ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION OF SULFOCILLIN ADMINISTERED PARENTERALLY IN MICE, RATS, RABBITS AND DOGS

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The plasma level and tissue distribution of sulfocillin administered by intravenous, intraperitoneal, intramuscular or subcutaneous routes into mice, rats, rabbits and dogs, were compared with those of carbenicillin. The drug level was measured as active penicillin by a biological assay method using *Bacillus subtilis* PCI 219 as a test organism. The recovery rates of penicillins from the mixture with phosphate buffer homogenates of the tissues *in vitro* varied depending on the individual tissues and animal species, and in many cases the recovery rate of sulfocillin was higher than that of carbenicillin. A single dosage of sulfocillin or carbenicillin in all administration routes produced the highest plasma and tissue levels 5~15 minutes after injection in all animal species tested. Measurable concentrations of penicillin in plasma and tissues were detected up to 2 hours after administration in mice and rats, whereas they were detectable for 4~6 hours after intramuscular injection in rabbits and dogs. The levels of sulfocillin and carbenicillin in the liver and kidney were higher than in plasma in mice and rats, but not in rabbits. On the other hand, the levels of both penicillins in the lung and spleen were lower than in plasma and no measurable concentration of penicillin was detected in the brain. Urinary and biliary excretions of sulfocillin and carbenicillin were also studied. Amounts of both penicillins recovered from the 6-hour urine or bile sample in rabbits were similar. However, when sulfocillin and carbenicillin were given in dogs, the recovery rates in urine and bile were markedly different. When sulfocillin was administered, as much as 20 % of the given dose was recovered in the 6-hour urine and about 50 % of dose was recovered in the 6-hour bile. On the other hand, with carbenicillin, about 65 % of the dose was recovered in the 6-hour urine and about 23 % of the dose was recovered in the 6-hour bile.

Sulfobenzylpenicillin (sulfocillin) exhibited strong antibacterial activity against Gram-positive and Gram-negative bacteria[1,2] and furthermore, this penicillin has been shown to have a significant feature of possessing antibacterial activity against *Pseudomonas aeruginosa* and benzylpenicillin-resistant *Staphylococcus aureus*. Also, this penicillin is relatively stable against staphylocooccal β-lactamase. This stability is likely to reflect to the activity of sulfocillin against benzylpenicillin-resistant staphylococci.

The present report concerns with plasma levels, urinary and biliary excretion and tissue distribution of sulfocillin and carbenicillin in mice, rats, rabbits and dogs.

Materials and Methods

Animals: Female CF 1/H mice weighing 24 to 26 g and 5 to 7 weeks old, female Donryu rats weighing 180~200 g and 8~10 weeks old, female rabbits weighing 2.4~2.6 kg and female dogs weighing 7.7~11.5 kg were used.

Penicillin: Sulfocillin (disodium salt) and carbenicillin (disodium salt) were prepared at Takeda Chemical Industries, Ltd., Osaka.

Method of administration: Each penicillin was dissolved in physiological saline and injected intravenously, intramuscularly and intraperitoneally in the rats, intravenously and intramuscularly in the rabbits and dogs, and subcutaneously in the mice.

Measurement of the penicillin concentration: At 15 and 30 minutes, and 1, 2, 4 and 6 hours after injection, the animals were sacrificed for the sampling of the plasma and visceral organs. The tissues were homogenized in 9 volumes of $^1/_{15}$ M phosphate buffer (pH 7.4) and the supernatants of the homogenates obtained by centrifugation were used for the assay of the penicillin concentrations, following the cup method using *B. subtilis* PCI 219 as a test organism. The urine and bile (obtained through bile duct cannula) collected at various times after administration were assayed, following dilution in 9 volumes of buffer solution, and the total amounts of penicillin excreted in the urine or bile were calculated by measuring the volume of urine or bile collected. The concentration of the penicillin in the plasma was similarly measured. From the standard curve of penicillin concentration in the plasma and phosphate buffer, the concentration of penicillin was calculated. The activity of penicillin in urine or bile was not affected by ten-fold dilution in buffer solution.

Recovery test of the penicillins from the organ homogenate: Lung, liver, spleen, kidney and brain from the nontreated animals were homogenized with 4 volumes of $^1/_{15}$ M phosphate buffer of pH 7.4. These tissue homogenates and the same volume of the penicillin solution of 20 mcg/ml were mixed, and the supernatant obtained by centrifugation of the emulsion at 3,000 r.p.m. for 10 minutes was used for the measurement of penicillin activity.

Results

1. Recovery Rate of the Penicillin from the Organ Homogenates

The results obtained from the various animal tissues are shown in Table 1.

Table 1. Mean recovery percentage of sulfocillin and carbencillin mixed with mouse, rat and rabbit tissue homogenates

Animal (Number of animals)	Penicillin	Recovery percentage				
		Lung	Liver	Spleen	Kidney	Brain
Mouse (n=10)	Sulfocillin	84.67 (72~101.7)	86.62 (75~102.8)	94.03 (85~104.2)	90.97 (73~101.7)	98.09 (83~101.7)
	Carbenicillin	87.02 (56~100.7)	79.6 (60~100)	78.8 (62~94)	79.83 (49.5~100.3)	97.16 (70~120)
Rat (n=10)	Sulfocillin	76.67 (60~90)	88.78 (68~108)	81.56 (62~131)	88.95 (60~104)	87.67 (63~122)
	Carbenicillin	71.94 (58~95)	88.78 (55.5~103)	68 (59~80)	61.94 (48~89)	73.89 (58~90)
Rabbit (n=3)	Sulfocillin	105.83 (97.5~115)	87.83 (80~92.5)	95.83 (72.5~115)	95.83 (70~115)	103 (89~120)
	Carbenicillin	116.17 (86~140)	100.17 (78~122.5)	120.67 (107~130)	112.5 (100~130)	121.17 (101~120)

Table 2. Mean plasma level and tissue distribution of sulfocillin and carbenicillin at a single subcutaneous injection of 20 mg/kg into mice

Penicillin (Number of animals)	Tissue	Mean concentration in mcg/g			
		1/4 hr.	1/2 hr.	1 hr.	2 hr.
Sulfocillin (n=6)	Lung	32.2 (26~32)	19.2 (14~37)	2.5 (0~3)	0.5 (0~3)
	Liver	169.5 (92~253)	170.8 (108~327)	42.8 (23~77)	8.0 (2~15)
	Spleen	9.0 (0~17)	6.0 (0~13)	16.3 (2~34)	5.0 (0~10)
	Kidney	107.0 (74~124)	79.0 (45~107)	39.3 (9~882)	5.0 (0~26)
	Brain	0	0	0	0
	Plasma	69.27 (44.0~85.0)	37.58 (30.0~60.5)	10.5 (8.0~13.6)	0.6 (0~1.7)
Carbenicillin (n=6)	Lung	43.9 (17~79)	14.8 (0~40)	1.0 (0~7)	0
	Liver	333.5 (176~628)	258.6 (36~540)	94.2 (4~266)	3.5 (0~14)
	Spleen	17.2 (11~27)	5.5 (0~12.4)	0	0
	Kidney	138.2 (75~219)	65.2 (19~91)	17.1 (0~42.5)	0
	Brain	0	0	0	0
	Plasma	59.47 (23.5~100.7)	33.82 (6.9~70.0)	7.1 (1.0~14.2)	0

Table 3. Mean plasma level of sulfocillin and carbenicillin at a single injection into rats

Penicillin	Dose (mg/kg)	Administration route	Number of test animal	Mean concentration in mcg/ml			
				1/4 hr.	1/2 hr.	1 hr.	2 hr.
Sulfocillin	100	IV	4	52.58 (30.3~68.0)	44.38 (24.0~85.0)	9.23 (5.6~18.2)	0
	100	IP	4	65.0 (51.0~81.0)	39.08 (29.0~49.0)	14.43 (10.3~17.7)	0
	100	IM	10	65.17 (29.0~126.0)	41.31 (22.7~55.8)	7.0 (2.1~17.3)	0
	50	IM	6	40.5 (24.5~62.5)	18.55 (12.3~24.5)	0.75 (0~2.0)	0
	20	IM	6	16.68 (7.0~26.3)	8.41 (3.3~18.0)	0.17 (0~1.0)	0
Carbenicillin	100	IM	6	93.08 (53.5~175.0)	29.83 (24.0~35.0)	5.13 (2.1~7.0)	0
	50	IM	3	74.67 (64.0~92.0)	29.7 (20.5~37.0)	2.17 (0~6.5)	0
	20	IM	3	16.83 (15.0~18.0)	6.5 (5.5~8.0)	0.39 (0~2.8)	0

Table 4. Mean tissue level of sulfocillin and carbenicillin at a single intramuscular injection of 100 mg/kg into rats

Penicillin (Number of animals)	Tissue	Mean concentration in mcg/g			
		1/4 hr.	1/2 hr.	1 hr.	2 hr.
Sulfocillin (n=7)	Lung	35.2 (22~39)	26.2 (19~25)	1 (0~6)	0
	Liver	247.2 (140~304)	221.5 (97~249)	44 (5~108)	0
	Spleen	3.3 (0~11)	3 (0~6)	1.7 (0~5)	0
	Kidney	417.3 (202~821)	263 (169~354)	90 (19~230)	7.5 (0~25)
	Brain	0	0	0	0
Carbenicillin (n=6)	Lung	44 (25~69)	17.2 (13~22)	0	0
	Liver	277.3 (210~504)	154.7 (97~228)	35 (9~71)	1.5 (0~9)
	Spleen	19 (6~42)	6.5 (0~22)	0	0
	Kidney	620.3 (331~1,082)	503.2 (161~759)	163.3 (26~74)	2.9 (0~8.8)
	Brain	0	0	0	0

Table 5. Mean plasma level of sulfocillin and carbenicillin at a single injection of 20 mg/kg into rabbits

Penicillin	Administration (Number of animals)	Mean concentration in mcg/ml						
		1/12 hr.	1/4 hr.	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.
Sulfocillin	IV (n=6)	109.5 (57.0~170.0)	53.5 (39.0~75.0)	20.4 (9.5~31.5)	8.13 (0~13.5)	2.63 (0~9.3)	0	0
	IM (n=6)	—	21.05 (16.0~26.8)	15.47 (13.3~18.5)	10.48 (8.5~15.8)	5.6 (2.3~11.0)	2.38 (0~6.3)	0.7 (0~3.4)
Carbenicillin	IV (n=3)	145.3 (93.8~218.8)	68.6 (25.8~132.5)	25.37 (8.0~52.5)	5.97 (0~14.4)	1.26 (0~3.8)	0	0
	IM (n=3)	—	18.43 (11.5~30.5)	13.93 (12.5~16.0)	8.47 (5.3~10.8)	6.1 (2.5~9.3)	3.53 (1.0~5.1)	3.43 (0~6.5)

Recovery rates of sulfocillin from the various animal tissues were 80~105 % and little variation in recovery rate was observed between animal species or tissues. Recovery rates of carbenicillin from the mouse and rat tissues were 60~97 % or lower compared to sulfocillin. Furthermore, high recovery rates (100~120 %) of carbenicillin were obtained in rabbit tissues.

Table 6. Mean plasma level and tissue distribution of sulfocillin and carbenicillin at a single intramuscular injection of 100 mg/kg into rabbits

Penicillin (Number of animals)	Tissues	Mean concentration in mcg/g	
		1/4 hr.	2 hr.
Sulfocillin (n=3)	Lung	3.67 (2.0~5.6)	0
	Liver	23.67 (17.7~29.4)	0
	Spleen	0	0
	Kidney	26.1 (18.5~30.7)	1.0 (0~3.0)
	Brain	0	0
	Plasma	58.67 (50.0~75.0)	4.83 (2.8~7.6)
Carbenicillin (n=3)	Lung	0.5 (0~1.5)	0
	Liver	2.4 (2.2~2.6)	0
	Spleen	0	0
	Kidney	31.1 (18.0~43.6)	8.93 (3.2~13.4)
	Brain	0	0
	Plasma	92.0 (87.5~95.0)	18.5 (9.9~23.6)

Table 7. Mean excretion of sulfocillin and carbenicillin in urine at a single injection of 20 mg/kg into rabbits

Penicillin	Administration route (Number of animas)	6 Hour total recovery (mg)	Percentage of dose
Sulfocillin	IV (n=6)	36.05 (12.16~62.13)	59.2 (18.42~94.1)
	IM (n=6)	33.75 (12.0~55.46)	51.74 (30.0~79.2)
Carbenicillin	IV (n=3)	33.75 (12.0~55.46)	51.74 (30.0~79.2)
	IM (n=3)	28.37 (19.2~42.9)	47.82 (30.97~55.0)

2. Mice

Plasma and tissue level of sulfocillin by subcutaneous injection of 20 mg/kg in mice reached a peak at 15 minutes after injection and rapidly decreased thereafter. At 2 hours after injection, the concentrations in plasma and tissues declined to very low levels. The concentrations in liver and kidney were higher than in the plasma, but the levels in other tissues were lower than in the plasma. Also, no distribution of the penicillin to the brain was observed (Table 2).

A similar pattern of distribution was produced in the mice injected with carbenicillin. Mean carbenicillin plasma level in mice (Table 2) is lower than that of sulfocillin. Tissue levels of the former are higher during the early sampling intervals but the data suggest a more rapid clearance of carbenicillin.

Table 8. Excretion of sulfocillin and carbenicillin in bile at a single intramuscular injection of 20 mg/kg into rabbits

Penicillin (Number of animals)	Recovery (mg)					Total recovery	
	0～1/2 hr.	1/2～1 hr.	1～2 hr.	2～4 hr.	4～6 hr.	mg	Percentage of dose (mean)
Sulfocillin (n=6)	0.244～0.570	0.877～1.518	0.897～8.448	0.304～2.408	0.070～1.32	3.17～10.52	5.86～19.48 (11.27)
Carbenicillin (n=3)	0.558～2.34	0.71～2.624	1.225～2.352	1.328～1.912	0.376～0.740	4.75～9.6	1.56～12.3 (7.28)

Table 9. Mean plasma level of sulfocillin and carbenicillin at a single injection of 20 mg/kg into dogs

Penicillin	Administration route (Number of animals)	Mean concentration in mcg/ml						
		1/12 hr.	1/4 hr.	1/2 hr.	1 hr.	2 hr.	4 hr.	6 hr.
Sulfocillin	IV (n=4)	34.2 (23.4～55.0)	15.8 (8.3～26.0)	8.95 (4.7～16.0)	4.7 (2.0～8.4)	15.5 (0～4.0)	0	0
	IM (n=2)	72.25 (52.0～92.5)	54.4 (40.0～68.8)	48.15 (30.0～46.3)	27.9 (22.0～33.8)	8.5 (8.2～8.8)	1.3 (1.3～3.0)	0.6 (0～1.2)
Carbenicillin	IM (n=2)	56.0 (52.8～59.2)	33.6 (30.4～36.8)	22.9 (15.4～30.4)	13.45 (8.5～18.4)	7.2 (3.7～10.7)	1.7 (0～3.4)	0.35 (0～0.7)

Table 10. Excretion of sulfocillin and carbenicillin in urine at a single injection of 20 mg/kg into dogs

Penicillin	Administration route (Number of animals)	Recovery (mg)					Total recovery	
		0～1/2 hr.	1/2～1 hr.	1～2 hr.	2～4 hr.	4～6hr.	mg	Percentage of dose (mean)
Sulfocillin	IV (n=4)	0.74～31.8	1.3～31.68	4.61～13.11	1.13～7.04	0～4.46	39.1～103.27	16.8～24.3 (20.73)
	IM (n=2)	2.5～10.24	4.03～10.73	7.32～7.81	2.56～4.4	0.97～4.42	15.65～21.82	15.65～21.82 (18.24)
Carbenicillin	IV (n=2)	28.6～75.74	18.69～27.9	23.52～33.48	20.89～26.75	3.48～10.99	127.72～142.32	59.43～71.16 (65.25)

Table 11. Excretion of sulfocillin and carbenicillin in bile at a single injection of 20 mg/kg into dogs

Penicillin	Administration route (Number of animals)	Recovery (mg)					Total recovery	
		0～1/2 hr.	1/2～1 hr.	1～2 hr.	2～4 hr.	4～6 hr.	mg	Percentage of dose (mean)
Sulfocillin	IV (n=4)	10.5～32.76	10.85～46.86	20.4～58.32	6.93～50.22	1.26～14.05	56.3～182.28	24.57～86.8 (50.84)
	IM (n=2)	16.1～18.4	12.0～30.0	24.57～37.05	17.94～19.24	7.98～12.09	68.2～112.67	47.4～72.97 (60.19)
Carbenicillin	IV (n=2)	8.5～10.62	9.7～12.4	10.08～18.95	5.72～15.12	1.19～4.64	37.31～59.18	18.65～27.5 (23.08)

3. Rats

The mean concentration of sulfocillin in plasma obtained after a single intravenous, intraperitoneal and intramuscular injection in rats is shown in Table 3. The peak concentration occurred in plasma after 15 minutes regardless of administration route or dose. There was a rapid decline and very low concentration was reached

at 2 hours after injection. Also, the same concentration of the penicillin in plasma was observed by the three administration routes at the same dose.

Peak tissue level of sulfocillin by intramuscular injection of 100 mg/kg in rats was found at 15 minutes after injection and a rapid decline took place thereafter. At 2 hours after injection, no more penicillin was observed in many tissue samples. The distributions of penicillin in the kidney and liver were higher than those in other tissues and plasma. The distributions of sulfocillin in the lung and spleen were lower than that in plasma. The penicillin was not found in the brain.

Similar patterns of distribution were found in the carbenicillin-injected rats but the level of penicillin in plasma and tissues was somewhat higher than that of sulfocillin.

4. Rabbits

The concentrations of sulfocillin and carbenicillin in plasma, bile and urine are shown in Tables 5~8.

Intravenous injection of 20 mg/kg of sulfocillin in rabbits produced a very high concentration at 5 minutes after the injection. Thereafter, the plasma level rapidly decreased and was not detected 4 hours later.

After intramuscular injection of 20 mg/kg of the penicillin, the peak level of sulfocillin in plasma was found at 15 minutes after injection and a gradual decline took place with a concentration of 0.7 mcg/ml at 6 hours after the injection. Sulfocillin was rapidly excreted in the urine and bile. At 20 mg/kg injected intravenously and intramuscularly, mean percentage amounts of 59.2 % and 51.74 % respectively was recovered from urine within 6 hours after injection. Peak biliary level reached 1~2 hours after injection. Within 6 hours after injection, the amount of penicillin recovered was about 11 % of the intramuscular dose.

Tissue levels of sulfocillin by intramuscular injection of 100 mg/kg in rabbits were studied. Although high levels of penicillin in the liver and kidney were observed at 15 minutes after injection, these levels were lower than in plasma. At 2 hours after injection, the penicillin was scarcely observed in several tissues.

Following parenteral injection of carbenicillin, the plasma level and excretion in the urine and bile were similar to those of sulfocillin. Carbenicillin was mainly present in the kidney but the renal level of the penicillin was lower than in plasma.

5. Dogs

The concentrations of sulfocillin and carbenicillin in plasma, bile and urine were determined as active penicillin. The results are shown in Tables 9~11.

The plasma level of sulfocillin reached a peak at 5 minutes after intravenous administration of the penicillin. Thereafter, it gradually declined and measurable concentrations were found for 2 hours. Following intramuscular injection of sulfocillin, the level of sulfocillin in plasma was higher than that attained after intravenous injection, during the period of observation. The peak level of sulfocillin in plasma was observed at 5 minutes after injection and it gradually declined. Following the intravenous or intramuscular injection of sulfocillin, the penicillin was mainly excreted in the bile. About 50 % and 60 % of the given dose was rccovered from the

bile within 6 hours after intravenous and intramuscular injections, respectively. About 20 % and 18 % of the given dose was excreted in urine within 6 hours after intravenous and intramuscular injections, respectively. On the other hand, carbenicillin was mainly excreted in urine following intravenous injection and about 65 % of the given dose was excreted in the urine within 6 hours after injection. Also, about 23 % of the given dose was excreted in the bile within 6 hours after the injection.

Discussion

Although it was not mentioned in this paper, the active metabolites in urine and bile from the animals administered sulfocillin and carbenicillin were studied by bioautography of thin-layer chromatography, and no active metabolite was observed. This observation was confirmed by NAKAI *et al.*[3] Following intramuscular injection of sulfocillin-^{14}C to bile duct cannulated rats, thin-layer and paper chromatographic examinations showed that 65~85 % of the radioactivity in urine and bile was identified as unchanged penicillin by both radioassay and bioassay and about 10~20 % was accounted for by the penicilloic acid. NISHIDA *et al.*[4] and SHIMIZU[5], observed that carbenicillin was excreted into the urine without conversion to active metabolites such as penicillin G, by thin-layer chromatography, bioautography and agar-gel electropholesis. From these observations, the concentration of the two penicillins in plasma, tissues, urine and bile were measured by biological method as each penicillin.

Except the urinary and biliary excretions in the dog, similar behaviors were noted for sulfocillin and carbenicillin. Both penicillins were rapidly absorbed from the injected site, and distributed to various tissue. The concentrations of sulfocillin especially in the liver and kidney reached high levels soon after injection. Also, a large amount of sulfocillin was rapidly excreted in urine similar to carbenicillin, in the rabbits. However, when sulfocillin was injected into dogs, the urinary excretion was smaller than the biliary excretion. On the other hand, when carbenicillin was injected into dogs, the amount of the penicillin excreted in the urine was larger than that in the bile. In the rabbit, the amount of penicillin excreted in the bile was smaller than that in the urine, the level of penicillin in the bile was high enough to show an antibacterial activity against many pathogens. These observations suggest that sulfocillin might be useful against urinary tract infections and biliary infections.

References

1) TSUCHIYA, K.; T. OISHI, C. IWAGISHI & T. IWAHI: *In vitro* antibacterial activity of disodium α-sulfobenzylpenicillin. J. Antibiotics 24 : 607~619, 1971
2) YAMAZAKI, T. & K. TSUCHIYA: *In vivo* antibacterial activity of disodium α-sulfobenzylpenicillin. J. Antibiotics 24 : 620~625, 1971
3) NAKAI, Y.; H. SIRAKAWA, T. FUJITA, Z. SUZUOKI & T. FUGONO: The metabolic fate of α-sulfobenzylpenicillin in rats and mice. Xenobiotica (in press)
4) NISHIDA, M.; T. MATUBARA, Y. MINE, M. OKUI & Y. YOKOTA: Fundamental studies on carbenicillin. Chemotherapy 17 : 1095~1105, 1969
5) SHIMIZU, K.: Studies on determining humoral levels of carbenicillin. Chemotherapy 17 : 1131~1133, 1969