TRANSFER OF INJECTED FOSFOMYCIN INTO HUMAN SKIN EXUDATES (BLISTER)

Hiroko Sugiyama[1], Hisashi Aoyama[1], Buon Koh[2]
and Izumi Komiya[3]

Department of Plastic Surgery, Aichi Medical University,
Nagakute, Aichi-gun, Aichi 480-11[1]
Department of Plastic Surgery, Chukyo Hospital, Nagoya 457[2]
Central Research Laboratories, Meiji Seika Kaisha, Ltd.
Yokohama 222[3], Japan

The transport of fosfomycin into three kinds of exudates (suction blister fluid, burn blister fluid, and exudates from excoriated skin wounds) from blood was studied after intravenous bolus injection of fosfomycin (50 mg/kg). Concentrations of fosfomycin in serum and in the above fluids or exudates were determined by bioassay using *Proteus* sp. (MB–837) as a test organism. Concentrations of fosfomycin in serum reached 246 ± 54.6 μg/ml (mean$\pm$SD) 15 minutes after injection, and decreased to 13.3 ± 10.0 μg/ml after 8 hr. Peak concentrations of the antibiotic in suction blister fluid, burn blister fluid, and exudates from excoriated wounds occurred after approximately 1 hour. According to calculations made by a modified deconvolution method, T_{max}(time of maximum concentration), C_{max}(maximum concentration), $AUC^{0-8 h}$ (area under the curve), apparent transport rate constant K_1 (serum to exudate), K_2 (exudate to serum) and K_1/K_2 were, respectively, 1.5 hr, 79.8 μg/ml, 391.8 μg·hr/ml, 0.631 hr^{-1}, 0.839 hr^{-1} and 0.752 for suction blister fluid, 1.3 hr, 80.9 μg/ml, 358.7 μg·hr/ml, 0.612 hr^{-1}, 1.10 hr^{-1}, and 0.556 for burn blister fluid, and 0.7 hr, 73.4 μg/ml, 229.2 μg·hr/ml, 0.986 hr^{-1}, 2.27 hr^{-1}, and 0.434 for exudates from excoriated skin wounds.

INTRODUCTION

Knowledge of antibiotic distribution in vascular and extravascular compartments provides a guide for the clinical use of antibiotics. Concentrations of antibiotic in serum have been useful in determining the adequacy of therapy ; however, it is not clear whether levels in serum reflect extravascular antibiotic activity. Data on concentrations of antibiotics in human interstitial fluids are scarce because of the difficulty and potential hazards in obtaining specimens. Studies of other extravascular fluids such as aqueous humor, synovial fluid, cerebrospinal fluid, and saliva, while helpful in infections involving these areas, are not representative of the levels in the interstitial fluids because of the specialized nature of the membranes and secretory mechanisms of the tissues involved.

In the study reported herein, the concentrations of fosfomycin in the interstitial fluids of human skin were investigated, using three kinds of fluids, suction blister fluid, burn blister fluid, and exudates from excoriated skin wounds. All data obtained were analysed pharmacokinetically.

MATERIALS AND METHODS

The study population comprised 21 patients–ten male and eleven female–between 14 and 74 years of age, weighing from 40 to 74 kg. All subjects gave informed consent to take part in the study after it was explained to them. Laboratory values reflecting liver and renal functions were within normal limits.

A razor knife was used to remove a thin layer of skin (1 mm thickness) from the abdominal region for grafting to burn wounds. After bleeding

had stopped, a bolus of fosfomycin (50 mg/kg) was injected intravenously over a five-minute. Samples of exudate were taken at 0.5, 1, 2, 3, 4, 6, and 8 hours after administration of fosfomycin. The exudate fluids(30 to 50 μg) were collected with a paper disk(Whatman 13 mm AA disk). Disks were weighed and then stored at −20℃ until determination of the drug concentration, which was performed within ten days of sampling.

Suction blisters were produced by application of negative (−200 mmHg) pressure to skin areas. Plastic suction cups (15 mm in diameter) were tightly strapped to abdominal area. The negative pressure was maintained for 2 hours, during which time the blister slowly developed. The suction blister fluid was withdrawn by injection syringe.

Burn blister fluid (0.5-1 ml) was removed by injection syringe from blisters formed on the surface of second-degree burn wounds of patients.

Blood samples were taken at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 hours after the fosfomycin injection. Serum was separated and each sample was stored at −20℃ until the determination of fosfomycin was made. The concentrations of fosfomycin were determined by the paper-disk bioassay method[1,2]. *Proteus* sp. (MB-838) was used as the test strain. Nutrient agar (Difco) was the test medium. Standard curves were prepared by use of known concentrations of fosfomycin in pooled human serum. After preincubation at 4℃ for two hours, the plates were incubated at 37℃ for 18 hours, and the zones of inhibition were read. Pharmacokinetic calculations were carried out by computer(HITAC 20). The serum concentrations of fosfomycin were analysed using a two-compartment model, while the concentrations in exudates, and in burn and suction blisters were analysed using a modified deconvolution method[3]. The model used for tissue distribution is shown schematically in Fig. 1. There is a balance between the intravascular drug amount (AM) and the amount of drug in the blister fluid(exudates) (BM) ; and the rate of transport from the central to the extravascular compartment is k_1 ; and in the opposite direction, k_2 :

$$\frac{d\mathrm{BM}}{dt} = k_1 \mathrm{AM} - k_2 \mathrm{BM} \qquad (1)$$

For BM=0 at 0 time, the integral equation obtained is as follows :

Fig. 1 Model for pharmacokinetic analysis
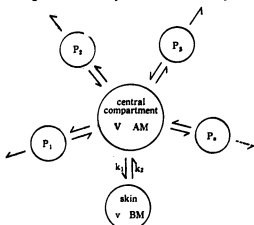
V : volume of central compartment
AM : drug amount of central compartment
v : volume of peripheral compartment
BM : drug amount of peripheral compartment
k_1, k_2 : transfer rate constant

$$\mathrm{BM}(t) = \int_0^t \mathrm{AM}(t-\theta) k_1 \cdot e^{-k_2 \theta} d\theta \qquad (2)$$

For concentration A(t) and distribution volume (V) within the central compartment, and B(t) and v as those respectively in the exudate, the following equation was obtained :

$$\mathrm{B}(t) = \int_0^t A(t-\theta) K_1 \cdot e^{-K_b \theta} d\theta \qquad (3)$$

Here, the apparent transport rate constant (K_1) is $k_1 \cdot V/v$.

In the integral equation shown in equation(3), serum levels of fosfomycin at arbitary times were substituted for the input function, A(t), while the concentration of the drug in the tissue was substituted for the output function, B(t). Apparent transport rate constants, K_1 and K_b, were obtained by the iterative least squares method.

RESULTS

Fosfomycin concentrations in serum and various exudates of burn patients after a bolus injection of fosfomycin.

Fosfomycin concentrations in serum after a bolus injection of the drug (50 mg/kg) are shown in Table 1 and Fig. 2. Fosfomycin concentrations in serum reached 246 ± 54.6 μg/ml 15 minutes after injection and decreased to 8.6 ± 4.0 μg/ml after 8 hr. Fosfomycin concentrations in suction blister fluid reached maximum level 1 hour after the injection (75.4 ± 33.3 μg/ml), and decreased gradually. In

Table 1 Fosfomycin(FOM) levels (μg/ml) in serum, suction blister, in burn blister and in exudates from excoriated skin wounds after intravenous bolus injection of FOM (50mg/kg)

Time after dose (hr)	Serum Mean±SD	(n)	Suction blister Mean±SD	(n)	Burn blister Mean±SD	(n)	Exudate fluid Mean±SD	(n)
0.25	246±54.6	(6)						
0.5	191.3±39.1	(6)	64.3±30.8	(7)	64.4±18.1	(7)	71.1±21.3	(13)
1	141±18.7	(6)	75.4±33.3	(12)	77±26.0	(7)	70.5±21.2	(10)
2	92.5±18.9	(4)	69.7±29.9	(11)	71.6±24.7	(7)	46.2±14.6	(8)
3	59.3±5.5	(4)	61.3±24.5	(6)	64.8±23.6	(8)	31.7±14.5	(6)
4	42.4±9.4	(4)	49.9±23.3	(7)	43.2±8.8	(6)	22.4±11.4	(7)
6	15.6±6.8	(4)	32.7±19.0	(7)	24.8±7.9	(6)	11.9±6.9	(9)
8	8.6±4.0	(4)	31.8±14.8	(6)	17.9±10.5	(6)	5.5±4.3	(8)

Table 2 Pharmakokinetic parameters of fosfomycin in serum after its intravenous bolus injection

Parameter	A	B	α	β	V_1	V_2	K_{12}	K_{21}	K_{10}
Units	μg/ml	μg/ml	hr⁻¹	hr⁻¹	1/kg	1/kg	hr⁻¹	hr⁻¹	hr⁻¹
Value	126.7	205.3	2.82	0.35	0.151	0.0614	0.767	1.88	0.529

Parameter	Vss	$V_{dβ}$	$t_{1/2}$	$t_{1/2}$	Cl_{tot}	$AUC^{0-\infty}$
Units	1/kg	1/kg	hr⁻¹	hr⁻¹	1/hr/kg	μg·hr/ml
Value	0.212	0.226	0.246	1.969	0.0796	641.7

Fig. 2 FOM concentrations in serum, in suction blister fluid, in burn blister fluid and exudate from excoriated skin wounds after its intravenous bolus injection (50mg/kg)

serum(□),
suction blister fluid(○),
burn blister fluid(●),
exudate from excoriated skin wound(△).
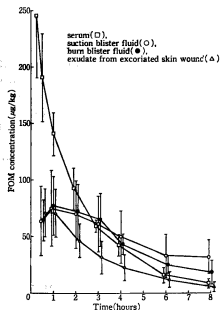
burn blister fluid, concentrations peaked after 1 hour. There was no significant difference between the concentrations in the two types of blister fluid.

On the other hand, fosfomycin concentrations in exudates from excoriated skin wounds increased rapidly to a maximum level from 0.5 to 1 hour after the injection.

Pharmacokinetic analysis of fosfomycin concentrations in serum and exudates of burn patients following bolus injection of fosfomycin.

Fosfomycin concentrations in serum after its bolus injection were analysed using a two-compartment model to obtain the pharmacokinetic parameter. The rate constants of distribution and elimination phases were 2.82 and 0.35 hr⁻¹. The half-life of the elimination phase ($t_{1/2β}$) was 1.969 hr. A distribution volume ($V_{dβ}$) of 0.226 l/kg and an AUC⁰⁻∞ of 641.7 μg·hr/ml are shown in Table 2. No significant differences between these parameters and those of healthy volunteers were found.

Fosfomycin concentrations in exudates were then analysed using the model as described in "Methods" and are shown in Fig. 3. In the case of suction blister fluid, T_{max} and C_{max} were calculated as 1.5 hr and 79.8 μg/ml, respectively(Table 3). $K_1(=k_1·V/v)$ and $K_2(=k_2)$ were 0.631 hr⁻¹ and 0.839 hr⁻¹. In the case of burn blister fluid, T_{max}, C_{max}, K_1 and K_2 were calculated as 1.3 hr, 80.9 μg/ml, 0.612 hr⁻¹ and 1.10 hr⁻¹, respectively. The

Table 3 Pharmacokinetic parameters of fosfomycin in suction and burn blister fluids and in exudate after its intravenous bolus injection (50 mg/kg)

	T_{max} hr⁻¹	C_{max} μg/ml	AUC⁰⁻⁸ μg·hr/ml	AUC⁰⁻∞ μg·hr/ml	K_1 hr⁻¹	K_2 hr⁻¹	K_1/K_2
Suction blister	1.5	79.8	391.8	436.9	0.631	0.839	0.752
Burn blister	1.3	80.9	358.7	423.9	0.612	1.10	0.556
Exudate	0.7	73.4	229.2	245.5	0.986	2.27	0.434

Fig. 3 Simulation curves of FOM concentrations in serum, suction blister fluid, burn blister fluid and exudates from excoriated skin wounds after its intravenous bolus injection (50 mg/kg)
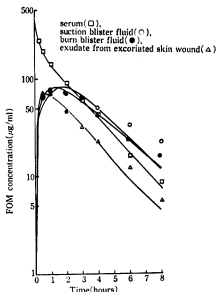
curves of fosfomycin levels in suction blister fluid and burn blister fluid were shown to be similar. In the case of exudates from excoriated skin wounds, T_{max} was 0.7 hr, and was shorter than that of suction blister fluid or burn blister fluid. Apparent transport rates K_1 and K_2 were 0.986 hr⁻¹ and 2.27 hr⁻¹, both of which were larger than those of the other blister fluids.

DISCUSSION

In the investigation of any antimicrobial drug the penetration into different tissues is of interest. Such investigations usually assay the amount of drug in biopsy material[4,5,6], which will be contaminated by blood or, in the cases of liver and kidney, by bile or urine as well. The information derived from such an exercise may be irrelevant. Attempts have been made to use animal models, i. e., the dog[7] or rabbit[8], in which cages have been implanted. The problems of using animal models and extrapolating to man are well known[9] and the fibrosed nature of these cages must lead one to suspect that they represent a pharmacokinetically "deep" compartment, especially since rather slow equilibration of radio-labelled compounds has been found[10]. Thus, the transfer of various antibiotics administered to the skin of patients has been studied using exudates (blister) induced artificially by the skin window technique (dermabrasion)[11,12,13], dermal suction[14,15], or application of canthalidin ointment[16,17,18,19].

The information obtained from the three tissue models used in this study provides a more accurate picture of the drug concentrations in interstitial fluid of patients. Two of the models used in this study are schematically shown in Fig. 4. The blister technique possibly represents a closed exudate. On the other hand, the excoriated skin technique represents a very "superficial" compartment that attains rapid equilibrium. T_{max}, C_{max}, and the transfer rate constant for suction blister fluid resembled those for burn blister fluid. There is the difference between T_{max} in blister fluid (1.5, 1.3 hr) and that of exudates from excoriated skin wounds(0.7 hr). These data of fosfomycin resemble those of cefmenoxime[20]. It seems possible that the volume of the blister fluid can result in delayed peak concentrations and falsely prolonged high concentrations as there must be a diffusion of the antibiotics into the fluid, which diffusion would require a certain amount of time. Pharmacokinetically, in suction blister fluid, the ratio of peripheral compartment volume to central compartment volume (V/b) is constant, so that the apparent transport ratio seems to be the true transport constant.

Staphylococcus aureus and *Pseudomonas aeruginosa* are commonly found in the skin wounds of burn patients in our hospital. Minimum inhibitory

Fig. 4 Model for pharmacokinetic analysis
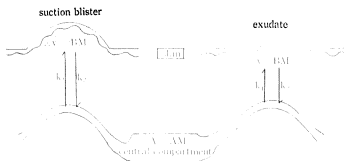
concentrations (MIC 80%) of fosfomycin against these organisms were reported to be 12.5 μg/ml for *Staphylococcus aureus* and 12.5 μg/ml for *Pseudomonas aeruginosa*[21,22]. In the present experiment, the drug concentrations in suction blister fluid and burn blister fluid exceeded these concentrations for approximately 8 hours ; and those in exudates from excoriated skin wounds, for 4 hours. These results indicate that fosfomycin should be a useful antibiotic for burn patients in preventing burn wound infection.

References

1) ZIMMERMAN, S. B. ; E. O. STAPLEY, H. WALLICK & R. BALDWIN : Phosphomycin IV. Susceptivity testing method and survey. Antimicrob. Agent. Chemotherapy-1969 : 303~309, 1969

2) KAWABATA, N. ; Y. SHIRAHA, S. DOI, K. UMEMURA & K. YAGINUMA : A study on serum level and urinary excretion of fosfomycin-Na in man with special reference to pharmacokinetic analysis. Jap. J. Antibiot. 21 : 549~560, 1978

3) KOMIYA, I. ; S. MURATA, K. UEMURA, N. TOMONO, S. KIKAI & M. FUJITA : Pharmacokinetics of dibekacin in rabbits and dogs. J. Pharm. Dyn. 4 : 362~373, 1981

4) BERGAM, T. : Pharmacokinetics of tissue penetration of antibiotics. Rev. Infect. Dis. 3 : 45~66, 1981

5) ALEXANDER, J. W. ; N. S. SYKES, M. M. MITCHELL & M. W. FISHER : Concentration of selected intravenously administrated antibiotics in experimental surgical wound. J. Trauma. 13 : 423~434, 1973

6) CHRISTHOLM, G. D. ; P. M. WATERWORTH, J. S. CALNAN & L. P. GARROD : Concentrations of antibacterial agents in interstitial tissue fluid. Br. Med. J. 1 : 569~573, 1973

7) RYLANDER, M. ; S. E. HOLM, R. NORRBY & J. E.

BRORSON : Studies on the pharmacokinetics of cefoxitin, cefuroxime, cephaloridine and cephalothin using subcutaneous tissue cages. Scand. J. Infect. Dis. 13 (Sup.) : 52~57, 1978

8) O'GRADY, F. : Animal models in the assessment of antimicrobial agents. J. Antimicrob. Chemother. 2 : 1~8, 1976

9) CALNAN, J. S. ; J. J. PFLUG, G. D. CHRISHOLM & L. M. TAYLOR : Pathophysiology of tissue fluid. Proc. Royal Soc. Med. 65 : 715~719, 1972

10) RAEBURN, J. A. : A method for studying antibiotic concentrations in inflammatory exudate. J. Clin. Path. 24 : 633~635, 1971

11) RAEBURN, J. A. : Antibiotic concentrations in inflammatory and interstitial fluids. Infection. 4 : 149~151, 1976

12) TAN, J. S. ; A. TROTT, J. P. PHAIR & C. WATANAKUNAKORN : A method for measurement of antibiotics in human interstitial fluid. J. Infect. Dis. 126 : 492~497, 1972

13) SCHREINER, A. ; K. B. HELLUM, A. DIGRANES & I. BERGMAN : Transfer of penicillin G and ampicillin into human skin blisters induced by suction. Scand. J. Infect. Dis. 14 : 233~237, 1978

14) SUGIYAMA, H. ; T. HATANO, K. TAKAYANAGI & H. AOYAMA : Transfer of cefoperazone into human skin fluid. Clin. Therapeutics 7 : 607~610, 1985

15) SHREINER, A. ; T. BERGAN, K. B. HELLIUM & A. DIGRANES : Pharmacokinetics of ampicillin in serum and in dermal suction blisters after oral administration of bacampicillin. Rev. Infect. Dis. 3 : 125~131, 1981

16) DURHAM, S. R. & R. WISE : Human pharmacology and tissue levels of CGP 7174/E : a new antipseudomonal cephalosporin. J. Antimicrob. Chemother. 6 : 734~747, 1980

17) SIMON, C. ; V. MALERCZYK & M. KLAUS : Absorption of bacampicillin and penetration into

body fluids (skin blister fluid, saliva, tears) in healthy volunteers. Scand. J. Infect. Dis. 14 : 228~292, 1978

18) SIMON, C.; V. MALERCZYK & F. NIXDORI : *In vitro* activity and clinical pharmacology of cefamandole in comparison to cephalothin. J. Antimicrob. Chemother. 4 : 85~90, 1978

19) WISE, R.; S. BAKER & R. LIVINGSTON : Comparison of cefotaxime and moxalactam pharmacokinetics and tissue levels. Antimicrob. Agents Chemother. 18 : 369~371, 1980

20) NISHIZAKI, A.; J. OKUDA, H. AOYAMA, Y. IZAWA & E. MIZUTA : Studies on transfer of injected

cefmenoxime to exudates(blister) of wounds of burn patients. Chemotherapy 33 : 1069~1074, 1985

21) GOTO, S.; I. DOGASAKI, Y. KANEKO, M. OGAWA, T. TAKITA & S. KUWAHARA : *In vitro* and *in vivo* antibacterial activity of fosfomycin. Chemotherapy 23 : 1653~1661, 1975

22) MIYAUCHI, K.; K. YOSHIDA, T. KASAI, T. SAITO, H. HIRANO, K. TOYAMA, M. YOSHIMURA, T. ISHII, M. ABE & S. KIKAI : Bacteriological studies on fosfomycin-antibacterial activity *in vitro* and *in vivo*-. Jap. J. Antibiot. 18 : 320~330, 1975

静注 Fosfomycin のヒト皮膚滲出液（水疱液）中への移行

杉 山 博 子・青 山 　 久

愛知医科大学形成外科

高 　 富 　 彦

中京病院形成外科

小 　 宮 　 泉

明治製薬株式会社中央研究所

体重 kg 当り 50 mg の FOM を one shot で静脈内に投与し、3種類の皮膚滲出液（吸引水疱液、熱傷水疱液、皮膚剥離創滲出液）への移行を調べ、薬動力学的解析を行なった。FOM 濃度の測定は *Proteus* sp.（MB-837）を測定菌とする生物学的方法によった。

血清中の FOM 濃度は投与後 15 分で 246±54.6 μg/ml に達し、8 時間後では 13.3±10.0 μg/ml であった。吸引水疱液、熱傷水疱液、皮膚剥離創滲出液内 FOM 濃度が最高濃度に達するのは大体 1 時間前後であった。

測定結果をもとに薬動力学的解析を行ない T_{max}, C_{max}, AUC^{0-8h}, K_1, K_2, K_1/K_2 を求めたところ、吸引水疱液では順に、1.5 hr, 79.8 μg/ml, 391.8 μg·hr/ml, 0.631 hr^{-1}, 0.839 hr^{-1}, 0.752 であった。熱傷水疱液では 1.3 hr, 80.9 μg/ml, 358.7 μg·hr/ml, 0.612 hr^{-1}, 1.10 hr^{-1}, 0.556 であり、剥離創滲出液ではそれぞれ 0.7 hr, 73.4 μg/ml, 229.2 μg·hr/ml, 0.986 hr^{-1}, 2.27 hr^{-1}, 0.434 という結果を得た。