DISTRIBUTION IN NORMAL AND INFLAMMATORY TISSUE OF A NEW SEMISYNTHETIC CEPHALOSPORIN, SK&F 75073

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SK&F 75073 is a new cephalosporin with broad spectrum antibacterial activity. SK&F 75073-^{14}C and cefazolin-^{35}S were administered separately to groups of rats as a single intramuscular dose of 20 mg/kg. Tissues with highest drug levels 15 minutes following dose were as follows: (SK&F 75073/cefazolin levels), kidney - 86/70 μg/g, liver - 33/22 μg/g, lung - 29/17 μg/g, heart - 23/10 μg/g, adrenal - 13/7 μg/g. Plasma levels at peak were 134 μg SK&F 75073/ml (half-life, 1.9 hours) and 72 μg cefazolin/ml (half-life, 0.75 hours). Dose excreted in 24 hours was: SK&F 75073, urine 66% and feces 27%; cefazolin, urine 96% and feces 2%. Both antibiotics were also administered, at 20 mg/kg, to rats with the carrageenan-induced inflammatory pouches. Exudate from these pouches contained from 2 to 10 times more SK&F 75073 than cefazolin. Radioassay and bioassay of these substances in the exudate gave similar results. Serum protein binding ranged from 96~98% for SK&F 75073 and 34~69% for cefazolin.

Data indicated that highly protein bound SK&F 75073 enters tissues and tissue fluid to a greater extent than the lesser bound but therapeutically proven antibiotic agent cefazolin.

Antibiotics are generally known to bind in varying degrees to plasma proteins, blood elements, interstitial and cellular proteins. This phenomenon, which has been shown to have various effects on serum concentrations, distribution, elimination rates, tissue penetration and biologic activity of these compounds, has been the subject of extensive reviews.[1,2,3] However, KUNIN[1] points out that generalizations of the effects of protein bound antibiotic on these parameters should not be made but each compound should be considered individually. For example, WATERMAN[4] showed that the cephalosporins, cephaloridine and cefazolin, 10% and 80% serum protein bound respectively, both, with equal ease, left the cardiovascular system to enter the interstitial fluid of dogs. NISHIDA[5] found higher levels of cefazolin compared to cephalothin, in the exudates of rat granuloma pouches although cefazolin is the more highly protein bound of the two substances.

SK&F 75073 is a new long-acting cephalosporin shown to possess broad spectrum *in vitro* and *in vivo* antibiotic activity and is highly protein bound[6]. The studies reported here compare the tissue distribution, excretion and inflammatory exudate levels of this highly bound antibiotic with that of the somewhat lesser bound cefazolin[7,8].

Experimental

SK&F 75073 was synthesized at Smith Kline & French Laboratories to contain the labeled carbon in the position shown by asterisk in Fig. 1. Radiochemical purity assayed by HPLC and TLC was 93.7 and 98% respectively, chemical purity by HPLC 100%. Final specific activity was 9.02 μCi/mg. Cefazolin was synthesized at Smith Kline & French Laboratories to contain the labeled sulfur in the position as shown in Fig. 2. Radiopurity by TLC was 95% and chemical purity by UV was 91.3%.

Final specific activity was 54.3 μCi/mg. Both labeled compounds were diluted with non-labeled drug before dosing.

Fig. 1. SK&F 75073-^{14}C

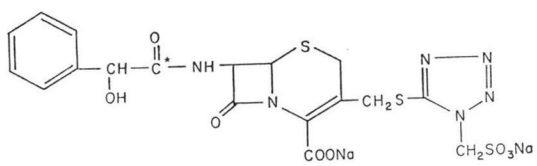
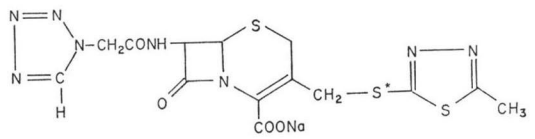


Fig. 2. Cefazolin-^{35}S



Tissue Distribution Study

Thirty male albino Sprague-Dawley rats from Charles River Farms (weight range 230~260 g) were administered an intramuscular dose of SK&F 75073-^{14}C such that each rat received 20 mg/kg. Thirty rats of the same strain (235~275 g) were each given a 20 mg/kg intramuscular dose of cefazolin-^{35}S. Three rats from each drug group were placed in metabolism cages for quantitative collection of urine and feces. Three rats from each drug group were anesthetized at designated time intervals with ether, exsanguinated from the inferior vena cava and tissues of interest were harvested.

Exudate Study

White rats of Wistar strain weighing between 149 and 375 g were utilized in these studies. A carrageenan solution (1% saline) was injected subcutaneously in the skin of the rat's back 18~22 hours prior to intramuscular injection of antibiotic. This treatment produced an inflamed pouch in the skin from which quantities of inflammatory exudate could be obtained. Rats were injected intramuscularly with SK&F 75073-^{14}C or cefazolin-^{35}S at a dose of 20 mg/kg. At specified intervals rats were anesthetized with ether and sacrificed by exsanguination from the heart. The inflammatory pouch was opened and exudate fluid was removed with a syringe and needle. Serum and exudate levels of radioactivity were measured. Antibiotic activity of serum and exudate were also measured by a bioassay disc procedure. Extent of *in vivo* serum protein binding of both SK&F 75073 and cefazolin was also determined in some of the rats.

Preparation of Samples for Determination of Radioactive Content

All tissues after removal were rinsed briefly under tap water, blotted dry, then weighed. Tissues were dissolved in 25% solution of tetramethylammonium hydroxide pentahydrate[a] (TMAH) from which aliquots were removed for counting in dioxane-based, liquid scintillation counting phosphor[b] which contained 5% of a thixotropic gel[c]. Gastrointestinal tract contents and feces containing either labeled carbon or sulfur were lyophilized, ground to a powder and oxidized using the Schoniger combustion technique. Labeled sulfur was trapped in 0.01 N NaOH and labeled carbon in 30% phenethylamine in methanol. Aliquots (1 ml) were counted in a premixed phosphor[b]. Urine was counted by direct addition to this phosphor. Plasma (0.2 ml) was placed into a scintillation vial containing 25% TMAH. After a minimum of three hours at 60~70°C, 15 ml of a 5% solution of a thixotropic gel[c] in phosphor[b] was added. When blood was counted, a 0.2-ml aliquot was first lysed with a surfactant[d]. After 0.5 hour, 0.1 ml hydrogen peroxide solution was added. The procedure for plasma counting was then followed.

Procedure for Determining Protein Binding

The amounts of SK&F 75073-^{14}C and cefazolin-^{35}S bound to plasma proteins were determined using a standard ultrafiltration technique.

Results and Discussion

A. Tissue Distribution, Blood-Plasma Levels and Excretion Studies

a) Southwestern Analytical Chemical, Inc., Austin, Texas
b) BRAY's Solution, New England Nuclear, Boston, Mass.
c) Cabosil, Cabot Corporation, Boston, Mass.
d) Zaponin, Coulter Diagnostics, Hialiah, Florida

Table 1. Concentration[1] of SK&F 75073-^{14}C in tissue of rats following a single intravenous dose of 20 mg/kg

Sacrifice time	μg/g tissue														
	Kidney	Spleen	Lung	Muscle	Testes	Heart	Fat	Eye	Adrenal	Brain	Liver	GI Tract[2]	Carcass	Blood μg/ml	Plasma μg/ml
5 min.	73.68 ±24.11	7.91 ±1.53	27.91 ±8.06	6.77 ±2.32	2.34 ±0.63	26.04 ±6.14	7.44 ±2.51	4.01 ±0.81	14.26 ±2.42	1.58 ±0.15	25.20 ±6.69	10.00 ±3.49	19.75 ±1.21	77.21 ±10.03	134.34 ±18.71
15 min.	85.51 ±1.60	9.27 ±0.93	29.31 ±1.45	9.48 ±0.47	4.55 ±0.64	23.08 ±0.57	10.91 ±0.23	5.11 ±0.53	12.63 ±1.94	1.59 ±0.18	32.50 ±2.30	17.89 ±5.46	18.61 ±0.63	73.53 ±4.10	128.61 ±2.89
30 min.	80.84 +10.69	7.49 ±0.47	25.50 ±1.96	9.75 ±0.32	6.94 ±1.55	17.98 ±1.63	9.28 ±1.35	5.73 ±0.47	10.51 ±0.52	1.40 ±0.24	25.42 ±1.68	28.00 ±5.07	14.70 ±0.87	63.92 ±3.28	111.45 ±3.85
1 hour	39.15 ±8.22	5.57 ±0.22	18.03 ±0.78	6.84 ±0.57	7.37 ±0.80	13.72 ±0.96	5.94 ±0.44	5.36 ±0.53	7.78 ±0.42	1.01 ±0.03	15.91 ±0.52	28.63 ±6.50	10.40 ±1.08	45.85 ±4.30	80.45 ±4.91
2 hour	25.76 ±3.90	3.11 ±0.29	10.87 ±1.42	4.06 ±0.20	6.73 ±0.74	7.41 ±1.52	4.83 ±0.66	3.99 ±0.99	5.23 ±0.75	0.70 ±0.04	9.19 ±0.98	44.03 ±5.65	6.71 ±3.82	26.40 ±3.19	44.44 ±6.42
3 hour	18.04 ±0.58	2.77 ±0.04	8.18 ±0.11	2.87 ±0.30	6.35 ±0.32	6.02 ±0.40	4.27 ±0.80	2.52 ±0.19	3.35 ±0.45	0.52 ±0.05	6.22 ±0.23	20.36 ±1.95	4.74 ±0.26	20.28 ±1.81	34.41 ±2.35
4 hour	15.58 ±1.38	2.22 ±0.23	7.71 ±1.21	2.67 ±0.25	5.66 ±0.39	4.83 ±0.65	3.69 ±0.77	2.48 ±0.27	2.76 ±0.32	0.46 ±0.05	5.40 ±0.81	16.99 ±8.43	4.27 ±0.46	16.72 ±0.56	29.59 ±2.21
6 hour	11.01 ±0.92	1.68 ±0.49	4.24 ±0.88	1.43 ±0.25	2.99 ±0.15	2.91 ±0.53	1.83 ±0.25	1.37 ±0.25	1.92 ±0.60	0.31 ±0.02	3.30 ±0.37	10.79 ±4.93	3.45 ±1.72	9.43 ±1.93	16.58 ±4.05
24 hour	3.35 ±0.25	0.20 ±0.25	0.60 ±0.06	0.19 ±0.03	0.30 ±0.02	0.32 ±0.02	0.18 ±0.01	0.17 ±0.01	0.22 ±0.02	0.08 ±0.02	0.32 ±0.04	0.58 ±0.16	0.43 ±0.03	0.82 ±0.04	1.37 ±0.03

Excretion as % dose/24 hour = Urine 66.40±10.44, Feces 27.24±8.21

1) Mean±S.D. of three rats

2) Less contents

Table 2. Concentration[1] of cefazolin-^{35}S in tissue of rats following a single intramuscular dose of 20 mg/kg

Sacrifice time	μg/g tissue														
	Kidneys	Spleen	Lungs	Muscle	Testes	Heart	Fat	Eyes	Adrenals	Brain	Liver	GI tract[2]	Carcass	Blood μg/ml	Plasma μg/ml
5 min.	45.67 ±3.39	3.75 ±0.58	12.46 ±0.24	3.87 ±0.19	1.69 ±0.27	8.40 ±1.15	2.97 ±1.18	2.29 ±0.05	7.63 ±1.47	1.23 ±0.45	13.55 ±0.65	6.78 ±1.23	7.36 ±0.62	32.64 ±2.44	56.58 ±0.92
15 min.	69.99 ±6.28	5.30 ±0.35	16.86 ±0.47	6.02 ±0.45	4.49 ±0.50	10.48 ±0.60	5.08 ±0.57	3.98 ±0.52	7.36 ±0.53	1.32 ±0.07	22.34 ±2.84	12.25 ±1.95	11.76 ±2.17	41.39 ±2.60	72.32 ±4.36
30 min.	54.87 ±5.93	4.73 ±0.19	17.65 ±1.18	5.20 ±0.10	7.16 ±0.61	10.30 ±0.31	4.97 ±0.43	4.54 ±0.08	8.35 ±0.64	1.46 ±0.11	16.75 ±3.38	13.25 ±1.90	9.83 ±0.76	34.94 ±0.87	62.21 ±0.10
1 hour	31.98 ±3.15	2.92 ±0.41	9.55 ±2.20	3.01 ±0.30	5.07 ±0.70	4.98 ±1.15	2.83 ±0.39	2.91 ±0.32	5.30 ±1.55	0.88 ±0.06	8.12 ±0.79	11.90 ±0.25	7.32 ±3.92	18.16 ±3.27	31.11 ±4.79
2 hour	5.63 ±1.73	0.57 ±0.18	2.65 ±0.92	0.74 ±0.22	1.51 ±0.24	1.44 ±0.49	0.83 ±0.11	1.17 ±0.11	1.33 ±0.29	0.38 ±0.11	1.80 ±0.40	7.46 ±4.48	1.65 ±0.23	4.14 ±1.00	6.82 ±1.77
3 hour	3.09 ±0.89	0.57 ±0.18	1.23 ±0.31	0.47 ±0.09	0.82 ±0.15	0.76 ±0.23	0.56 ±0.20	0.83 ±0.27	1.28 ±0.52	0.36 ±0.13	1.20 ±0.48	3.99 ±0.54	0.82 ±0.21	1.71 ±0.48	2.68 ±0.77
4 hour	1.86 ±0.13	0.47 ±0.03	0.95 ±0.06	0.30 ±0.05	0.56 ±0.07	0.51 ±0.05	0.29 ±0.02	0.56 ±0.05	0.62 ±0.07	0.35 ±0.07	1.15 ±0.12	3.34 ±0.07	0.67 ±0.03	1.15 ±0.11	1.66 ±0.17
6 hour	2.19 ±0.72	0.61 ±0.11	1.03 ±0.13	0.39 ±0.04	0.61 ±0.08	0.58 ±0.09	0.36 ±0.05	0.68 ±0.07	0.88 ±0.18	0.39 ±0.02	1.23 ±0.20	3.19 ±0.47	0.89 ±0.16	1.24 ±0.17	1.74 ±0.23
24 hour	0.72 ±0.05	0.26 ±0.09	0.30 ±0.04	0.10 ±0.02	0.13 ±0.01	0.14 ±0.01	0.11 ±0.03	0.14 ±0.01	0.36 ±0.05	0.17 ±0.01	0.19 ±0.01	0.70 ±0.07	0.35 ±0.04	0.15 ±0.02	0.21 ±0.02

Excretion as % dose/24 hour=Urine 95.97±0.82, Feces 2.23±1.96

1) Mean±S.D. of three rats

2) Less contents

SK&F 75073:

Data for SK&F 75073 are found in Table 1. Most of the tissues analyzed showed the highest concentration of SK&F 75073 at 15 minutes following intramuscular injection. At this time, the highest concentrations were found in kidney, liver, lung, heart and adrenal. Tissue levels then fell at the same rates as the fall in plasma levels, however, 24 hours after dosing, tissue levels were still measurable. As with other cephalosporins, penetrations into brain was minimal, however, all tissues studied showed the presence of SK&F 75073. Plasma and blood concentrations were measured separately and were at highest concentrations 5 minutes following intramuscular injection. This indicated a rapid absorption of the compound from the injection site. Plasma levels were higher than blood levels such that very little drug was associated with the red cells (approximately $1 \sim 2\%$ of the drug during the first 4 hours). Plasma levels fell with a half-life of 1.9 hours during the first 6 hours. Urinary excretion accounted for 66% of the dose and fecal excretion 27%, during the 24-hour measurement period. The sizeable fecal excretion following intramuscular dose administration indicated biliary secretion of the compound.

Cefazolin:

Data for cefazolin are found in Table 2. Cefazolin, like SK&F 75073 showed a maximal concentration in most tissues 15 minutes following dose, with kidney, liver, lung, heart and adrenal showing the highest concentration. However, the tissue concentration of cefazolin for these tissues was lower than was shown for SK&F 75073, despite the administration of equal doses (SK&F 75073 concentration for the highest 5 tissues averaged 68% higher than cefazolin levels for these same tissues). Brain levels

Table 3. Levels of SK&F 75073 and cefazolin in inflammatory exudates and serum after an intramuscular dose of either SK&F 75073-^{14}C or cefazolin-^{35}S (20 mg/kg) in rats (3 animals per group)

Sacrifice time	μg/ml							
	Exudate				Serum			
	SK&F 75073		Cefazolin		SK&F 75073		Cefazolin	
	Radioassay	Bioassay	Radioassay	Bioassay	Radioassay	Bioassay	Radioassay	Bioassay
2 hour	16.80[1] ±11.67	15.30[1] ±11.60	9.85 ±2.26	8.57 ±2.13	43.98[1] ±8.02	47.95[1] ±14.78	5.94 ±2.03	4.24 ±3.12
4 hour	8.05 ±1.49	7.97 ±1.18	7.13 ±1.74	6.33 ±1.79	14.25 ±2.61	16.37 ±3.59	3.66 ±0.86	N.D.[2]
6 hour	10.05 ±1.54	8.83 ±0.67	3.80[1] ±0.76	3.20[1] ±0.52	7.01 ±1.38	7.87 ±1.45	1.63[1] ±0.01	N.D.
24 hour	4.00 ±0.35	3.17 ±0.49	0.39 ±0.04	N.D.	0.57 ±0.06	N.D.	0.33 ±0.44	N.D.

[1] two animals per group. [2] N.D. - none detectable.

Table 4. *In vivo* protein binding[1] and "free" levels in serum of rats following intramuscular administration of SK&F 75073-^{14}C or cefazolin-^{35}S, 20 mg/kg

Sacrifice time	% Bound		μg/ml "Free" in serum	
	SK&F 75073	Cefazolin	SK&F 75073	Cefazolin
15 min.	96.4±0.7[2]	67.3± 5.3[3]	4.2[2]	21.02[3]
30 min.	97.2±0.2[2]	69.0± 3.0[3]	2.94[2]	16.67[3]
1 hour	97.9±0.1[3]	60.6± 3.2[2]	1.34[3]	12.46[2]
2 hour	98.4±0.1[3]	34.2±15.6[2]	0.44[3]	6.97[2]

[1] Measured by ultrafiltration. [2] Three animals per group. [3] Four animals per group.

were minimal but all tissues showed measurable amounts of cefazolin present 24 hours after dosing. Blood levels of cefazolin were lower than plasma levels and indicated little red cell-drug association. Plasma levels peaked 15 minutes following dosing and fell with a half-life of 45 minutes, during the first 6 hours. Comparison of plasma levels of cefazolin with those of SK&F 75073 during the first hour showed that SK&F 75073 plasma levels averaged two times greater than cefazolin; at 24 hours post-dose SK&F 75073 plasma levels averaged seven times greater than those of cefazolin. Urinary excretion of cefazolin was 96% and fecal excretion 2% of dose during the first 24 hours after dosing.

B. Exudate Studies

Concentration of either SK&F 75073 or cefazolin in serum and inflammatory-pouch exudates are found in Table 3. Serum levels of SK&F 75073 were higher than those of cefazolin in these rats for each of the 4 sacrifice periods measured. Serum levels measured by bioassay were similar to those measured by radioassay (except when levels were below the sensitivity of the bioassay) which indicated that the radioactive material in the serum was bioactive. Levels of SK&F 75073 in the exudate were two times higher than those of cefazolin at the 2-hour measurement period and remained higher through the 24th hour when the concentration of SK&F 75073 was 10 times higher than that of cefazolin. Similarity between radioassay and bioassay of exudate indicated that the radioactive material that left the plasma to enter the exudate, was bioactive. The extent of *in vivo* protein binding in a group of similar rats was measured during the period when plasma concentration of SK&F 75073 and cefazolin was greatest (Table 4). SK&F 75073 in the serum ranged from 96 to 98% protein bound while cefazolin ranged from 34 to 69%.

Therefore, in spite of a higher serum protein binding of SK&F 75073 with a resultant smaller amount of circulating "free" drug, as compared to cefazolin, more SK&F 75073 was able to leave the intravascular space and enter the exudate.

These studies demonstrated that the high degree of serum protein binding of SK&F 75073 did not confine it to the intravascular space or prevent it from entering tissues and tissue fluids and at quantities greater than that of cefazolin.

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