BIOLOGICAL AND CHEMOTHERAPEUTIC STUDIES ON THREE SEMISYNTHETIC CEPHAMYCINS

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Three semisynthetic cephamycin antibiotics (7α-methoxy-cephalosporins), SK&F 73678, SK&F 83088 (CS-1170) and cefoxitin, have been found to possess favorable biological and chemotherapeutic properties. All three cephamycins are active *in vitro* against strains of *Staphylococcus aureus* and a variety of gram-negative bacilli. Beta-lactamase producing organisms including indole-producing *Proteus* spp., *Enterobacter* spp. and *Serratia* strains as well as certain anaerobic bacteria were found to be susceptible to these antibiotics. SK&F 73678 showed somewhat better MIC values than cefoxitin against multiple strains of bacteria. Strains of *Pseudomonas aeruginosa* and group D streptococci are essentially insensitive to these compounds. Their binding to serum proteins is relatively low. In mice, cefoxitin showed the most favorable pharmacokinetics with respect to peak serum level, serum half-life and urinary recovery. These cephamycins protected mice experimentally infected with a variety of bacterial strains. All three compounds are rapidly bacteriolytic to the logarithmically growing *Escherichia coli* and belatedly so to *Staphylococcus* strains with complete sterilizing effect. SK&F 73678 and SK&F 83088 showed activity and potency comparable to or better than cefoxitin and thus can be considered candidates for clinical study.

The discovery of a new family of antibiotics, the naturally occurring 7α-methoxy cephalosporins or cephamycins has been reported independently by two research groups[12,16]. Among these fermentation isolates, cephamycin C was found to possess broad gram-negative activity along with significant resistance to inactivation by beta-lactamases[4,16]. Intensive chemical modification designed to improve antibacterial spectrum and especially the gram-positive potency and beta-lactamase stability has resulted in the synthesis of the chemical analogue, cefoxitin. Early biological studies on cefoxitin proved it to have desirable properties[9,15,19]. Further chemical efforts in other laboratories have led to the synthesis of other analogues. Of these, SK&F 73678[6] and CS-1170[13] appeared to deserve further evaluation on the basis of their preliminary *in vitro* results. This communication presents data obtained from *in vitro* and *in vivo* studies with three semisynthetic cephamycin analogues. The chemical structures for the three cephamycins studied are shown in Fig. 1.

Materials and Methods

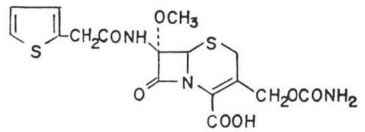
Antibiotics

Cefoxitin, and CS-1170 were kindly supplied as research samples by Merck Sharp & Dohme Research Laboratories. SK&F 73678 was prepared in the Smith Kline & French Laboratories.

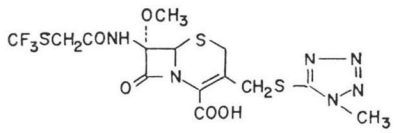
Bacterial Cultures

Bacterial strains used for susceptibility determinations were cultures regularly employed in our primary screening of cephalosporins and penicillins and clinical isolates obtained from various geographical locations in the United States.

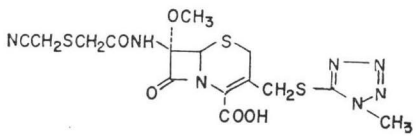
Fig. 1. Structures of three semisynthetic cephamycins



Cefoxitin



SK&F 73678



CS-1170

MIC Determinations

The minimal inhibitory concentrations (MIC) were determined by the agar dilution method on Trypticase Soy agar or by the microdilution technique in MUELLER-HINTON broth. Both media were buffered to pH 7.0 by the addition of 10% MCILVAINE's citric acid-buffer. In the broth medium, MIC's were determined with and without pooled, inactivated human serum in the medium. When serum was present, it constituted 50% of the medium. For the agar-dilution assay, the cephamycins were incorporated into the melted Trypticase Soy agar in serial two-fold dilutions. Appropriate dilutions of bacterial cultures grown overnight were used to inoculate the plates with the aid of a Steers' inocula replicating device[17]. MIC values were determined after overnight incubation at 37°C and were defined as those concentrations which completely inhibited colony-formation. When activities of the compounds were determined by the broth dilution method, a semiautomated microdilution technique (Microtiter, Cooke Engineering Co.) was used for both making the serial two-fold (200 to 0.2 μg/ml) dilutions of cephamycins and adding the inocula (approximately $1.5 \sim 5 \times 10^5$ cells/ml of medium) to each well of the microdilution trays. After overnight incubation at 37°C, the MIC's were determined by visual examination of growth with the lowest concentration of compound completely inhibiting growth defined as the MIC.

Microbiological Assay

The concentrations of the cephamycins in blood and urine samples were determined by bioassay using the disc agar-diffusion method with *Bacillus subtilis* ATCC 6633 as the indicator organism. For serum assays, samples and standards were diluted in pooled mouse serum, whereas for urine assays the diluent was 0.01 N phosphate buffer (pH 6.0). Plates were incubated overnight at 30°C.

In Vitro Serum Protein Binding

The percentage of antibiotic activity bound by serum proteins (pooled mouse or human) was estimated by comparing standard dose-response assay curves obtained for the compounds diluted in buffer (pH 6.0) or in serum. A disc agar-diffusion assay was employed for these determinations[20].

In Vivo Efficacy Studies

The *in vivo* efficacy of the three cephamycins was studied, using procedures previously described[2]. Groups of ten male albino Swiss-Webster mice weighing $18 \sim 20$ g, were infected intraperitoneally with the number of bacterial cells which produce uniformally lethal infections in nontreated animals. Challenge organisms were diluted in 5% hog gastric mucin. Infected mice were treated subcutaneously at one and five hours post-infection with four-fold increments of drug concentrations dissolved in isotonic sodium chloride solution. Death and survivors were recorded for a period of three days of observation. The total dose of each cephamycin that protected 50% of infected mice (ED_{50}) was calculated by the method of LITCHFIELD and WILCOXON[10]. The microorganisms used for these experimental therapeutic studies were strains of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* (inducible beta-lactamase producer) and *Proteus morganii* (constitutive beta-lactamase producer).

Pharmacokinetic Studies

Serum levels and urinary recovery of the cephamycins were determined in mice after subcutaneous administration of 20 mg/kg. Blood samples were obtained at the indicated intervals by decapitation of duplicate pooled groups of ten mice each. The pooled blood was allowed to clot at 4°C and the collected serum was kept frozen (−20°C) until assayed. The apparent serum half-life was determined

from a semi-log plot of antibiotic concentrations *versus* time. Urinary antibiotic recovery during the first four-hour period after dosing was determined in duplicate groups of ten mice. Mice were placed in metabolism cages and urine samples were collected in containers packed in dry ice. Urine volumes were measured and the samples were stored at −20°C prior to assay. The disc agar-diffusion assay described above was employed to determine drug concentration in blood and urine samples.

Mode of Action Study

The rate of bacteriolysis for strains of *E. coli, S. aureus* and *Ent. cloacae* of the three cephamycins was compared in peptone water-glucose broth (pH 7.3) at 37°C. When the cultures reached optical density of 0.4~0.6 (rapid logarithmic growth phase) measured in a Bausch and Lomb spectrophotometer (Spectronic 70) at 500 nm, antibiotics were added to obtain final concentrations of 50, 25, 12.5 or 6.3 μg/ml. Optical density readings were taken hourly for 6 hours and at 24 hours.

Results and Discussion

In Vitro Studies

The *in vitro* antibacterial activities of the three semisynthetic cephalosporins, were compared initially against 15 bacterial strains by agar dilution method, used in our laboratory for primary evaluation of cephalosporins and penicillins. The minimal inhibitory concentrations (MIC) obtained are presented in Table 1. All three compounds possess a broad-spectrum antibacterial activity including staphylococci and gram-negative bacilli. SK&F 73678 and CS-1170 have similar MIC values against these bacterial strains. These findings are in agreement with the published results for SK&F 73678[6] and for CS-1170[13]. The MIC values for cefoxitin are, in general, 2~4 times higher than those of the two other cephamycins. The three antibiotics showed poor or no activity against *Pseudomonas aeruginosa, Streptococcus faecalis* and the methicillin-resistant *Staphylococcus aureus.*

Table 1. *In vitro* activity of three semisynthetic cephamycin antibiotics against fifteen bacterial strains

Organism	Minimal inhibitory concentration (μg/ml)		
	Cefoxitin	SK&F 73678	CS-1170
*Staph. aureus** HH 127	1.6	0.4	0.8
Staph. aureus SK&F 23390	1.6	0.4	0.8
*Staph. aureus*** Villaluz (M.R.) SK&F 70390	100	25	25
Strep. faecalis HH 34358	100	50	100
E. coli SK&F 12140	6.3	1.6	1.6
*E. coli** HH 33779	12.5	3.1	3.1
Kleb. pneumoniae SK&F 4200	6.3	1.6	1.6
*Kleb. pneumoniae** SK&F 1200	1.6	0.4	0.8
Salmonella paratyphi ATCC 12176	1.6	0.4	0.4
Proteus mirabilis PM 444	3.1	1.6	1.6
*Pseudo. aeruginosa** HH 63	>200	200	>200
*Serratia marcescens** ATCC 13880	12.5	3.1	3.1
*Proteus morganii** 179	12.5	1.6	3.1
*Enterobacter aerogenes** ATCC 13048	25	3.1	6.3
*Enterobacter cloacae** HH 31254	6.3	0.8	0.8

* β-Lactamase producer

** Methicillin resistant & β-lactamase producer

Table 2. Comparative *in vitro* activities of cefoxitin, SK&F 73678 and CS-1170 against non-beta-lactamase and beta-lactamase producing strains of *Staphylococcus aureus* and *Escherichia coli*

Organism	No. of strains tested	Drug	Number of strains inhibited at concentration (μg/ml)									
			≤0.4	0.8	1.6	3.2	6.3	12.5	25	50	100	Median
Staphylococcus aureus[a]		Cefoxitin				5	7					6.3
Non-beta-lactamase producer	12	SK&F 73678		2	9	1						1.6
		CS-1170		1	8	3						1.6
Staphylococcus aureus[b]		Cefoxitin					13					6.3
Beta-lactamase producer	13	SK&F 73678		2	6	5						1.6
		CS-1170		1	3	9						3.2
Escherichia coli[c]		Cefoxitin			2	3	3					3.1
Non-beta-lactamase producer	8	SK&F 73678		2	3	2	1					1.6
		CS-1170		4	2	2						1.2
Escherichia coli[d]		Cefoxitin			1	1	7	6	1		1	6.3
Beta-lactamase producer	17	SK&F 73678		2	4	6	2	2	1			3.1
		CS-1170		3	4	8			1	1		3.1

a) Penicillin G sensitive; b) Penicillin G resistant; c) Ampicillin sensitive; d) Ampicillin resistant

Some of the *Bacteroides fragilis* strains tested (results not shown) showed some sensitivity to these cephamycins, but this activity was not of a high order. The results obtained with cefoxitin are in general agreement with those published in the literature[5,9].

All three semisynthetic cephamycins showed only slightly less activity against the beta-lactamase producing strains than with the non-producer strains. Table 2 demonstrates the results of such an experiment. The median MIC values are basically the same for the penicillin G sensitive as for the penicillin G resistant *S. aureus* isolates. As in the previous experiments, cefoxitin was found to be 2~4 times less active than the other compounds.

The MIC values for 213 bacterial isolates were obtained for cefoxitin and SK&F 73678 in broth and in the presence of 50% human serum (Tables 3 and 4). CS-1170 was not included in this study due to limited supplies. When comparing the distribution of MIC's of the two cephamycins, it can be seen that, in general, SK&F 73678 has better MIC's than cefoxitin, particularly in broth. In the presence of human serum, the activities of the two compounds against *P. mirabilis*, indole-producing *Proteus* spp., *Providencia* spp. and *S. marcescens* appeared to be virtually equivalent. The *Herellea* strains were not susceptible to either cephamycin. Against the *S. aureus* strains (penicillin G sensitive and resistant) SK&F 73678 was found to be about twice as potent as was cefoxitin. The presence of serum in the medium shifts the MIC values higher for both compounds, but SK&F 73678 still retains its superiority over cefoxitin. Both semisynthetic cephamycins proved to be highly resistant to most of the staphylococcal beta-lactamases. Against *E. coli* strains (many of them ampicillin resistant), SK&F 73678 is slightly superior to cefoxitin in broth but the two cephamycins appear to be equal in the presence of serum.

The *Enterobacter* strains produced a widely scattered MIC distribution with a definite advantage to SK&F 73678. Several strains were resistant to both compounds making them less impressive against this genera. Against the 25 *K. pneumoniae* strains, SK&F 73678 was found to be consistently superior

Table 3. Comparative *in vitro* activities of cefoxitin and SK&F 73678 against bacterial species in MUELLER HINTON Broth

Organism	No. of Strains	Drug	Cumulative percent strains inhibited at concentrations (μg/ml)										Median
			≦0.4	0.8	1.6	3.2	6.3	12.5	25	50	100	200	>200	
S. aureus[a]	25	Cefoxitin	4	8	60	92	100							1.6
		SK&F 73678	16	68	92	100								0.8
E. coli	24	Cefoxitin				38	71	88	92	96	100			6.3
		SK&F 73678	8	38	75	88	96	100						1.6
Ent. aerogenes	21	Cefoxitin				10	14	19		50	81	90	100	50
		SK&F 73678	5	4	43	81	86	90			95		100	3.2
Ent. cloacae	21	Cefoxitin				10			38	52	76		100	50
		SK&F 73678		10	19	38	57	67		71	81	90	100	6.3
K. pneumoniae	25	Cefoxitin			12	88		100						3.2
		SK&F 73678	8	52	80	92		100						0.8
P. mirabilis	24	Cefoxitin				63	88	92		100				3.1
		SK&F 73678			21	67	83	92		100				3.1
P. spp. indole positive[b]	25	Cefoxitin		4	8	32	56	88	96	100				6.3
		SK&F 73678	8	12	40	60	80	92		100				3.1
S. marcescens	25	Cefoxitin				4	36	56	64		68	88	100	12.5
		SK&F 73678		4	20	40	56		64	68	72	88	100	6.3
Citrobacter spp.	10	Cefoxitin			10	40		80		90	100			12.5
		SK&F 73678	10	60	90						100			0.8
Providencia spp.	9	Cefoxitin		11	44	89						100		3.2
		SK&F 73678		44	56	78		89				100		1.6
Herellea spp.	4	Cefoxitin								75	100			50
		SK&F 73678								50	100			75

a) 20 of these 25 strains are β-lactamase producers.

b) *P. morganii, P. vulgaris* & *P. rettgeri* are represented.

Table 4. Comparative *in vitro* activities of cefoxitin and SK&F 73678 against bacterial species in the presence of 50% pooled human serum/MUELLER HINTON Broth

Organism	No. of Strains	Drug	Cumulative percent strains inhibited at concentrations μg/ml										Median
			≦0.4	0.8	1.6	3.2	6.3	12.5	25	50	100	200	>200	
S. aureus[a]	25	Cefoxitin				36	88	96	100					6.3
		SK&F 73678			4	68	88	100						3.2
E. coli	24	Cefoxitin				21	63	75	88	92	96	100		6.3
		SK&F 73678			4	38	67	83	88	92	96	100		6.3
Ent. aerogenes	21	Cefoxitin					5	10		14	48	81	100	200
		SK&F 73678			5	10	24	33	62	76	86	95	100	25
Ent. cloacae	21	Cefoxitin			5		10		14	43	62	76	100	100
		SK&F 73678			10	14	29	43		52	62	76	100	50
K. pneumoniae	25	Cefoxitin				16	88	100						6.3
		SK&F 73678			8	68	92	96	100					3.2
P. mirabilis	24	Cefoxitin				13	92		100					6.3
		SK&F 73678				17	75	92			100			6.3
P. spp. indole positive[b]	25	Cefoxitin		4	8	20	40	80	96	100				12.5
		SK&F 73678	4	8	16	24	44	64	84	96	100			12.5
S. marcescens	25	Cefoxitin					24	56	60	72	88	96	100	12.5
		SK&F 73678				12	56	60	64	68	72	100		6.3
Citrobacter spp.	10	Cefoxitin				30		40		60	80	100		50
		SK&F 73678			30	60	70	80				100		3.2
Providencia spp.	9	Cefoxitin				44	78	89					100	6.3
		SK&F 73678			22	33	67	89					100	6.3
Herellea spp.	4	Cefoxitin								75	100			50
		SK&F 73678									75	100		100

a) 20 of these 25 strains are β-lactamase producers.

b) *P. morganii, P. vulgaris* & *P. rettgeri* are represented.

to cefoxitin even in the presence of human serum. Of the 25 indole-producing *Proteus* strains, only three were resistant to both compounds in broth as well as in serum-containing medium. The sensitivity of *S. marcescens* strains to both compounds shows a biphasic distribution; sixteen of the 25 strains examined are quite sensitive to the two cephamycins, whereas the remaining nine strains are completely resistant to them. Thus, the MIC data obtained with a large number of bacterial isolates show that SK&F 73678 and cefoxitin are wide spectrum antibacterials. Many earlier papers came to this conclusion for cefoxitin[3,5,8,9,11,14,15,18,19]. SK&F 73678, on the basis of the above-described comparative study, has a slight but consistent superiority to cefoxitin by having a somewhat broader antibacterial spectrum and greater potencies.

In Vivo Studies

The therapeutic effectiveness of the three semisynthetic cephamycins was compared along with that of cefazaflur (SK&F 59962), the structural analogue of SK&F 73678, against *S. aureus* and three gram-negative bacterial acute systemic experimental infections in mice. The ED_{50} values for subcutaneous administration of the four compounds are presented in Table 5. Cefazaflur is a parenteral cephalosporin, reported to be highly active against *S. aureus, E. coli* and *K. pneumoniae* infections[10]. Against *S. aureus* infection, cefazaflur tended to be more active than the cephamycins. Closest to cefazaflur (ED_{50} 3.4 mg/kg) is CS-1170 (ED_{50} 4.4 mg/kg), followed by SK&F 73678 (ED_{50} 7.2 mg/kg) and the poorest was cefoxitin with an ED_{50} value of 15.7 mg/kg. In *E. coli* and *K. pneumoniae* infections, the ED_{50} values of CS-1170 were almost identical with those of cefazaflur. Against *E. coli* infection, SK&F 73678 was found to be 2~4 times more potent than cefoxitin whereas against *K. pneumoniae* infection both compounds are almost equivalent and somewhat weaker than in other infections. Cefazaflur failed to protect mice infected with indole-positive *Proteus* at 50 mg/kg, the highest level tested, whereas all three cephamycins proved to be highly efficacious. Compound CS-1170 was the most successful with an ED_{50} value of 3.6 mg/kg, followed by cefoxitin (ED_{50} 7.2 mg/kg) and SK&F 73678 (ED_{50} 17 mg/kg).

Table 5. Efficacy (ED_{50}) of subcutaneously administered cefoxitin, SK&F 73678, CS-1170 and cefazaflur in experimental acute bacterial infections of mice

Test organism	Test No.	ED_{50} (mg/kg)				Challenge LD_{50}
		Cefoxitin	SK&F 73678	CS-1170	Cefazaflur	
Escherichia coli #12140	1	8.7	4.4	—	—	472
	2	—	3.4	—	2.4	517
	3	6.3	2.1	1.6	1.6	117
Klebsiella pneumoniae #4200	1	21.5	25.0	—	—	375
	2	—	16.0	—	7.2	156
	3	11.3	7.2	6.3	6.3	75
Proteus morganii #179[a]	1	7.2	17.0	3.6	>50.0	60
Staphylococcus aureus #127[b]	1	15.7	7.2	4.4	3.4	313

[a] Constitutive beta-lactamase producer
[b] Inducible beta-lactamase producer

Serum Levels and Urinary Recovery in Mice

The antibiotic concentrations of the three cephamycins were determined in serum and urine of mice dosed subcutaneously with 20 mg/kg of compound (Table 6). It can be seen from the data in Table 6 that the average serum levels, 15 minutes after dosing, were basically the same for all three

Table 6. Antibiotic concentrations in mouse serum, half-lives and excretion in urine following subcutaneous injection of cephamycins at 20 mg/kg

Cephamycins	Average serum concentration (μg/ml) at min.				$T_{1/2}$ (min)	Urinary recovery (%) at 4 hrs.	Serum binding (%)
	15	30	60	120			
Cefoxitin	26.4	31.6	12.8	T	23	37	25
SK&F 73678	25.3	11.9	4.4	T	18	11	38
CS-1170	27.3	24.4	5.4	T	18	28	24

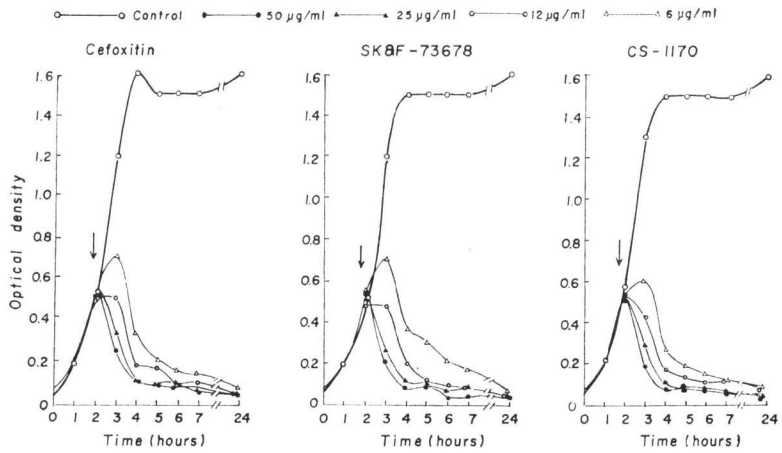
T=Trace activity

compounds. However, cefoxitin's level continued to rise resulting in a somewhat higher and sustained serum concentration than those of the other two compounds. Only trace microbiological activity was found in the two hour serum samples. Urine antibiotic recovery followed, in general, the course of serum concentrations; the highest level was found with cefoxitin, next was CS-1170 and the lowest was for SK&F 73678. Examination of urine samples suggested that all three cephamycins were excreted as intact molecules and no active metabolites were observed. The calculated biological serum half-life of cefoxitin was found to be 23 minutes while that of both SK&F 73678 and CS-1170 were 18 minutes. The extent of binding to mouse serum proteins can be considered as low for all three compounds, 24% for cefoxitin and CS-1170, and 38% for SK&F 73678.

Mode of Action Studies—Bacteriolytic Properties of the Three Cephamycins

Three *S. aureus* strains, three *E. coli* and one *Ent. cloacae* strain were employed for the lytic experiments. One strain each of *S. aureus* and *E. coli* were beta-lactam sensitive and the other strains were beta-lactamase producers to various degrees. The *Ent. cloacae* strain is also a strong cell-bound, constitutive beta-lactamase producer. The growth curves were established in static broth culture. A typical experiment is shown in Fig. 2 with *E. coli* 211, a beta-lactamase-producing, ampicillin-resistant strain. Antibiotics were added to the cultures in the active logarithmic growth phase at final concentrations of 50, 25, 12 and 6 μg/ml. The growth in the control non-treated cultures continued to produce the characteristic growth curve. In the antibiotic treated cultures, growth

Fig. 2. Lytic action of cefoxitin, SK&F 73678 and CS-1170 on a β-lactamase producing *E. coli* 1211. Arrow shows time of drug addition to culture.



stopped almost immediately with a concomitant lysis which was complete 3~4 hours after antibiotic addition. There was no regrowth, and all the treated cultures were sterile upon examination at 24 hours. All three cephamycins showed, basically, equivalent potencies. Similar results were obtained with the three *S. aureus* strains (not shown), although lysis commenced approximately five hours after antibiotic addition and was complete within 24 hours of incubation. Sterilization was achieved with two staphylococcal strains but regrowth of the third strain only with cefoxitin at the three lower concentrations was observed. The three strains of staphylococci showed a low level of spontaneous lysis after the stationary growth phase. *Ent. cloacae* was lysed in a similar fashion but regrowth was observed at all concentrations.

On the basis of the data presented in this paper, it can be concluded that on balance, the three cephamycins studied are almost equivalent. The general spectrum of activity for these cephamycins is similar and includes organisms that are strong beta-lactamase producers. SK&F 73678 and CS-1170 are more potent *in vitro* than cefoxitin especially against staphylococci. CS-1170 protected mice against various bacterial pathogens at a lower dose than was required for SK&F 73678 or cefoxitin. Cefoxitin appeared to have the best pharmacokinetic profile in mice when compared with the other two cephamycins. Cefoxitin is presently undergoing clinical trial and GEDDES *et al.*[7] have expressed the opinion that it will be a useful drug in the management of serious infections of the abdominal cavity and urinary tract. It has certain shortcomings, namely, its irritation upon intramuscular injection and the tendency to cause phlebitis when administered intravenously. The two experimental cephamycins, SK&F 73678 and CS-1170 have not been studied in man, however, on the basis of the available data merit further work.

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References

1) ACTOR, P.; J. V. URI, J. R. GUARINI, I. ZAJAC, L. PHILLIPS, C. S. SACHS, R. M. DEMARINIS, J. R. E. HOOVER & J. A. WEISBACH: A new parenteral cephalosporin. SK&F 59962; *In vitro* and *in vivo* antibacterial activity and serum levels in experimental animals. J. Antibiotics 28: 471~476, 1975
2) ACTOR, P.; J. V. URI, L. PHILLIPS, C. S. SACHS, J. R. GUARINI, I. ZAJAC, D. A. BERGIS, G. L. DUNN, J. R. E. HOOVER & J. A. WEISBACH: Laboratory studies with cefatrizine (SK&F 60771), a new broad-spectrum orally-active cephalosporin. J. Antibiotics 28: 594~601, 1975
3) BRUMFITT, W.; J. KOSMIDIS, J. M. T. HAMILTON-MILLER & J. N. G. GILCHRIST: Cefoxitin and cephalothin: antimicrobial activity, human pharmacokinetics and toxicology. Antimicr. Agents & Chemoth. 6: 290~299, 1974
4) DAOUST, D. R.; H. R. ONISHI, H. WALLICK, D. HENDLIN & E. O. STAPLEY: Cephamycins, a new family of β-lactam antibiotics: antibacterial activity and resistance to β-lactamase degradation. Antimicr. Agents & Chemoth. 3: 254~261, 1973
5) DARLAND, G. & J. BIRNBAUM: Cefoxitin resistance to beta-lactamase: a major factor for susceptibility of *Bacteroides fragilis* to the antibiotic. Antimicr. Agents & Chemoth. 11: 725~734, 1977
6) DEMARINIS, R. M.; J. V. URI & J. A. WEISBACH: Synthesis and *in vitro* antibacterial activity of 7-trifluoromethylthioacetamido cephamycins related to SK&F 59962 (cefazaflur). J. Antibiotics 29: 973~975, 1976
7) GEDDES, A. M.; L. P. SCHNURR, A. P. BALL, D. MCGHIE, G. R. BROOKES, R. WISE & J. ANDREWS: Cefoxitin: a hospital study. Brit. Med. J. 1977-1: 1126~1128, 1977
8) HAMILTON-MILLER, J. M. T.; D. W. KERRY & W. BRUMFITT: An *in vitro* comparison of cefoxitin, a semi-synthetic cephamycin with cephalothin. J. Antibiotics 27: 42~48, 1974

9) KOSMIDIS, J.; J. M. T. HAMILTON-MILLER, J. N. G. GILCHRIST, D. W. KERRY & W. BRUMFITT: Cefoxitin, a new semisynthetic cephamycin: an *in vitro* and *in vivo* comparison with cephalothin. Brit. Med. J. 1973-4: 653~655, 1973

10) LITCHFIELD, J. T., Jr. & F. WILCOXON: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96: 99~113, 1949

11) MOELLERING, R. C.; M. DRAY, Jr. & L. J. KUNZ: Susceptibility of clinical isolates of bacteria to cefoxitin and cephalothin. Antimicr. Agents & Chemoth. 6: 320~323, 1974

12) NAGARAJAN, R.; L. D. BOECK, M. GORMAN, R. L. HAMILL, C. E. HIGGENS, M. M. HOEHN, W. M. STARK & J. G. WHITNEY: β-Lactam antibiotics from *Streptomyces*. J. Am. Chem. Soc. 93: 2308~2310, 1971

13) NAKAO, H.; H. YANAGISAWA, B. SHIMIZU, M. KANEKO, M. NAGANO & S. SUGAWARA: A new semisynthetic 7α-methoxycephalosporin, CS-1170: 7β-[[(cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Antibiotics 29: 554~558, 1976

14) NEU, H. C.: Cefoxitin, a semisynthetic cephamycin antibiotic: antibacterial spectrum and resistance to hydrolysis by gram-negative β-lactamases. Antimicr. Agents & Chemoth. 6: 170~176, 1974

15) ONISHI, H. R.; D. R. DAOUST, S. B. ZIMMERMAN, D. HENDLIN & E. O. STAPLEY: Cefoxitin, a semisynthetic cephamycin antibiotic: resistance to β-lactamase inactivation. Antimicr. Agents & Chemoth. 5: 38~48, 1974

16) STAPLEY, E. O.; M. JACKSON, S. HERNANDEZ, S. B. ZIMMERMAN, S. A. CURRIE, S. MOCHALES, J. M. MATA, H. B. WOODRUFF & D. HENDLIN: Cephamycins, a new family of β-lactam antibiotics. I. Production by actinomycetes, including *Streptomyces lactamdurans*, sp. n. Antimicr. Agents & Chemoth. 2: 122~131, 1972

17) STEERS, E.; E. L. FOLTZ, B. S. GRAVES & J. RIDEN: An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. & Chemoth. 9: 307~311, 1959

18) UNE, T. & S. MITSUHASHI: Antimicrobial evaluation of cefoxitin: a new semisynthetic cephamycin. Arzneim.-Forsch. 27: 89~93, 1977

19) WALLICK, H. & D. HENDLIN: Cefoxitin, a semisynthetic cephamycin antibiotic: susceptibility studies. Antimicr. Agents & Chemoth. 5: 25~32, 1974

20) WICK, W. E.; D. A. PRESTON, W. A. WHITE & R. S. GORDEE: Compound 64716, a new synthetic antibacterial agent. Antimicr. Agents & Chemoth. 4: 415~420, 1973