IN VIVO ANTIBACTERIAL ACTIVITY OF DISODIUM α-SULFOBENZYLPENICILLIN

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The therapeutic activities of a new semi-synthetic penicillin, disodium α-sulfobenzylpenicillin (sulfocillin) in comparison with carbenicillin were studied in mice infected intraperitoneally with either *Staphylococcus aureus* (benzylpenicillin sensitive as well as resistant strains), *Streptococcus pyogenes*, *Diplococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* or *Klebsiella pneumoniae*. Both sulfocillin and carbenicillin were more effective by the parenteral route than by the oral route. Sulfocillin proved to be more effective than carbenicillin in the mice challenged with *D. pneumoniae* and *P. aeruginosa* when both penicillins were given three times after infection, while their effects by the single dose on the animals challenged with other microorganisms such as benzylpenicillin-sensitive *S. aureus*, *S. pyogenes* and *K. pneumoniae* were comparable. Sulfocillin was equieffective in the treatment of the animals infected with benzylpenicillin resistant or sensitive strains of *S. aureus*, whereas carbenicillin was somewhat less effective against the resistant strain.

Sulfocillin is a new semi-synthetic penicillin[1], with strong antibacterial activity against benzylpenicillin-resistant staphylococci and exhibiting high stablity to staphylococcal β-lactamase and to low pH (*e.g.* pH 2), more so than carbenicillin. In addition, its antipseudomonal activity was similar to that of carbenicillin[2].

The present communication deals with the therapeutic activity of sulfocillin against experimental Gram-positive and Gram-negative bacterial infections in mice.

Material and Method

Penicillins: Disodium sulfocillin and disodium carbenicillin, prepared by Takeda Chemical Industries, Ltd., Osaka, Japan and sodium ampicillin supplied by Wyeth Laboratories, U. S. A., were used. Sterile physiological saline solutions of each penicillin were prepared freshly on use.

Animals: Used were four weeks old male, CF 1/H mice, weighing 19~21 g.

Infection: The following organisms suspended in 0.5 ml of 5 % mucin solution were injected intraperitoneally in mice: *Staphylococcus aureus* 308 A-1; *S. aureus* 42 from the clinics; *Streptococcus pyogenes* E-14; *Escherichia coli* O-111; *Pseudomonas aeruginosa* U 31; *Proteus vulgaris* and *Klebsiella pneumoniae*. Only in the case of *Diplococcus pneumoniae*, 0.5 ml of bacterial suspension in nutrient broth was used. Each organism cultivated was prepared for infection in the following manner:

S. aureus 308 A-1, which was reported to produce coagulase, α, β-hemolysin and grew on monnitol-salt agar, was cultivated in Brain Heart Infusion (BHI) broth overnight and was diluted by 10^{-1} with 5 % mucin.

S. aureus 42, clinically isolated benzylpenicillin-resistant strain which was made available through the courtesy of Miss Y. SHIMIZU, Central Clinical Laboratory, Osaka University Hospital, was cultivated overnight in BHI broth for dilution by 10^{-1} with 5 % mucin.

S. pyogenes producing β-hemolysin was cultivated on Trypticase soy agar supplemented with 10 % beef blood (blood TSA). Two mg of bacterial suspension per ml of nutrient broth was diluted by 10^{-5} with the same medium. This bacterial suspension further diluted by 10^{-1} with 5 % mucin.

D. pneumoniae type I was cultivated on blood TSA. Tow mg of the bacterial suspension per ml of nutrient broth was diluted by 10^{-6} with the broth.

P. aeruginosa U 31, kindly supplied by Dr. U. HONMA, the Institute of Medical Science, Tokyo University, was cultivated overnight in KING B broth and diluted by 0.33×10^{-2} with nutrient broth. The bacterial suspension was further diluted by 10^{-1} with 5 % mucin.

E. coli O-111 cultivated overnight in BHI broth was diluted by 0.50×10^{-1} with nutrient broth. The bacterial suspension was further diluted by 10^{-1} with 5 % mucin.

P. vulgaris cultivated overnight in BHI broth was diluted by 10^{-2} with nutrient broth. The bacterial suspension was further diluted by 10^{-1} with 5 % mucin.

K. pneumoniae cultivated overnight in BHI broth was diluted by 10^{-1} with nutrient broth. The bacterial suspension was further diluted with 5 % mucin.

Therapeutic procedure: The animals challenged intraperitoneally with the respective microorganisms were divided as groups of 5 mice per each dosage level of test agent. Therapy consisted of a single subcutaneous, intraperitoneal, intravenous or oral dose of penicillin given immediately after challenge expect in the case of *D. pneumoniae* and *P. aeruginosa* infections. The mice infected with the latter two microorganisms were similarly treated three times, 3, 6 and 21 hours after infection in the former, and immediately, 1 and 2 hours after infection in the latter. Death of the animals by infection was recorded daily, and the 50 percent effective dose (ED_{50}=mg/kg) was determined 7 days after infection by the method of REED and MUENCH[8].

Results

The therapeutic results obtained allowed comparison of the relative therapeutic activity of sulfocillin and carbenicillin against the bacterial species used.

Sulfocillin and carbenicillin by either route was similar in the therapeutic activities against mice infected with *S. aureus* 308 A-1. However, both penicillins by the parenteral route were more effective than by the oral route (Table 1).

As shown in Table 2, the therapeutic effect of sulfocillin by the subcutaneous route was similar in mice infected with two staphylococcal strains, irrespective of the acquired resistance to benzylpenicillin. Carbenicillin, however, was slightly less effective in the mice infected with a benzylpenicillin-resistant staphylococci than in those infected with a benzylpenicillin-sensitive strain. Furthermore, larger doses of ampicillin was required to provide an equivalent survival rate in the mice infected with benzylpenicillin-resistant *S. aureus*.

The therapeutic results with *S. pyogenes* infections are shown in Table 3. Sulfocillin and carbenicillin afforded a similar degree of death-protection of the infected animals regardless of the route of administration. By parenteral administration both penicillins were more effective than by oral administration.

In the mice infected with *D. pneumoniae*, sulfocillin and carbenicillin with a single dose were considerably less effective than in the case of animals infected with staphy-

Table 1. Therapeutic effect of sulfocillin and carbenicillin against *Staphylococcus aureus* 308 A-1 infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	3.125				3.125			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	18.3	—	—	123.1	40.0	10.0	56.7	142.9
	22.3	17.9	—	142.9	35.7	10.0	—	226.9
	31.7	—	—	178.8	17.9	10.0	52.6	226.9
	—	15.4	—	112.7	44.7	15.2	40.0	142.9
	40.0	14.5	33.6	142.9	—	—	—	—
Average	28.08	15.93	33.6	140.08	34.58	11.3	49.77	184.9

Note: Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 10^{-1}. Antibiotic was administered as a single dose immediately after challenge.

Table 2. Therapeutic effect of sulfocillin, carbenicillin and ampicillin administered subcutaneously to CF 1/H mice infected with *Staphylococcus aureus* 308 A-1 and *Staphylococcus aureus* 42 (clinically isolated penicillin resistant strain)

Penicillin	Sulfocillin		Carbenicillin		Ampicillin	
Organism	*Staph. aureus* 308 A-1	*Staph. aureus* 42	*Staph. aureus* 308 A-1	*Staph. aureus* 42	*Staph. aureus* 308 A-1	*Staph. aureus* 42
In vitro sensitivity (mcg/ml)	3.125	6.25	3.125	25	0.05	100
ED_{50} (mg/kg)	14.0	5.6	58.0	46.5	0.5	20.0
	20.0	16.0	44.9	17.9	0.35	27.6
	10.0	17.9	22.5	28.4	0.23	14.1
	—	16.8	—	64.0	0.17	—
	—	13.7	—	103.9	—	—
	—	16.8	—	55.2	—	—
	—	—	—	67.2	—	—
Average	14.67	14.47	32.66	54.73	0.31	20.57
Relative value	1	0.99	1	1.67	1	66.35

Note: Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 10^{-1}. Challenge dose of each experiment was distributed from 31.6 to 156 LD_{50}. Antibiotic was administered as a single dose immediately after challenge.
Relative value was compared with ED_{50} value of penicillin against *St. aureus* 308 A-1 infection.

lococci or streptococci. The therapeutic ED_{50} of both penicillins with a single dose administered immediately after infectious procedure was more than 200 mg/kg. The remarkable therapeutic effects, however, were obtained, when penicillins were administered three times at the 3, 6 and 21 hours after infection. The result of the repeated administration of penicillins against exprimental pneumococcal infection is illustrated in Table 4. Sulfocillin was more active than carbenicillin by subcutaneous administration and was slightly more active than carbenicillin by intravenous administration. When administered intraperitoneally and orally, the effects of both penicillins were comparable. The ED_{50} of carbenicillin by subcutaneous, intraperitoneal or intravenous routes were similar, but that by the oral route was higher than by the parenteral routes.

Sulfocillin and carbenicillin with single doses in mice infected with *P. aeruginosa*

Table 3. Therapeutic effect of sulfocillin and carbenicillin against *Streptococcus pyogenes* E-14 infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	0.78				0.78			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	6.2	2.23	—	68.4	5.0	—	8.0	63.5
	3.5	—	3.5	47.6	3.5	0.9	3.5	47.6
	1.8	0.7	3.1	47.6	8.1	4.0	—	—
	3.5	1.25	3.8	64.0	2.8	—	7.0	—
	3.4	—	8.9	50.3	—	2.0	8.4	89.9
	—	—	—	—	8.1	1.7	11.2	61.5
Average	3.68	1.39	4.83	55.58	5.5	2.15	7.62	65.63

Note : Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 10^{-6}. Antibiotic was administrated as a single dose immediately after challenge.

Table 4. Therapeutic effect of sulfocillin and carbenicillin against *Diplococcus pneumoniae* type I infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	1.56				1.56			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	10.7	50.0	17.7	89.3	56.2	43.9	80.0	178.6
	6.98	76.9	32.5	116.3	59.5	34.2	100.0	119.0
	21.7	38.2	74.6	259.7	40.0	44.6	89.3	160.0
	19.5	36.2	62.1	200.0	—	—	—	—
Average	14.72	50.33	46.73	166.33	51.9	40.9	89.77	152.53

Note : Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in TSB at the concentration of 10^{-6}. Antibiotic was administered as 3 doses at 3, 6 and 21 hours after challenge.

produced poor results as was in the case of a diplococcal infection. However, when both penicillins were given three times, immediately, 1 and 2 hours after infection, showed marked therapeutic effects were obtained. Table 5 shows the respective results obtained with the penicillins by various routes in mice infected with *P. aeruginosa*. Sulfocillin administered by various routes was generally more active than carbenicillin on a dosage basis.

The data in Table 6 provide comparison of the ED_{50} of sulfocillin and carbenicillin in the mice infected with *E. coli*. Both penicillins by parenteral routes were more effective than by oral routes.

As shown in Table 7, sulfocillin and carbenicillin in mice infected with *P. vulgaris* was comparable in their therapeutic effects. Also, both penicillins by the parenteral route were more effective than by the oral route.

The therapeutic effects of sulfocillin and carbenicillin against *K. pneumoniae* infections were strikingly inferior to than against the other microorganisms infection though either agent was equieffective against *K. pneumoniae in vitro*. The ED_{50} values of both penicillins were approximately similar.

Table 5. Therapeutic effect of sulfocillin and carbenicillin against *Pseudomonas aeruginosa* N 18 infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	3.125				3.125			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	2.3	1.39	4.96	4.96	32.5	13.7	19.8	72.5
	9.9	—	13.7	10.1	18.5	10.5	10.5	23.0
	7.2	3.7	8.6	—	20.2	9.6	20.2	—
	10.1	—	12.5	—	17.7	—	8.8	—
	4.4	5.6	9.6	7.8	12.5	12.5	—	—
	7.3	8.8	11.2	—	—	—	—	—
Average	6.87	4.87	10.08	7.62	20.28	11.5	14.85	47.75

Note : Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 0.33×10^{-3}. Antibiotic was administered in 3 doses at 0, 1 and 2 hours after challenge.

Table 6. Therapeutic effect of sulfocillin and carbenicillin against *E. coli* O-111 infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	6.25				6.25			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	—	2.7	11.2	24.8	6.2	4.2	20.0	70.2
	5.0	3.4	10.0	35.7	3.1	3.5	—	89.9
	11.9	—	6.5	51.9	8.0	—	16.0	64.0
	11.2	—	18.0	114.3	8.7	3.6	14.2	70.2
	13.0	7.0	13.8	28.4	16.0	—	16.1	49.7
	11.2	—	—	35.7	7.1	3.4	23.5	58.0
	—	2.1	7.0	40.0	6.2	1.98	8.4	53.5
	12.4	7.9	16.8	46.5	16.1	11.2	20.0	51.1
Average	10.78	4.62	11.91	47.16	8.93	4.65	16.89	63.3

Note : Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 0.5×10^{-2}. Antibiotic was administered as a single dose immediately after challenge.

Discussion

The therapeutic efficacy of sulfocillin in several experimental Gram-positive and Gram-negative bacterial infections in mice has been demonstrated. From our studies sulfocillin proves to be more effective than carbenicillin in the treatment of mice infected with *D. pneumoniae* or *P. aeruginosa*. Also, sulfocillin is compared with equivalent dose of carbenicillin in mice infected with *S. aureus*, *S. pyogenes*, *E. coli*, *P. vulgaris* or *K. pneumoniae*. Though the *in vitro* antibacterial activities of both penicillins are similar against the microorganisms excepting *K. pneumoniae*, sulfocillin or carbenicillin was variable in their therapeutic activities against experimental infections. Several factors, such as the growth rate, the pattern of distribution or the biological characteristics of the infectious organisms seem to be involved in the variation of the effect.

SASAKI[4] reported that benzylpenicillin was therapeutically effective with three repeated administrations every three hours in mice infected with *D. pneumoniae*, whereas with a single dose it was not as effective. NISHIDA *et al.*[5] observed the therapeutic effect of carbenicillin with multiple doses at 30 minutes, 1 and 2 hours after infection in mice infected with *P. aeruginosa*. ENGLISH[6] reported the therapeutic effect of carbenicillin with

Table 7. Therapeutic effect of sulfocillin and carbenicillin against *Proteus vulgaris* infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	1.56				1.56			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	51.9	40.0	61.5	112.7	49.7	32.0	69.6	128.0
	56.3	31.7	68.4	198.8	71.4	51.9	—	89.9
	112.7	56.7	174.9	225.4	56.7	61.5	61.5	136.8
	51.9	40.0	47.6	71.4	100.6	47.6	64.0	179.8
Average	68.2	42.1	88.1	152.08	69.6	48.25	65.03	133.63

Note: Five mice of each group were injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 10^{-3}. Antibiotic was administered as a single dose immediately after challenge.

Table 8. Therapeutic effect of sulfocillin and carbenicillin against *Klebsiella pneumoniae* infection in CF 1/H mice

Penicillin	Sulfocillin				Carbenicillin			
In vitro sensitivity (mcg/ml)	25				25			
Administration route	SC	IP	IV	Oral	SC	IP	IV	Oral
ED_{50} (mg/kg)	198.8	—	—	256.0	207.8	128.0	179.8	512.0
	71.4	60.6	100.6	—	225.4	71.4	128.0	397.5
	127.0	71.4	128.0	190.5	142.9	121.1	146.8	320.0
	160.0	93.0	201.3	220.7	—	—	—	—
Average	139.3	75.0	143.3	222.4	192.03	106.83	151.53	409.83

Note: Five mice of each group werc injected intraperitoneally with 0.5 ml of the test organism suspension in 5% mucin at the concentration of 10^{-6}. Antibiotic was administered as a single dose immediately after challenge.

three time administrations in mice infected with *E. coli*.

According to the *in vitro* antibacterial activity reported by TSUCHIYA *et al.*[2] sulfocillin was similarly active against both the penicillin sensitive and resistant strains staphylococci. With the mice infected with benzylpenicillin-sensitive or resistant staphylococci, similar doses of sulfocillin produced an equivalent therapeutic activity. This evidence suggested that sulfocillin is useful not only against *P. aeruginosa* infections but also against benzylpenicillin resistant staphylococcus infections.

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