

## Antibiotic Uptake by Alveolar Macrophages of Smokers

W. LEE HAND,\* RICHARD M. BOOZER, AND NEVA L. KING-THOMPSON

Veterans Administration Medical Center (Atlanta), Decatur, Georgia 30033,\* and Department of Medicine, Emory University School of Medicine, Atlanta, Georgia 30303

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Cigarette smoking, particularly when associated with chronic pulmonary disease, increases the risk of respiratory tract infection. Thus, we elevated the uptake of antibiotics by alveolar macrophages (AM) obtained by bronchoalveolar lavage from persons who smoke and have associated pulmonary abnormalities, circumstances which adversely affect certain macrophage functions. The entry of radiolabeled drugs into AM was determined by a velocity-gradient centrifugation technique, and uptake was expressed as the ratio of cellular to extracellular antibiotic concentration (C/E). Cefamandole and penicillin G were taken up poorly by the AM obtained from smokers ( $C/E \leq 1$ ). Cellular levels of isoniazid, gentamicin, and tetracycline were similar to their extracellular concentrations. The lipid-soluble drugs lincomycin, chloramphenicol, and rifampin were concentrated severalfold by the AM from smokers ( $C/E = 3$  to 11). Ethambutol also entered macrophages readily ( $C/E = 11$ ). Erythromycin and clindamycin were massively concentrated by the AM from smokers ( $C/E = 23$  to 56). The AM of smokers accumulated a lipid-soluble antibiotic (rifampin) and actively transported agents (erythromycin propionate, clindamycin) more avidly than did the AM of nonsmokers. Augmented uptake of these antibiotics by the AM of smokers may be related to structural and functional alterations induced by smoking.

People who smoke cigarettes, especially those with associated chronic obstructive pulmonary disease, are more susceptible to respiratory infections than those who do not smoke (3, 15). It is possible that smoking and its associated respiratory tract abnormalities may predispose smokers to pulmonary infection in part by interfering with the function of pulmonary alveolar macrophages (AM), the predominant phagocytes in the lungs (5, 10, 13).

The usefulness of antibiotics in treating certain pulmonary infections, particularly those due to facultative intracellular organisms, may depend to some extent upon the ability of the drug to enter phagocytes and kill viable intracellular organisms. With this in mind, we recently studied the uptake of 12 antibiotics by AM from healthy, nonsmoking volunteers (6). Interactions between antibiotics and phagocytic cells might be especially important in the therapy of those bacterial infections which complicate chronic pulmonary disease states. Therefore, we have now determined the uptake of antibiotics by AM from patients with various pulmonary diseases, all of whom were smokers.

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### MATERIALS AND METHODS

**Bronchoalveolar lavage for collection of AM.** Human AM were obtained from volunteers by bronchoalveolar lavage performed during fiberoptic bronchoscopy as previously described (6). These hospitalized patients were all cigarette smokers on whom bronchoscopy was performed for various clinical indications. Informed consent was obtained by the investigator who performed the procedure. Bronchoalveolar lavage of a lower lobe or right middle lobe subsegment with 50 ml of sterile saline was performed five times for a total

volume of 250 ml. When the location of the pulmonary pathology (mass lesion, bleeding, etc.) could be demonstrated, lavage was performed in an uninvolved lung segment. Total and differential cell counts and viability determinations were performed as previously described (1, 16).

**Radiolabeled antibiotics for uptake studies.** The following radiolabeled antibiotics were used in the study: [ $^{14}\text{C}$ ]rifampin; [ $^3\text{H}$ ]clindamycin hydrochloride; [ $^3\text{H}$ ]lincomycin hydrochloride; chloramphenicol-[dichenoacetyl-1-2- $^{14}\text{C}$ ]; [ $7\text{-}^3\text{H}$  (N)] tetracycline; [ $^{14}\text{C}$ ]cefamandole nafate; [ $\text{N-}^{14}\text{CH}_3$ ]erythromycin; [ $\text{N-}^{14}\text{CH}_3$ ]erythromycin propionate; benzyl[ $^{14}\text{C}$ ] penicillin potassium; [ $\text{G-}^3\text{H}$ ]isoniazid (isonicotinic acid hydrazide); [ $\text{G-}^3\text{H}$ ]gentamicin sulfate; and [ $^{14}\text{C}$ ]ethambutol dihydrochloride. These antibiotic preparations were obtained from commercial sources or donated by various pharmaceutical companies.

**Antibiotic uptake by AM of smokers.** AM were suspended in tissue culture medium 199 containing 15% normal serum at a concentration of  $5 \times 10^6$  cells per ml. The cells were then incubated with radiolabeled antibiotics at a concentration of  $\sim 2.5 \times 10^{-5}$  M, which is a clinically appropriate serum level for these drugs. At intervals, samples of the incubation mixture were removed, and AM were separated from the radioactive antibiotic in solution by velocity-gradient centrifugation in a microcentrifuge tube (6, 7, 14). Uptake of antibiotics by AM was determined by centrifugation of the phagocytes through a water-impermeable silicone oil barrier into formic acid, which dissolves the cells. The lower layer containing the radiolabeled antibiotic in cells and the upper layer containing the extracellular antibiotic were counted in a liquid scintillation counter (model LS-350; Beckman Instruments, Inc., Fullerton, Calif.). Antibiotic uptake was expressed as the ratio of cellular to extracellular antibiotic concentration (C/E).

**Statistical analysis of data.** Differences between experimental groups were determined by means of a two-sample *t* test with a Tektronix 4051 computer (Tektronix, Inc., Beaverton, Oreg.).

\* Corresponding author.

RESULTS

The 15 subjects were patients undergoing bronchoscopy for diagnostic purposes. All were males ranging in age from 26 to 72 years, with a median age of 59 years and a mean age of 57 years. All were heavy cigarette smokers; their exposure ranged from 20 to 100 pack-years. In eight cases, bronchoscopy was performed because of suspected malignancy. In the other patients, hemoptysis or inflammatory disease of uncertain etiology was the indication for the procedure.

**Characteristics of the cell population.** The number of cells ( $3.14 \pm 0.77 \times 10^7$ ) obtained from the lower respiratory tract in these smokers was relatively large. In a companion study performed during the same time period, a much smaller number of cells ( $5.93 \pm 0.65 \times 10^6$ ) was collected by bronchoalveolar lavage of normal, nonsmoking volunteers (6). Approximately 90% of the cells collected from smokers were AM. Cell viability was  $\geq 95\%$ , as judged by trypan blue exclusion.

**Uptake of antibiotics by AM.** Cefamandole and penicillin G were taken up poorly by the AM from smokers ( $C/E \leq 1$ ) (Table 1). Gentamicin, tetracycline, and isoniazid achieved cellular levels which were similar to or slightly greater than their extracellular concentrations. The more lipid-soluble drugs lincomycin, chloramphenicol, and rifampin were concentrated severalfold by the AM of smokers ( $C/E = 3$  to 11). Ethambutol, a drug which is accumulated by complex mechanisms in other phagocytes (14, 17), also readily entered the AM of smokers ( $C/E = 11$ ).

The most striking results occurred in studies with erythromycin, erythromycin propionate, and clindamycin, which were massively concentrated by AM ( $C/E = 23, 32,$  and  $56,$  respectively). In previous studies, we have demonstrated that these antibiotics enter phagocytes by means of active transport mechanisms (6, 7, 14, 17). With clindamycin, transport is by means of the cell membrane nucleoside system (7, 19).

Antibiotic uptake studies with AM of healthy, nonsmoking volunteers (6) were performed in our laboratory during the same time period as the experiments with the AM of smokers. Each of the antibiotics entered the AM of smokers at least as well as it did the cells of the nonsmokers. Although the uptake of most of the antibiotics was the same in both cell populations, the AM of smokers accumulated several antibiotics to a greater extent than did the AM of nonsmokers. Thus, the actively transported agents clindamycin and erythromycin propionate reached higher levels in the AM of smokers than in the AM of nonsmokers (Fig. 1). The erythromycin base was also concentrated more avidly by the AM of smokers, but only two experiments were performed with cells from nonsmokers, precluding a meaningful comparison of the groups. Rifampin, a highly lipid-soluble drug which enters phagocytes by simple solubility partition (14, 17), also reached a significantly higher level in the cells of smokers (Fig. 1).

DISCUSSION

Cigarette smokers, especially those with associated chronic obstructive pulmonary disease, are at increased risk of respiratory tract infection (3, 15, 20). Since AM are the major phagocytic cells in the lung, their function is of particular importance in protection against and recovery from bacterial infection in the lower respiratory tract (4). Relationships among AM, bacteria, and antibiotics might be crucial in defense against those bacterial infections which

TABLE 1. Uptake of antibiotics (C/E) by AM from cigarette smokers with pulmonary disease

Time of incubation (min)	Mean $\pm$ SEM C/E (no. of expts)											
	Clindamycin	Erythromycin propionate	Erythromycin	Ethambutol	Rifampin	Chloramphenicol	Lincomycin	Isoniazid	Tetracycline	Gentamicin	Cefamandole	Penicillin G
5	28.83 $\pm$ 3.33 (3)	5.13 $\pm$ 0.57 (3)	3.45 $\pm$ 1.65 (4)	2.40 (1)	4.16 $\pm$ 0.74 (5)	3.40 (1)	1.00 (1)	1.70 (1)	0.66 (1)	0.49 (1)	0.58 (1)	0.10 $\pm$ 0.00 (2)
15	50.90 $\pm$ 2.13 (3)	20.16 $\pm$ 3.14 (3)	6.30 $\pm$ 1.76 (4)	1.67 $\pm$ 0.73 (2)	6.72 $\pm$ 1.18 (4)	3.74 (1)	1.90 (1)	1.39 $\pm$ 0.45 (3)	1.11 (1)	1.05 $\pm$ 0.15 (2)	1.05 $\pm$ 0.15 (2)	0.32 $\pm$ 0.08 (2)
30	51.24 $\pm$ 4.24 (5)	24.72 $\pm$ 1.71 (4)	13.23 $\pm$ 3.56 (4)	2.94 $\pm$ 0.96 (2)	7.88 $\pm$ 0.86 (6)	3.92 (1)	2.25 (1)	1.36 $\pm$ 0.17 (3)	1.21 (1)	1.15 $\pm$ 0.25 (2)	0.67 (1)	0.31 (1)
60	56.10 $\pm$ 3.72 (4)	28.10 $\pm$ 2.34 (4)	23.32 $\pm$ 5.23 (5)	5.40 $\pm$ 2.20 (2)	9.78 $\pm$ 0.51 (5)	3.19 (1)	3.54 (1)	1.26 $\pm$ 0.03 (3)	1.30 (1)	1.15 $\pm$ 0.35 (2)	0.72 (1)	0.45 $\pm$ 0.09 (2)
120	47.92 $\pm$ 5.98 (4)	32.40 $\pm$ 6.04 (3)	22.13 $\pm$ 3.80 (3)	10.83 $\pm$ 3.87 (2)	11.00 $\pm$ 0.62 (5)			1.43 $\pm$ 0.03 (2)		2.06 (1)		0.35 (1)

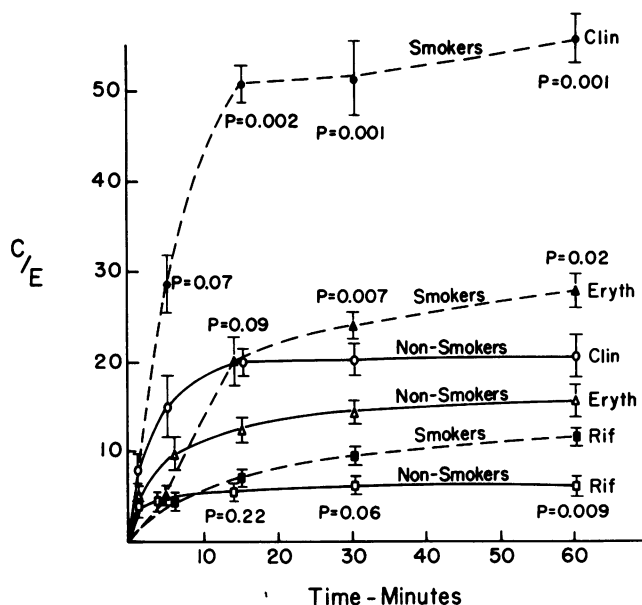


FIG. 1. Uptake of clindamycin (Clin), erythromycin propionate (Eryth), and rifampin (Rif) by AM of smokers and nonsmokers. Results are presented as the mean  $\pm$  standard error of the mean of observations at each time point. The *P* values reflect differences between studies with AM from smokers and nonsmokers. Data for nonsmokers are from reference 6.

are frequent complications of chronic obstructive pulmonary disease. Treatment of these infections should utilize antibiotics which are able to enter phagocytic cells and inactivate surviving intracellular organisms. Thus, it is relevant to determine how cells from individuals who are cigarette smokers with pulmonary abnormalities which might adversely affect AM function take up antibiotics and how these drugs influence AM antimicrobial activity.

In the present study, we evaluated the uptake of antimicrobial agents by AM from smokers with underlying lung disease and compared these results with those of our studies in normal individuals (6). All tested antibiotics entered the AM of the patients at least as well as they entered the AM of normal nonsmokers. Thus, as in studies with the AM of nonsmokers,  $\beta$ -lactam antibiotics entered the AM of smokers poorly, and both gentamicin and isoniazid achieved cellular concentrations in the AM of smokers similar to those in the extracellular fluid. Lipid-soluble antibiotics (e.g., rifampin) and actively transported agents (clindamycin, erythromycin antibiotics) were efficiently concentrated (several- to manyfold) by the AM of smokers. It was of interest that the AM of smokers concentrated these antibiotics more avidly than did the AM of nonsmokers. Thus, rifampin, clindamycin, and erythromycin propionate reached higher levels in the AM of smokers.

It is probable that cigarette smoking per se is responsible for the augmented accumulation of lipid-soluble and actively transported antibiotics by the AM of smokers. There are several pieces of evidence which favor this possibility. First, no single, unifying respiratory tract disease was present in all of the smokers. Second, in preliminary studies with AM of healthy, young cigarette smokers, we found that antibiotic uptake values were similar to those reported in the present study. Third, reported structural and functional alterations of AM from healthy smokers (2, 8-13, 16, 18, 21, 22) could account for the augmented accumulation of specific antibiotics. Thus, the increased lipid content of the AM of

smokers may be responsible for enhanced uptake of rifampin (a lipid-soluble drug) by these cells. Previous demonstrations of stimulated metabolic pathways and altered membrane function in the AM of smokers are compatible with our findings of enhanced uptake of erythromycin and clindamycin, antibiotics which enter phagocytes by active cell membrane transport systems. Our observations demonstrating satisfactory antibiotic uptake by the AM of smokers are encouraging with respect to therapy of infection in patients with underlying lung disease. However, it is now imperative to actually determine the effects of these drugs on organisms which persist after being ingested by the AM of such patients.

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