Pulmonary Absorption Studies Utilizing *In Situ* Rat Lung Model: Designing Dosage Regimen for Bronchial Delivery of New Drug Entities

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Abstract □ The absorption of 7-methylsulfinylxanthone-2-carboxylic acid, 7-(methylthio)xanthone-2-carboxylic acid, and their sodium salts from the respiratory tract of anesthetized rats was studied after intratracheal administration of 0.1 ml of a solution or suspension containing the drug. At various times after administration, the lungs and trachea were removed and assayed radiochemically for unabsorbed drug. Sodium 7-(methylthio)xanthone-2-carboxylate from solution was absorbed approximately 20 times faster than sodium 7-methylsulfinylxanthone-2-carboxylate from a solution. The absorption from solutions was three to four times faster than the absorption from suspensions. For inhalation aerosol dosage forms intended for prophylactic use, the drug entity with slower systemic absorption probably would be more desirable than the drug entity with rapid absorption. Rapid systemic absorption following inhalation of powder or liquid aerosols would lead to more frequent dosing if the biological activity is related to the drug concentration in the tracheobronchial tissues. Therefore, the powder or liquid inhalation aerosols of organic acids rather than the corresponding sodium salts may be preferable for designing a dosage regimen. However, if the dosage form is intended for utilizing the bronchodilator activity of the compound, the drug entity with rapid absorption is more desirable. Therefore, in the treatment of asthmatic attacks, liquid or powder inhalation aerosols of the sodium salt of the rapidly absorbing drug entity are preferable. The absorption rates were directly proportional to concentration when the initial concentration of sodium 7-methylsulfinylxanthone-2-carboxylate was varied over a 333-fold range. The effect of the pH of the drug solution administered intratracheally to the rat lung indicated a sharp increase in the pulmonary absorption at a pH near the pKa. The results suggested that structurally related xanthones are absorbed possibly by passive diffusion across the lipoidal region of the pulmonary membranes and that the absorption of organic acids and organic electrolytes is mainly controlled by the lipid solubility of the unionized species.

Keyphrases □ Xanthone-2-carboxylic acids, substituted—pulmonary absorption after intratracheal administration, solution and suspension compared, rat lungs □ Absorption, pulmonary—substituted xanthone-2-carboxylic acids and sodium salts, intratracheal administration, solution and suspension compared, rat lungs □ Bronchial delivery—substituted xanthone-2-carboxylic acids and sodium salts, intratracheal administration, solution and suspension compared, rat lungs □ Dosage forms—solutions and suspensions of substituted xanthone-2-carboxylic acids and sodium salts, pulmonary absorption, rat lungs

The delivery of drugs to the tracheobronchial tree has been used for centuries. The use of inhalation aerosols, however, has not gained as much popularity as many conventional dosage forms. The drugs are generally subjected to systemic absorption through the mouth, nose, trachea, lungs, and GI tract after aerosol inhalation. The determination of the site and extent of drug delivery becomes difficult because of the complications resulting from the various mechanisms of deposition, clearance, and retention of aerosol particles. In spite of these disadvantages, the bronchial route of drug administration is the most direct route to the tracheobronchial tree. In some situations, bronchial administration is the only practical way of delivering a drug to the respiratory tract because of the loss of important biological activity by acid or enzymatic hydrolysis in the stomach, first-pass metabolism in the liver, or poor absorption in the GI tract.

Although the major objectives of inhalation therapy are to provide the drug directly to the site of action and to treat the conditions within the tracheobronchial tree, the potential absorption of the drug across alveolar capillary membranes must be considered. The knowledge of the absorption rates of new drug entities becomes extremely important if the biological activity is directly related to drug concentration at the site of action. From a dosage regimen viewpoint, the slow systemic absorption of a drug may be more desirable than rapid systemic absorption from the lungs. Therefore, the pulmonary absorption of potential drug entities should be compared quantitatively before the most suitable drug species is selected.

A quantitative method of measuring the absorption of various nonvolatile organic solutes from the anesthetized rat lung was introduced recently (1–11). The results of these studies indicated that the respiratory tract epithelium conforms to the classical lipid pore model of biological membranes. Lipid-soluble compounds were absorbed more rapidly than lipid-insoluble compounds, and the latter substances crossed the membranes at rates inversely related to their molecular size. With most compounds, absorption appeared to occur by passive diffusion. Notable exceptions were the absorption of phenolsulfonphthalein (8) and cromolyn sodium (9), which were absorbed in part by a saturable carrier-type transport process and in part by diffusion.

This investigation utilized the *in situ* rat lung model for quantitative determination of the absorption rates of two structurally related xanthone-2-carboxylic acids and their sodium salts. It was hoped that the comparisons would provide useful information in the selection of the most suitable drug entity for an appropriate dosage regimen for bronchial delivery. Furthermore, this study was expected to elucidate the processes involved in the absorption of these compounds from the lungs.

EXPERIMENTAL

Chemicals—Monobasic and dibasic sodium phosphate[1] (anhydrous), citric acid[1], sodium citrate[1], calcium chloride[1], sodium chloride[2], potassium chloride[2], dextrose[2] (anhydrous), and acetic acid[3] were analytical reagent grade.

The two xanthones, 7-methylsulfinylxanthone-2-carboxylic acid[4]

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Table I—Apparent First-Order Rate Constants and Half-Times of Absorption of the Sodium Salts and Free Acids of Two Xanthones

Compound	Primary Absorption Rate Constant, min^{-1}	Primary Half-Time of Absorption, min	Secondary Rate Constant, min^{-1}	Secondary Half-Time of Absorption, min
7-Methylsulfinylxanthone-2-carboxylic acid	0.0304	23	0.0112	61.7
Sodium 7-methylsulfinylxanthone-2-carboxylate	0.0534	13	—	—
7-(Methylthio)xanthone-2-carboxylic acid	—	—	0.231	3.0
Sodium 7-(methylthio)xanthone-2-carboxylate	—	—	0.232	3.0

and 7-(methylthio)xanthone-2-carboxylic acid[4], were used as received. The radiochemical purity of 7-methylsulfinylxanthone-2-carboxylic acid and its sodium salt was greater than 95%. The chemical purity as determined by quantitative TLC, using chlorform–methanol–acetic acid (95:5:1), was greater than 99.5%. The radiochemical purity of 7-(methylthio)xanthone-2-carboxylic acid was greater than 98%, and its chemical purity was 99.7%.

Naphthalene[1], 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene[5], 2,5-diphenyloxazole[5], methanol[2], dioxane[6] (spectrograde), and toluene[2] (scintillation grade) were used in making the scintillation cocktail.

Doses—Doses for the sodium 7-(methylthio)xanthone-2-carboxylate were prepared by dissolving radioactive and nonlabeled compounds in pH 7.4 phosphate buffer. Sodium 7-methylsulfinylxanthone-2-carboxylate doses were prepared by dissolving the radioactive and nonlabeled materials together in appropriate buffer solutions. The doses of the free acids were prepared by coprecipitating the free acid from a solution of radioactive and nonlabeled materials by adding an equimolar quantity of hydrochloric acid and then adding the buffer species to obtain the appropriate buffer concentration and pH. The particle-size distribution of suspensions resulting from the two compounds were similar.

The buffers contained 0.134 *M* sodium chloride, 0.011 *M* potassium chloride, 0.0001 *M* calcium chloride, 0.02 *M* glucose, and 0.008 *M* buffer salts. Sodium 7-methylsulfinylxanthone-2-carboxylate was used for studying the effects of concentration and pH on pulmonary absorption. For pH 3, 3.5, and 4 experiments, acetate buffer was used. For pH 5 and 7.4 experiments, citrate–phosphate and phosphate buffers, respectively, were used. All buffers were of the same molarity (0.008 *M*). Except as indicated under the concentration effect, the dose per rat for the solutions was 10 μg; for the suspensions, it was 1 mg.

Procedure in Animals—Young male Sprague–Dawley-derived rats[7], 160–190 g, were used. Animals were anesthetized with pentobarbital and placed on their backs on a small animal operating board[8]; the limbs were secured. After exposing the trachea through a longitudinal incision along the ventral aspect of the neck, the trachea was cut traversely, halfway through between the fourth and fifth tracheal rings caudal to the thyroid cartilage.

Medical grade tubing[9] [0.07 cm (0.027 in.) i.d., 0.09 cm (0.039 in.) o.d., and 6.3 cm (2.5 in.) in length] was inserted around the needle of a 100-μl syringe[10] so that 1.2 cm remained exposed from the needle. The tubing was inserted through the tracheal incision to a depth of 2.5 cm below the tracheal incision, so the tip of the tubing was slightly above the bifurcation of the trachea. With the tubing in this position, the solution or suspension was injected over 1–2 sec. The tubing was then withdrawn completely, and the animal was maintained under light anesthesia for the remainder of the experiment. The body temperature of the animal was maintained at 36 ± 1° throughout the experiment by heat from a 100-w incandescent lamp in a reflector suspended over the animal at about 20 cm.

Absorption of the xanthones was allowed to occur for various times. One minute before the end of the absorption period, removal of the lungs was begun. The trachea just below the incision was tied by means of a nylon thread, and the lungs were quickly removed along with the trachea up to just above the tied end. Any surrounding tissue was then trimmed, and the lungs were cut into two parts so as not to exceed the sample weight of the combustor. Each part was placed on a 7-cm diameter filter paper[11] and dried overnight in a petri dish using a vacuum oven at 50°.

Measurement of Radioactivity—Both parts of the dried lungs from each animal, along with the filter paper, were pelletized and then combusted[12]. The pelletized samples were ignited on a platinum filament in the combustion chamber and burned completely. The vapors from each sample passed through a refrigerated condenser and then into a scintillation vial. After complete combustion, the flask and condenser were flushed with five injections of distilled water to cleanse the system of radioactivity. The wash was also passed into the scintillation vial. The condenser was again flushed with water and added along with the scintillation fluid to the scintillation vial. The composition of the scintillation fluid was: naphthalene, 100 g; 2,5-diphenyloxazole, 5 g; 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, 0.3 g; dioxane, 720 ml; toluene, 135 ml; and methanol, 45 ml.

To minimize radioactivity overlap, a blank pellet was combusted in a similar fashion between each pair of samples. Radioactivity was determined by a scintillation counter[13]. Background counts were determined from combustion of blank filter paper and subtracted from the sample counts. Quench corrections were made by comparing the counts gained from spiking both the blanks and the samples with a known amount of radioactivity. The ratio of the average spike from blanks to the average spike from samples determined the quench correction applied to the samples.

To determine the combustion efficiency of the oxidizer, two sets of standards were prepared from the same solution used for dosing the animals. Each standard of the first set was prepared by placing a known volume of solution on a filter paper, and then pelletizing and combusting it. The second set of standards were noncombustion standards. The combustion efficiency equaled the counts per minute of the combustion standard divided by the counts per minute of the noncombustion standard. This value then represented the percent of radioactivity retained in the combustion procedure. The counts per minute value of the sample was multiplied by the reciprocal of the combustion efficiency.

The percent radioactivity remaining in the lungs for any given time point was determined from the counts per minute obtained from the noncombustion standards. The counts per minute values obtained from the two lung samples for each rat were combined to give the radioactivity remaining at that time point. The percent radioactivity remaining was determined from the counts per minute of the lung sample divided by the counts per minute of the dose given intratracheally.

Octanol–Water Partition Coefficient—The octanol[1]–water partition coefficients were determined by equilibrating at 23° 100 ml of 0.008 *M* HCl in which the compound was dissolved with 0.5 ml of 1-octanol. The initial concentration of 7-(methylthio)xanthone-2-carboxylic acid (tritiated) was 0.009 μg/ml, and the initial concentration of 7-methylsulfinylxanthone-2-carboxylic acid (tritiated) was 0.004 μg/ml. The samples were shaken on a wrist-action shaker[14] until equilibrium was reached. Samples from both phases were taken, and concentrations were determined by the radiochemical method.

RESULTS AND DISCUSSION

The results of pulmonary absorption studies with sodium 7-

[5] Arapahoe Chemicals, Boulder, Colo.
[6] Matheson, Coleman & Bell, Norwood, OH 45212
[7] Simonsen Laboratories Inc., Gilroy, CA 94020
[8] Americal Hospital Supply Corp., McGaw Park, IL 60085
[9] Lined with Teflon (du Pont), Becton, Dickinson and Co., Rutherford, N.J.
[10] Hamilton Co., Whittier, Calif.
[11] Whatman No. 1.
[12] Tri-Carb sampler oxidizer model 305, Packard Instruments.
[13] Nuclear Chicago, Des Plaines, IL 60018
[14] Burrell Corp., Pittsburgh, Pa.

Table II—Effect of Concentration on Absorption of Sodium 7-Methylsulfinylxanthone-2-carboxylate from Rat Lungs

Initial Concentration	Number of Animals	Time for Absorption, min	Mean Percent Unabsorbed	Range	*SE*
3 μg/ml	6	5	75.91	64.9–84.7	2.72
100 μg/ml	10	5	72.31	66.7–82.5	1.74
0.5 mg/ml	5	5	81.18	76.5–88.3	2.46
1 mg/ml	9	5	83.25	76.2–88.7	1.27

methylsulfinylxanthone-2-carboxylate solution and 7-methylsulfinylxanthone-2-carboxylic acid suspension are given in Fig. 1. The drug from the solution appeared to be absorbed by a first-order process. The semilogarithmic plots of pulmonary absorption from the suspension did not follow a linear relationship with time. This finding indicated that the dissolution of the drug from a suspension was superimposed on the absorption process. The slower rate of absorption in the secondary phase might indicate that the rate of dissolution rather than the rate of absorption may be the rate-limiting step.

Estimates of the absorption rate constant and half-time for absorption are given in Table I. The half-time of absorption from solutions was 13 min. However, the half-time of absorption from suspensions after the initial rapid phase was 61.7 min. According to these results, it would be possible to obtain different rates of drug clearance from the lungs by proper selection of the drug species.

Drugs intended for bronchial delivery from powders or inhalation aerosols are generally developed for topical treatment within the respiratory tract. For a suitable dosage regimen of these dosage forms, rapid systemic absorption from the lungs may not be desirable if the compound has only prophylactic activity. For a powder inhalation aerosol dosage form or propelled[15] aerosol dosage form, absorption data from solutions and suspensions suggest that the free acid of the drug would be absorbed at a slower rate compared to the sodium salt because the absorption from the lungs would be limited by the slower dissolution from the solid particles of the free acid. The slower systemic absorption from the lungs would provide drug concentrations for longer periods for the mast cell protective activity. Based on these considerations, the free acid rather than the sodium salt may be more desirable.

The results of pulmonary absorption studies with sodium 7-(methylthio)xanthone-2-carboxylate solution and 7-(methylthio)-xanthone-2-carboxylic acid suspension are given in Fig. 2. The semilogarithmic plots of the absorption data from solutions as well as suspensions suggest that the absorption process is biphasic. Because of the extremely rapid rate of absorption of the drug in the primary phase, this model did not permit the determination of primary rates of absorption. At the end of 90 sec, only about 20% drug was remaining in the lungs after intratracheal administration of a solution.

The secondary half-time of absorption of this compound both from solutions and suspensions was 3 min. Although the absorption from suspensions was somewhat slower in the primary phase, probably because of the dissolution step from the suspended drug in the free acid form, the amount remaining in the lungs at the end of 10 min was less than 10%. Rapid absorption of this compound in combination with bronchodilator activity provides a useful dosage regimen for the treatment of asthmatic attacks.

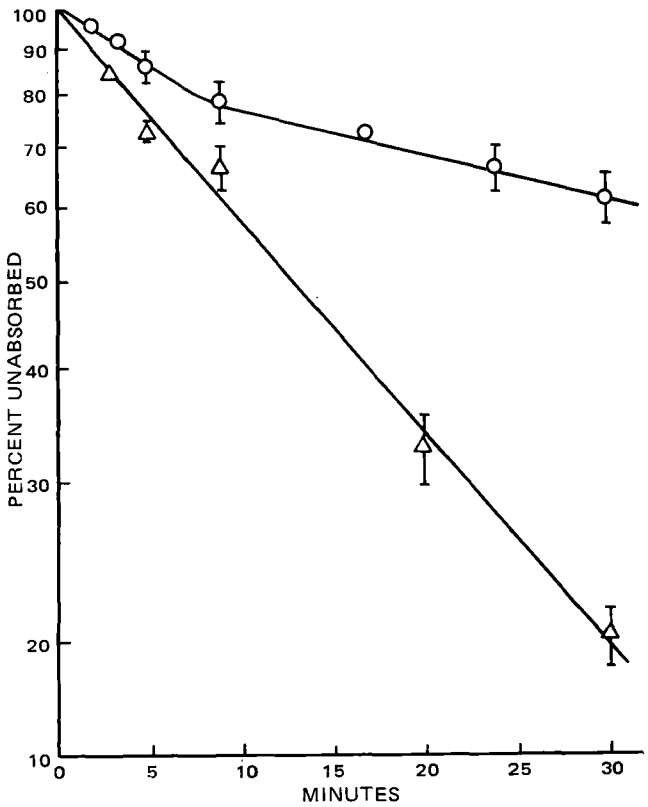

Figure 1—*Absorption of 7-methylsulfinylxanthone-2-carboxylic acid after intratracheal administration of a sodium salt solution (Δ) and an acid suspension (O) to the lungs of anesthetized rats. Data points are averages of at least three rats. Vertical bars represent standard error; the absence of vertical bars indicates that the standard error was too small to be shown.*

[15] Freon.

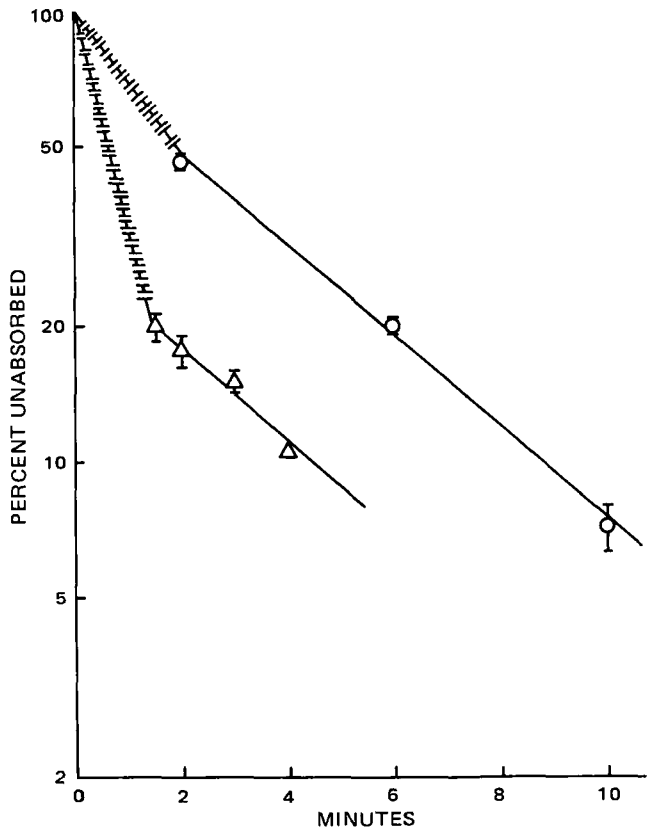

Figure 2—*Absorption of 7-(methylthio)xanthone-2-carboxylic acid and its sodium salt after intratracheal administration of a sodium salt solution (Δ) and an acid suspension (O) to the lungs of anesthetized rats. Data points are averages of at least three rats. Vertical bars represent standard error; the absence of vertical bars indicates that the standard error was too small to be shown.*

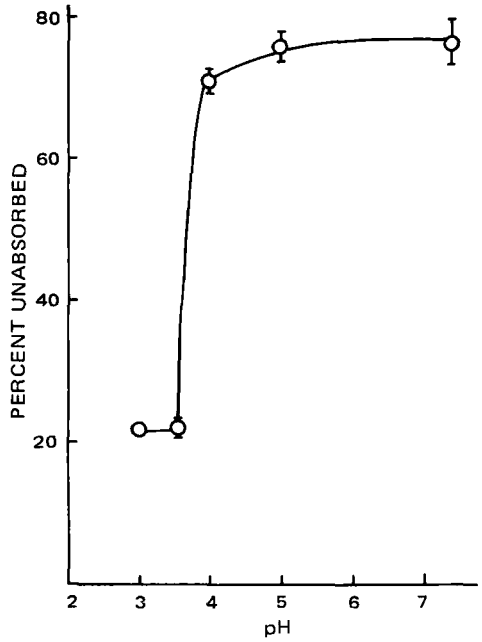

Figure 3—*Effect of pH of the solution administered intratracheally to rat lungs on absorption of sodium 7-methylsulfinylxanthone-2-carboxylate. Data points are averages of at least three rats. Vertical bars represent standard error; the absence of vertical bars indicates that the standard error was too small to be shown.*

The apparent first-order absorption of sodium 7-methylsulfinylxanthone-2-carboxylate and the biphasic absorption of sodium 7-(methylthio)xanthone-2-carboxylate from solution are similar to the absorption of cardiac glycosides from the rat lungs (7). The pulmonary absorption of digoxin and digitoxin was apparent first order, while ouabain and dihydroouabain appeared to fit a biexponential function. These differences in the mode of absorption of structurally related xanthones can probably be explained in terms of the large differences in the partition coefficients between the aqueous and the lipoidal phases. These data appear to agree with the octanol–water partition coefficient data. The octanol–water partition coefficient of 7-methylsulfinylxanthone-2-carboxylic acid was 58 compared to 2493 obtained for 7-(methylthio)xanthone-2-carboxylic acid. These differences in octanol–water partition coefficients and in the pulmonary absorption are in agreement with earlier (4, 5) observations that compounds with higher lipid solubility are absorbed faster than the compounds with lower lipid solubility.

In Table II, the effect of concentration on absorption of sodium 7-methylsulfinylxanthone-2-carboxylate from the rat lung administered in the form of a solution is given. Over a 3-μg/ml–1-mg/ml range, the absorption did not vary significantly. Since the percent unabsorbed remained essentially constant over a wide concentration range, the amount absorbed being proportional to the concentration, the results suggested that the absorption occurred mainly by a nonsaturable process such as passive diffusion.

The results of the percent sodium 7-methylsulfinylxanthone-2-carboxylate unabsorbed at 5 min as a function of pH of the drug solution administered intratracheally to rat lungs are given in Fig. 3. These results indicate a relatively small change in absorption in the pH 4–8 range. There was a sharp increase in the pulmonary absorption when the pH of the administered solution was changed to 3.5. The pKa of this compound, as determined by the solubility method, was 3.8. A considerable increase in the unionized species at the absorption site was reflected by a rapid increase in absorption. These results further support the hypothesis that pulmonary absorption of these organic electrolytes and organic acids is mainly controlled by the lipid solubility of the unionized species.

REFERENCES

(1) G. F. Moss and J. T. Richie, *Toxicol. Appl. Pharmacol.*, **17**, 699(1970).

(2) S. J. Enna and L. S. Schanker, *Am. J. Physiol.*, **223**, 409(1972).

(3) *Ibid.*, **223**, 1227(1972).

(4) J. A. Burton and L. S. Schanker, *Proc. Soc. Exp. Biol. Med.*, **145**, 752(1974).

(5) J. A. Burton and L. S. Schanker, *Steroids*, **23**, 617(1974).

(6) J. A. Burton and L. S. Schanker, *Xenobiotica*, **4**, 291 (1974).

(7) R. C. Lanman, R. M. Grillilan, and L. S. Schanker, *J. Pharmacol. Exp. Ther.*, **187**, 105(1973).

(8) S. J. Enna and L. S. Schanker, *Life Sci.*, **12**, 231(1973).

(9) T. S. Gardiner and L. S. Schanker, *Xenobiotica*, **4**, 725(1974).

(10) E. W. Mitchell and L. S. Schanker, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **32**, 258(1973).

(11) *Ibid.*, **33**, 422(1974).

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