoriated skin reached 32.4±12.7 μg/ml at 1 hr which was the highest concentration among the three kinds of exudates as shown in Table 1 and Fig. 2. Cefmenoxime concentrations in the exudates from burn wounds were then determined and were found to be lower than those in the other two kinds of exudates. The drug concentration in the exudate from burn wounds peaked at 21.3±6.4 μg/ml at 1 hr and then decreased to less than 1 μg/ml by 8 hr (Fig. 2). On the other hand, the cefmenoxime concentrations in burn blister fluid increased gradually after antibiotic administration and reached 22.8±8.8 μg/ml at 1 hr; it then decreased slowly to a higher level than that found in serum at the same time point (Table 1 and Fig. 2).

Pharmacokinetic analysis of cefmenoxime concentrations in serum and exudates of burn patients following bolus injection of the drug. Changes in cefmenoxime concentrations in serum after its bolus injection were analyzed using a two-compartment open model method to obtain the pharmacokinetic parameters. The rate constants (α, β) of distribution and elimination phases were 5.01 and 0.47. The half-life of the elimination phase, $t_{1/2(\beta)}$, was 1.53 hr. A distribution volume ($V_{d\beta}$) of 0.33 l/kg and an AUC of 346 μg·hr/ml are shown in Table 2. No significant difference between these parameters and those of healthy volun-

Table 2 Pharmacokinetic parameters of cefmenoxime in serum after its bolus injection (50 mg/kg)

Drug	α (hr^{-1})	β (hr^{-1})	$t_{1/2(\beta)}$ (hr)	$V_{d\beta}$ (1/kg)	AUC ($\mu g \cdot hr/ml$)
Cefmenoxime[a]	5.01±3.03	0.47±0.10	1.53±0.28	0.33±0.09	346±28

[a] Numbers represent the arithmetic means ± standard deviation.

Table 3 Pharmacokinetic parameters of cefmenoxime in exudates after its bolus injection (50 mg/kg)

	C_{max} (μg/ml)	T_{max} (hr)	$t_{1/2}$ (hr)	k_2[b] (hr^{-1})	F[c]
Exudate from excoriated skin[a] (n=15)	34.9±7.8	1.39±0.46**	1.58±0.18*	0.78±0.36**	0.47±0.10**
Exudate from burn wound[a] (n=8)	19.7±3.3**	1.47±0.47*	1.59±0.23	0.71±0.33	0.28±0.07
Burn blister fluid[a] (n=8)	22.9±6.6**	2.03±0.35	1.94±0.45	0.40±0.12	0.43±0.14*

[a] Numbers represent the geometric means ± standard deviation.
[b] Transport rate constant from exudate to serum.
[c] Apparent transport ratio.
(* $P<0.05$, ** $P<0.01$)

teers[14] was found.

Changes in cefmenoxime concentrations in exudates were then analyzed using our modified two-compartment open model method as described in methods and are shown in Table 3. In the case of exudates from excoriated skin, C_{max} and T_{max} were calculated to be 34.9 μg/ml and 1.39 hr, respectively (Table 3). The F value was found to be 0.47. In the case of exudates from burn wounds, C_{max}, T_{max}, and F were calculated to be 19.7 μg/ml, 1.47 hr and 0.28, respectively (Table 3). The curves of cefmenoxime levels in exudates from excoriated skin wounds and burn wounds were shown to be similar. Both of them attained a peak concentration at about 1.4 hr and decreased with almost the same half-life of 1.6 hr.

Cefmenoxime concentrations in burn blister fluid increased gradually after drug administration, and elimination of the antibiotic was slower than in the case of the other two exudates. The concentration peaked at 2.0 hr and then decreased gradually with a half-life of 1.9 hr. The values of C_{max}, T_{max} and F were calculated to be 22.9 μg/ml, 2.03 hr and 0.43, respectively (Table 3).

As shown in Table 3, the T_{max} of cefmenoxime for burn blisters was significantly longer than that for exudates from excoriated skin and burn wounds, and the k_2 value for the burn blister was significantly smaller than that obtained for the other exudates. We suspect that the transfer of cefmenoxime from serum to burn blister is slower than from serum to the other two exudates although we could not obtain k_1 values for any of the three kinds of exudates.

III. DISCUSSION

RAEBURN[4] reported that the acute inflammatory exudate induced by dermabrasion contains the highest level of clindamycin during the first hour of inflammation (2.1 mg/l). While the chronic exudates from leg ulcers contain gradually increasing concentrations of clindamycin, reaching a level of 2.2 mg/l at 3 hr. The exudates from burn wounds and excoriated skin in our study probably correspond to the acute inflammatory exudate, while the burn blister fluid may correspond to the chronic exudates reported by RAEBURN[4]. Thus the time-concentration curves of drugs may differ according to the kind of exudate. Since cefmenoxime has a relatively high protein-binding capacity, the differences in T_{max}, the half-lives, and initial levels of cefmenoxime in each exudate should be studied in the future both histologically and biochemically in connection with the concentration of protein in these exudates.

DURHAM *et al.*[9] concluded that dermabrasion fluid was a closer representation of interstitial fluid, whereas blister fluid with its higher protein and cell content was likely to be more closely related

to an inflammatory exudate. Electrolyte, protein, and cell contents in exudates from cantharidin-induced blisters were similar to those in burn blister fluid.

Staphylococcus aureus and *Pseudomonas aeruginosa* are commonly found in the traumatized area of burn patients both in our hospital and in other institutions. Cefmenoxime minimum inhibitory concentrations (50%) against these organisms were reported to be 8 μg/ml for *Staphylococcus aureus* and 16 μg/ml for *Pseudomonas aeruginosa* by VINCENT *et al.*[15]. In the present experiments, the drug concentration in the exudates examined was over these concentrations (8-16 μg/ml) for approximately 3 to 4 hr after antibiotic administration, so these results indicate that cefmenoxime should be a useful antibiotic for burn patients to avoid infection of their burn wounds. Furthermore, if pharmacokinetic consideration[16] is made of the transport of the antibiotic from serum into exudates as described above, a dose planning of cefmenoxime administration may make possible the control of infection not only on the surface of wounds, but also in the exudates from them. The present study also suggests the use of this antibiotic for the treatment of *impetigo*.

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静注 Cefmenoxime(CMX)の熱傷患者皮膚滲出液(水疱液)中への移行

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セファロスポリン系薬剤の一つである Cefmenoxime (CMX) を熱傷患者に静脈内投与して，皮膚剥離創滲出液，熱傷創滲出液および熱傷水疱液中への移行を調べた。CMX は 37 名の熱傷患者に体重 1 kg 当り，50 mg を one shot に静脈内注射した。血清中，滲出液中 CMX 濃度は bioassay 法によって測定した。

静注後，血清 CMX 濃度は投与後 15 分後に 189±29.7 μg/ml (mean±SD) となり，投与後 8 時間目には 4.8±1.3 μg/ml に低下した。2-compartment open model で解析すると $t_{1/2(\beta)}$ は 1.53 hr，$V_{d\beta}$ は 0.33 l/kg，AUC は 346 μg·hr/ml であった。

皮膚剥離創滲出液，熱傷創滲出液，熱傷水疱液中 CMX 濃度はともに静注後 1 時間目前後にピーク値を示した。その C_{max}，T_{max}，漸定的に求めた移行定数 F は皮膚剥離創滲出液，熱傷創滲出液，熱傷水疱液の順に各々，34.9，19.7，22.9 μg/ml；1.39，1.47，2.03 hr；0.47，0.28，0.43 であった。

以上のことから，薬動力学的には皮膚剥離創滲出液と熱傷創滲出液は相似形を示すが，熱傷水疱液とはその動態が若干異なることがわかった。