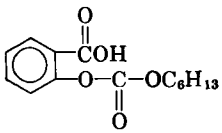
Pharmacology of the Hexylcarbonate of Salicylic Acid

By A. MISHER, H. J. ADAMS, J. J. FISHLER, and R. G. JONES

Gastric absorption of aspirin, salicylate, or acetic acid can result in increased mucosal permeability to H^+ ions leading to gastric bleeding. The pharmacology of the hexylcarbonate derivative of salicylic acid was investigated because its physical-chemical properties are such as could reduce the compound's availability to the gastric mucosa. It was found that the compound possesses antipyretic, analgesic, and anti-inflammatory activity qualitatively identical to those of aspirin. Its acute toxicity is less than that of aspirin in rats and mice and its gastric irritation potential is markedly lower in rats and dogs.

IT HAS BEEN SHOWN that the gastric absorption of aspirin, salicylate, or acetic acid can result in damage to the mucosa, making it more permeable to small ions including H^+ ions, and that the absorption of moderate quantities of H^+ ions through the insulted mucosa leads to gastric bleeding (1, 2).

The fact that aspirin administration is often associated with gastric irritation and hemorrhage is well documented in the literature (3). The authors' studies with carbonate derivatives of various drugs (4) suggested that salicylic acid might lend itself to prodrug formation, with retention of the antipyretic, analgesic, and anti-inflammatory activities, but with altered physical-chemical properties. Of the derivatives prepared, the *n*-hexyl-*O*-carboxyphenylcarbonate (SK&F 26070) (I) showed the most favorable profile being about only one-twentieth as soluble as aspirin in 0.1 *N* HCl at 37°, not producing acetic acid on hydrolysis, and having a much altered oil/water solubility coefficient (5). Changes such as these could reduce the compound's availability to the gastric mucosa.



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These studies were conducted in order to determine the impact of these changes on the pharmacological profile of this compound and its potential to cause gastric irritation.

EXPERIMENTAL

Acute Oral Toxicity in Mice and Rats—Male Carworth Farms (CF_1-S) mice, weighing 18–24 Gm., and male Charles River rats, weighing 100–159 Gm., were randomly divided into groups of 10 and dosed orally with suspensions of aspirin or SK&F 26070. The maximum dose volume was 20 ml./Kg. Control animals received either 0.5% tragacanth or 1% methylcellulose (400 cps.) at corresponding dose volumes. Daily observations were made until no deaths had occurred for 2 consecutive days following the fifth day. Food and water were available *ad libitum*.

Gastric Irritation in Rats—Male Charles River rats, weighing 144–213 Gm., were fasted 18 hr. prior to use. They were then randomly divided into groups of 10 and dosed orally with aspirin or SK&F 26070 suspended in 1% methylcellulose (400 cps.). Drug concentrations were adjusted so that each animal received a dose volume of 10 ml./Kg.; 20 control animals received an equal dose volume of the vehicle. Two hours postdrug, the animals were sacrified with ether. The stomachs were excised, opened along the greater curvature, and gently rinsed with warm water. They were then examined under low magnification with a binocular microscope and rated positive or negative for hemorrhage and for ulceration, these evaluations were done on a "blind" basis.

Gastric Irritation in Dogs—Mongrel dogs of either sex, weighing 10–15 Kg., were randomly assigned identification numbers and divided into groups of four. Each group received one of the following treatments: aspirin, 25 mg./Kg.; aspirin, 75 mg./Kg.; SK&F 26070, 25 mg./Kg.; SK&F 26070, 75 mg./Kg.; empty gelatin capsules (control group). Each animal received six consecutive oral doses, spaced 12 hr. apart. The animals were sacrificed 4–5 hr. after the last dose. The stomachs were removed, opened, and the degree of irritation graded on a "blind" basis.

Antipyretic Activity in Rats—Eighty male Charles River rats were randomly divided into six drug groups of 10 and a control group of 20 animals. Immediately after a control rectal temperature had been taken, each animal received subcutaneously in the nape of the neck 1 ml./Kg. of a freshly prepared 20% suspension of brewer's yeast in isotonic saline. Rectal temperatures were taken 5 hr. later, and each animal then received a dose of drug or an equivalent dose volume of vehicle. Both aspirin and SK&F 26070 were administered orally at 50, 100, and 200 mg./Kg., suspended in 0.5% tragacanth, at a dose volume of 20 ml./Kg. Rectal temperatures were taken again 2 hr. postdrug. Food was withheld throughout the test period, but water was available *ad libitum*.

The results were evaluated quantitatively: if the final rectal temperature of an animal was at least 1° below the mean of the control group, the animal's

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response was negative. ED_{50}'s for the antipyretic activity of the two compounds were calculated by the Logit chi-square method of Berkson (6).

Analgesic Activity in Rats—The inflamed paw method of Randall and Selitto, which has been shown to be sensitive enough to detect the mild nonnarcotic analgesics (7), was used for this study. Male and female Dierolf Farms rats, weighing 80–110 Gm., were fasted 16 hr. before use. Inflammation was produced by the injection of 0.1 ml. of a freshly prepared 20% suspension of brewer's yeast into the plantar surface of the rat's left hind foot. Two and one-half hours after the yeast injection, pain threshold determinations (predrug control values) were made on the inflamed paw. Animals were then randomly divided into groups of five and dosed orally. Drugs were prepared as suspensions in 0.5% tragacanth, and concentrations were adjusted so that each animal received a dose volume of 20 ml./Kg.; 10 control animals received 20 ml./Kg. of vehicle. Threshold determinations were made at 30, 60, 120, 180, and 240 min. postdrug.

To determine pain thresholds, pressure was applied to the plantar surface of the inflamed paw at a constant rate of 20 mm. Hg/sec. by means of a Plexiglas cone attached to the plunger of a 10-ml. syringe; the syringe was connected to a pressure gauge, control valves, and a source of air pressure. The pain threshold is expressed as the pressure (in mm. Hg) required to induce struggle in the animal. Analgesic activity is expressed as the difference (Δ mm. Hg) between the pressure required to induce this behavior after drug administration as compared to the measurement before drug administration.

Anti-inflammatory Activity—Anti-inflammatory activity was tested in the rat in which polyarthritis was induced by the intradermal injection of adjuvant; the method used was similar to that described by Ward and Cloud (8) and by Glenn (9). Paw volumes were recorded by means of a modification of the method described by Winter and Nuss (10).

On the morning of Day 1, the plantar surface of the right hind paw of male Carworth Farms rats (initial weight 145–213 Gm.) was injected intradermally with adjuvant (0.1 ml. of a 2.5 mg./ml. suspension of *Mycobacterium butyricum*[1] in mineral oil). Immediately after the injection, animals were randomly divided into groups of 10, and the volume of each injected paw was measured by immersing the paw to the level of the lateral malleolus in a mercury bath which was connected through a closed, fluid-filled tube to the Microcord 44[2] recorder. Volumes could be measured accurately with reliability to the hundredth of a milliliter.

The drugs were administered immediately after this initial paw volume measurement, and again 3 hr. later. The doses of aspirin were 100 and 200 mg./Kg., b.i.d., the doses of SK&F 26070 were 148 and 293 mg./Kg., b.i.d. and were equimolar to those of aspirin. The drugs were suspended in 0.5% methylcellulose (4,000 cps.) and administered orally, b.i.d., at a dose volume of 10 ml./Kg. for 19 days.

From Day 2 through Day 19 the second dose was administered approximately 5 hr. after the first dose. One adjuvant control group received 0.5% methylcellulose (4,000 cps.) orally, b.i.d., at a dose volume of 10 ml./Kg. for 19 days. To provide "normal" values for the parameters measured, a group of 10 rats that received neither adjuvant nor treatment was used.

On Day 20, plasma inflammation units were determined according to the method outlined by Glenn and Kooyers (11). The animals were anesthetized with ether, and blood was obtained in heparinized syringes by cardiac puncture. The blood samples were placed into heparinized glass centrifuge tubes and centrifuged at about 1500 r.p.m. for 30 min. Plasma samples were withdrawn, and inflammation units were determined after diluting the plasma 1:200 with sterile physiological saline.

The initial turbidity readings of the diluted solutions were determined in a Coleman No. 9 nephelometer, set at full sensitivity, against the nephalos standard, $N = 19$. The diluted solutions were subsequently placed in a water bath at 56° for 30 min., cooled to room temperature, and turbidity again determined. The differences between initial and final nephelometric readings are expressed as "corrected inflammation units."

RESULTS

Acute Oral Toxicity in Mice and Rats—As shown in Tables I and II, milligram for milligram, aspirin is about twice as toxic orally in both mice and rats as SK&F 26070; on a molar basis, aspirin is significantly more toxic ($p = 0.05$) than SK&F 26070. With both drugs, most or all deaths occurred within 24 hr. postdrug.

TABLE I—ACUTE ORAL TOXICITY IN MICE

Drug	Dose, mg./Kg. (10 Animals/ Group)	Mortality, %	LD_{50} 95% Confidence Limits[a]
SK&F 26070	1000	0	2680 (2158–3777)
	1450	10	mg./Kg. 10.1 (8.1–
	2200	20	14.4) moles/Kg.
	3000	60	$\times 10^{-3}$
	4250	100	
Aspirin	750	0	1264 (1091–1478)
	1000	20	mg./Kg. 7.0(6.0–
	1250	50	8.2) moles/Kg. $\times$
	1500	70	10^{-3}
	2000	100	

[a] LD_{50}'s and 95% confidence limits were calculated by the Logit chi-square method of Berkson.

TABLE II—ACUTE ORAL TOXICITY IN RATS

Drug	Dose, mg./Kg. (10 Animals/ Group)	Mortality, %	LD_{50} 95% Confidence Limits[a]
SK&F 26070	2080	0	3447 (2680–4253)
	3000	40	mg./Kg. 13.0
	4300	70	(10.2–16.0) moles/
	6200	100	Kg. $\times 10^{-3}$
Aspirin	625	0	1366 (1003–1744)
	1250	40	mg./Kg. 7.6 (5.6–
	2000	80	9.7) moles/
	2500	100	Kg. $\times 10^{-3}$

[a] LD_{50}'s and 95% confidence limits were calculated by the Logit chi-square method of Berkson.

[1] Difco Laboratories, Detroit, Mich.
[2] Photovolt Corp., New York, N. Y.

TABLE III—GASTRIC IRRITATION IN RATS

Drug	Dose, mg./Kg., p.o. (10 Animals/Group)	Incidence of Gastric Hemorrhage, %	Incidence of Gastric Erosions %
SK&F 26070	84.4	30	0
	148	20	0
	259	30	0
Aspirin	57	70	20
	100	100	30
	175	100	90
1% Methylcellulose	10 ml./Kg.	0	0

Gastric Irritation in Rats—At equimolar dose levels, the incidence of hemorrhagic stomachs in rats that received SK&F 26070 was markedly lower than in rats that received aspirin (Table III). Even at the highest dose tested (259 mg./Kg.), SK&F 26070 produced no gastric erosions; the molar equivalent dose of aspirin, on the other hand, caused gastric erosions in 90% of the animals.

Gastric Irritation in Dogs—In dogs, the effects of the drugs were studied after repeated administration—six doses spaced 12 hr. apart—rather than after a single administration as in rats. The results are even more pronounced than in rats. The stomachs of all eight dogs that received SK&F 26070 were normal in appearance and indistinguishable from those of control animals. All four dogs that received the 25 mg./Kg. dose of aspirin and one of those that received the 75 mg./Kg. dose showed localized superficial mucosal irritation or inflammation; the other three dogs at the high dose showed mucosal irritation and isolated hemorrhage. On a molar basis, the high dose of SK&F 26070, which produced no gastric irritation, is about twice that of the low dose of aspirin, which caused gastric irritation in all four animals.

Antipyretic Activity in Yeast-Fevered Rats—At each dose level, the percent of animals tested that exhibited an antipyretic response to the drugs was identical for both SK&F 26070 and aspirin: 50 mg./Kg.—60%; 100 mg./Kg.—80%; 200 mg./Kg.—100%. Therefore, the two compounds appear equipotent, on a mg. for mg. basis, in antipyretic activity in this test procedure.

Analgesic Activity in Rats—At 200 mg./Kg., aspirin and SK&F 26070 caused a maximum increase in the pain threshold of the inflamed paw of 31 mm. Hg and 34 mm. Hg, respectively; at 400 mg./Kg., aspirin produced a maximum increase of 87 mm. Hg and SK&F 26070 effected a 72 mm. Hg increase. These data indicate that SK&F 26070 exhibits analgesic activity comparable to that of aspirin.

Anti-inflammatory Activity: Paw Volume Change—The onset of the polyarthritis was indicated by an increase in volume of the injected paw after an initial inflammatory phase of about 10 days in which the volume first increased and then decreased. In the vehicle control group, the onset of polyarthritis occurred between Days 9 and 11 (see Table IV). Statistical analysis of the data seems to indicate that the onset of drug activity occurred earlier with SK&F 26070 than with aspirin.

On Days 15–19, both dose levels of SK&F 26070 and of aspirin elicited similar degrees of inhibition of paw volume increase. On Days 17 and 19, the inhibition of paw volume increase by the high dose

TABLE IV—ANTI-INFLAMMATORY ACTIVITY: REDUCTION IN VOLUME OF INJECTED PAW

Compound	Dose, b.i.d. (10 Animals/Group) mg./Kg.	Mean Paw Volumes (ml.) on Days 1	3	5	7	9	11	13	15	17	19	ED_{50} with 95% Fieller Confidence Limits Moles/Kg. $\times 10^{-3}$	mg./Kg. b.i.d.
SK&F 26070	148	0.94	1.22	1.11	1.06[a]	1.03	1.11[a]	1.12[a]	1.20[a]	1.28[a]	1.30[a]	0.98	261.0
	293	0.90	1.16[a]	1.10	1.02[a]	0.94[a]	1.06[a]	1.01[a]	1.05[a]	1.11[a]	1.12[a]	(—[b])	(—[b])
Aspirin	100	0.95	1.18	1.17	1.13	1.09	1.23	1.17[a]	1.22[a]	1.31[a]	1.36[a]	0.99	178.3
	200	0.95	1.16	1.13	1.10	1.02[a]	1.12[a]	1.12[a]	1.04[a]	1.12[a]	1.12[a]	(0.67–2.6)	(120.7–468.4)
0.5% Methylcellulose, 4,000 cps.	10 ml./Kg.	0.95	1.26	1.09	1.15	1.14	1.34	1.36	1.48	1.59	1.62	—	—
Nonarthritic control	No treatment	0.68[a]	0.78[a]	0.71[a]	0.74[a]	0.67[a]	0.79[a]	0.73[a]	0.68[a]	0.66[a]	0.68[a]	—	—

[a] Significantly different from the methylcellulose group ($p = 0.05$). [b] Limits could not be calculated.

TABLE V—ANTI-INFLAMMATORY ACTIVITY: PLASMA INFLAMMATION UNITS IN RATS

Compound	Dose, (10 Rat/Group) mg./Kg.	Mean Corrected Inflammation Units Determined from Plasma Withdrawn on Day 20	ED_{50} with 95% Fieller Confidence Limits Moles/Kg. × 10^{-3} b.i.d.	mg./Kg. b.i.d.
SK&F 26070	148	49.2	0.92	245.0
	293	21.5	(0.62–3.86)	(165.1–1027.9)
Aspirin	100	41.8	0.84[a]	151.3[a]
	200	20.3		
0.5% Methocel, 4,000 cps.	10 ml./Kg.	57.2	—	—
Nonarthritic control	No treatment	5.1	—	—

[a] Limits could not be calculated.

of both drugs was significantly greater ($p = 0.05$) than elicited by the low dose of the two drugs. ED_{50}'s for inhibition of paw volume increase (doses that decreased by 50% the difference in paw volumes between the vehicle control and nonarthritic control groups) were calculated from the data of Day 19. The mean paw volumes for each day and the ED_{50}'s for Day 19 are listed in Table IV.

The mean paw volume of the nonarthritic control group remained relatively constant during the 19 days of this study. Apparently the extremeties do not increase in size in proportion with the weight gain of the rat during this interval of growth.

Plasma Inflammation Units—The mean "corrected plasma inflammation units," determined on Day 20, are shown in Table V. The "corrected plasma inflammation units" for the low dose of SK&F 26070 and aspirin are not significantly different from the vehicle control group. The mean "corrected inflammation units" for the high dose for both compounds were similar and statistically significantly different from that of the vehicle control group. ED_{50}'s were calculated and are shown in Table V.

DISCUSSION

Orally in the mouse and rat, SK&F 26070 is less toxic, both on a milligram for milligram and on a molar basis, than aspirin. It is also significantly less irritating to the gastric mucosa of the rat and dog, even when administered repeatedly as was done with dogs. Although one might conclude that the lower toxicity and reduced gastric irritation are due to an incomplete or a slower rate of absorption, a previous study showed that SK&F 26070 is absorbed as rapidly and completely as aspirin following oral administration to dogs (5).

SK&F 26070 exhibits antipyretic, analgesic, and anti-inflammatory activities, and its pharmacological profile is, therefore, qualitatively similar to that of aspirin. One can infer from this and from the *in vitro* studies of hydrolysis of the compound (5) that the prodrug is cleaved after oral administration (and possibly after absorption) and that the pharmacological activities are due to the resultant salicylate formation. SK&F 26070's reduced gastric liability may be due to a reduced availability of the compound to the gastric mucosa because of the physical properties previously discussed.

Although SK&F 26070's acute toxicity and gastric irritation potential are less than those of aspirin, it appears, on a molar basis, as potent as aspirin in reducing both paw volume and plasma inflammation units in the chronic study of adjuvant-induced arthritis in rats.

SUMMARY

SK&F 26070, the hexylcarbonate of salicylic acid, possesses antipyretic, analgesic, and anti-inflammatory activities qualitatively identical to those of aspirin.

Its acute toxicity is less than that of aspirin, and its gastric irritation potential is markedly lower. Upon chronic administration, SK&F 26070 is as potent as aspirin in reducing both paw volume and plasma inflammation units in rats with adjuvant-induced arthritis.

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Keyphrases

Salicylic acid hexylcarbonate
Pharmacological activity—salicylic acid hexylcarbonate
Nephelometry—plasma analysis
Gastric irritation—salicylic acid hexylcarbonate