PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 752–756 (1974)

Absorption of Antibiotics from the Rat Lung[1] (37889)

JERRY A. BURTON AND LEWIS S. SCHANKER

Department of Pharmacology, University of Missouri–Kansas City, 5100 Rockhill Road, Kansas City, Missouri 64110

Antibiotic agents have been administered into the respiratory tract of man in the treatment of severe bronchopulmonary infections (1, 2). Although the drugs have been given mainly for their local action within the lungs, qualitative evidence is available to indicate that they are absorbed systemically to a significant extent. For example, after inhalation of aerosols or dusts of benzylpenicillin (3–8), streptomycin (9), kanamycin (10, 11), or colistin (12, 13) in humans, the compounds have been detected either in the blood or the urine.

Despite continued usage of antibiotic aerosols, quantitative information on the rate or extent of pulmonary absorption of the drugs has not become available. The present study in the rat describes quantitatively the pulmonary absorption of five antibiotic agents: benzylpenicillin, tetracycline, erythromycin, doxycycline, and chloramphenicol. Comparison of the absorption rates with various physicochemical properties of the compounds provides information concerning the mechanism of absorption of these substances.

Materials and Methods. Procedure in animals. To investigate the absorption of antibiotics from the lung, male Charles River-derived rats weighing 150–200 g were anesthetized with pentobarbital and prepared surgically according to a method described previously (14). Briefly, the trachea was exposed through a ventral midline incision in the neck. A 2.5-cm length of PE 240 tubing, which served as a tight-fitting tracheal cannula, was inserted through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage to a depth of 0.6 cm. Solutions of antibiotics (0.006–50 m*M*) were prepared by adding radioactively labeled compound together with unlabeled compound to Krebs–Ringer phosphate solution (pH 7.4), in which Ca ion had been lowered to one-fifth the usual concentration to avoid turbidity (15). One-tenth milliliter of solution was injected into the lungs through PE 20 tubing attached to a calibrated 100-μl syringe. The injection tubing was inserted through the tracheal cannula to a point 1 mm above the bifurcation of the trachea, the drug solution injected over a 1- to 2-sec interval, and the tubing quickly withdrawn. The incision in the skin was then closed with a wound clip and body temperature maintained at 37 ± 1° by heat from a 40-W incandescent lamp suspended above the animal.

Tissue extraction and drug assay. At the end of an absorption period, the lungs and attached trachea were excised from the animal, weighed, and placed in a 15-ml Tenbroeck glass homogenizer together with sufficient distilled water to make a total weight of 4 g. After homogenization, 100-mg samples of the tissue homogenate were transferred into glass liquid scintillation counting vials, and to each vial was added 0.2 ml of 60% perchloric acid together with 0.4 ml of 30% hydrogen peroxide. The vials were then heated at 70° for 1 hr. To the resulting

[1] Supported by Public Health Service Research Grant GM-15483 from the National Institute of General Medical Sciences. Portions of this paper appeared in a dissertation submitted by J. A. Burton to the School of Graduate Studies, University of Missouri-Kansas City, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. A preliminary report on portions of this work has appeared previously in Fed. Proc. 29, 544, 1970.

tissue digest was added 20 ml of a liquid scintillation medium of the following composition: 6 g 2,5-diphenyloxazole, 573 ml toluene, and 427 ml ethylene glycol monomethyl ether. Radioactivity was measured using a Packard model 3375 Tri-Carb liquid scintillation spectrometer equipped with automatic external standardization. Net counts per minute were corrected for quench by comparison with a standard quench correlation curve determined for the liquid scintillation medium used. When known amounts of various labeled antibiotics were added to lung tissue and the assays carried out as described above, recoveries were complete (98–102%).

The above tissue extraction and drug assay procedure was used for all antibiotics except chloramphenicol. For the latter drug, the lungs and trachea were placed in a Tenbroeck glass homogenizer with sufficient distilled water to make a total fluid volume (tissue water plus distilled water) of 11 ml (14). After thorough homogenization, 1 ml of concentrated HCl and 1.2 ml of 50% trichloroacetic acid solution were added, and the sample was again homogenized. The homogenate was then poured into a centrifuge tube and centrifuged for 20 min at 600*g*. Of the resulting clear supernatant fluid, 0.5 ml was placed in a scintillation counting vial containing 15 ml of a scintillation medium of the following composition: 4 g 2,5-*bis*-2(5-*tert*-butylbenzoxazolyl)-thiophene, 80 g naphthalene, 400 ml ethylene glycol monomethyl ether, and 600 ml toluene. When known amounts of chloramphenicol were added to lung tissue, the extraction carried out by the above procedure, and radioactivity measured by liquid scintillation spectroscopy as described above, the recovery was 84% (SE ± 1 in six determinations). Accordingly, results were corrected for the incomplete recovery.

Partition coefficients. Chloroform/water partition coefficients were calculated from the distribution of an antibiotic after shaking an aqueous solution of compound (pH 7.4) with the organic solvent at 23°. Mechanical shaking for 1 hr was sufficient for equilibration of all the compounds. The organic solvent and the aqueous phase, 0.05 *M* sodium pyrophosphate buffer (pH 7.4), had been previously shaken together until mutually saturated. Coefficients were expressed as the mean of six to nine determinations in which the SE was 2–4% of the mean value.

Drugs. Antibiotics were obtained from the following sources: chloramphenicol (dichloroacetyl-1,2-^{14}C), sp act 9.43 μCi/mg, and tetracycline-7-^{3}H, sp act 833 μCi/mg, New England Nuclear Corp.; benzylpenicillin-^{14}C, sp act 71.4 μCi/mg, Amersham-Searle; doxycycline hydrochloride hemiethanolate hemihydrate, Charles Pfizer & Co.; and benzylpenicillin potassium, Nutritional Biochemicals Corp. Erythromycin-*N*-methyl-^{14}C, sp act 7.1 μCi/mg, and unlabeled erythromycin were kindly provided by Eli Lilly & Co; doxycycline-^{3}H, sp act 352 μCi/mg, by Charles Pfizer & Co.; chloramphenicol by Parke, Davis & Co.; and tetracycline HCl by American Cyanamid Co. With the above radioactively labeled compounds, it has been shown by the manufacturers that 96–98% of the radioactivity has the same chromatographic behavior as the unlabeled compounds.

Results and Discussion. Results of pulmonary absorption studies with five antibiotics are shown in Fig. 1. When the percentage of unabsorbed drug was plotted semilogarithmically against time, the data for each compound conformed to a straight line, suggesting first-order kinetics for the absorption process. Half-times for absorption and apparent first-order rate constants, calculated from the slopes of the lines, are listed in Table I. It can be seen that the time required for 50% of a dose to be absorbed ranged from 1.9 to 33 min for the five compounds. Chloramphenicol was absorbed most rapidly followed by doxycycline, erythromycin, and tetracycline, with benzylpenicillin showing the slowest absorption rate.

Recent work in this laboratory (14, 15) has suggested that the respiratory tract epithelium of the rat has permeability characteristics similar to those of the classical lipoid-pore type of biologic membrane. Lipid-soluble compounds are absorbed more rapidly than lipid-insoluble compounds, and the latter substances cross the membrane at

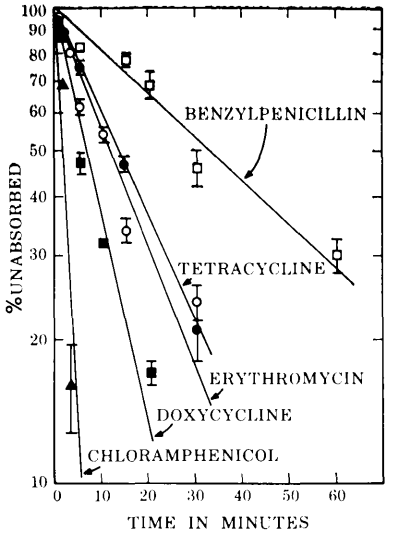

FIG. 1. Relative rates of absorption of antibiotic compounds from the rat lung. Krebs–Ringer phosphate solution (0.1 ml), containing a compound, was administered intratracheally to anesthetized rats prepared with a tight-fitting tracheal cannula. Initial concentrations were: chloramphenicol-^{14}C, 1 m*M*; doxycycline-^{3}H, 0.06 m*M*; erythromycin-^{14}C, 1 m*M*; tetracycline-^{3}H, 1 m*M*; and benzylpenicillin-^{14}C, 1 m*M*. Each point is the mean of 3–16 animals. Vertical brackets indicate SE, and absence of brackets indicates that SE was too small to be shown.

rates inversely related to their molecular size. The antibiotics of the present study have molecular weights ranging from 323 to 734 and have widely different lipoid solubilities as judged by the chloroform/water partition coefficients listed in Table I. On comparing partition coefficients, molecular weights, and pulmonary absorption rates, it appears that lipoid solubility is more important than molecular size in determining the relative speed with which these compounds cross the respiratory tract epithelium (Table I). For example, doxycycline and tetracycline have the same molecular weight, but doxycycline was absorbed 2 times more rapidly than tetracycline in accord with the 3-fold greater partition coefficient of doxycycline. Moreover, chloramphenicol and benzylpenicillin have similar molecular weights, but chloramphenicol was absorbed 17 times faster than the penicillin in accord with the 25-fold greater partition coefficient of chloramphenicol. The relationship was not perfect, however, since erythromycin with the highest partition coefficient had an absorption rate that ranked only midway in this list of compounds.

A previous report from this laboratory presented evidence that the anionic dye phenol red is absorbed from the rat lung not only by diffusion but also in part by a carrier-type transport process (16). This process, which becomes saturated at high concentrations of the dye, is inhibited by certain organic anions including benzylpenicillin. The question arises whether benzylpenicillin is also absorbed in part by a carrier process and, if so, whether it is the same process that transports phenol red. In looking for evidence of a saturable transport process, the pulmonary absorption rate of

TABLE I. Absorption of Antibiotic Compounds from the Rat Lung.

Compound	Mol. wt.	Chloroform/water (pH 7.4) partition coefficient × 10^4	Rate of absorption[a]	
			Half-time (min)	Rate constant × hr^{-1}
Chloramphenicol	323	2,460	1.9	21.9
Doxycycline	444	3,769	7.0	5.9
Erythromycin	734	12,578	12.0	3.5
Tetracycline	444	1,257	14.0	3.0
Benzylpenicillin	333	96	33.0	1.3

[a] Apparent first-order rate constant is equal to 0.693 divided by the half-time in hours. Half-time values were calculated from the curves in Fig. 1.

TABLE II. Effect of Concentration on Absorption of Antibiotic Agents from the Rat Lung.

Compound	Time (min)	Initial concn (m*M*)	No. of rats	% of Dose absorbed[a]
Benzylpenicillin	60	0.01	6	63 ± 2
		1	9	66 ± 2
		10	3	74 ± 1
		50	3	64 ± 2
Erythromycin	10	0.1	3	44 ± 4
		1	3	46 ± 5
		10	3	49 ± 3
Doxycycline	5	0.006	3	37 ± 2
		0.06	6	42 ± 3

[a] Mean ± SE.

the penicillin was measured over a wide range of initial concentrations. As shown in Table II, when the concentration of the antibiotic was varied 5000-fold, from 0.01 to 50 m*M*, the 1-hr absorption rate was directly proportional to concentration, the percentage absorption remaining essentially constant. The failure to find evidence of a saturable, carrier-type transport process for benzylpenicillin is interesting not only because of the inhibitory action of the compound on the pulmonary absorption of phenol red but also because the dye and the antibiotic appear to be transported by a common carrier process in other tissues such as liver, kidney, and choroid plexus (17).

Concentration studies with two other antibiotics gave results similar to those seen with benzylpenicillin (Table II). For example, doxycycline, which exists partly as an anion and partly as a zwitter ion at pH 7.4, when studied over a 10-fold range of concentration, showed no evidence of saturation, the percentage absorption remaining constant. Similarly the cationic compound erythromycin showed no evidence of saturation over a 100-fold range of concentration.

If results of the present study in the rat apply also to man, it would mean that administration of these antibiotics into the lungs for treatment of respiratory tract infections will result in most of the drug being absorbed into the circulation in a relatively short time. This could have important therapeutic and toxicologic implications.

Summary. Five antibiotics were administered intratracheally as solutions to anesthetized rats. The times necessary for 50% absorption ranged from 1.9 to 33 min. Chloramphenicol was absorbed most rapidly followed by doxycycline, erythromycin, and tetracycline, with benzylpenicillin showing the slowest rate. A comparison of pulmonary absorption rate, molecular weight, and lipoid (chloroform)/water partition coefficient of the drugs indicated that lipoid solubility is more important than molecular size in determining the relative absorption rates. The absorption process for benzylpenicillin, doxycycline, and erythromycin did not become saturated when drug concentrations were raised 10–5000-fold, suggesting that these antibiotics are absorbed mainly by a process of simple diffusion.

The authors are indebted to Mrs. Jean C. Henderson for her excellent technical assistance.

1. Miller, W. F., Arch. Intern. Med. **131**, 148 (1973).

2. Dautreband, L., "Microaerosols," p. 260. Academic Press, New York (1962).

3. Mutch, N., and Rewell, R. E., Lancet **248**, 650 (1945).

4. Knott, F. A., and Clark, W. H., Lancet **248**, 468 (1945).

5. Prigal, S. J., and Speer, F. D., Bull. N. Y. Med. Coll. 8, 21 (1945).

6. Prigal, S. J., McGavack, T. H., Speer, F. D., and Harris, R., J. Amer. Med. Ass. **134**, 932 (1947).

7. Talpin, G. V., and Thompson, H. T., Amer. J. Syph., Gonor., Vener. Dis. **32**, 124 (1948).

8. Gaensler, E. A., Beakey, J. F., and Segal, M. S., Ann. Intern. Med. **31**, 582 (1949).

9. Prigal, S. J., Tchertkoff, V., and Brooks, A. M., Dis. of Chest **17**, 304 (1950).

10. Bilodeau, M., Roy, J. C., and Giroux, M., Ann. N. Y. Acad. Sci. **132**, 870 (1966).

11. Ayres, S. M., Griesbach, J., and Giannelli, S., Jr., Curr. Ther. Res. **14**, 153 (1972).

12. Benincasa, T., and Moiraghi-Ruggenini, A., Minerva Otorinolaryngol. **11**, 392 (1961).

13. Halliday, N. P., Clin. Trials J. **4**, 771 (1967).

14. Enna, S. J., and Schanker, L. S., Amer. J. Physiol. **222**, 409 (1972).

15. Enna, S. J., and Schanker, L. S., Amer. J. Physiol. **223**, 1227 (1972).

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

17. Schanker, L. S., *in* "Metabolic Transport" (L. E. Hokin, ed.), p. 543. Academic Press, New York (1972).

Received Oct. 24, 1973. P.S.E.B.M., 1974, Vol. 145.