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Penetration of Clarithromycin into Lung Tissues from Patients Undergoing Lung Resection

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The concentrations of clarithromycin and its active principal metabolite, 14-(*R*)-hydroxy-clarithromycin, were determined in lung tissue obtained during lung resection and compared with concomitant concentrations in plasma. Concentrations of the parent and metabolite were determined by high-performance liquid chromatography. The 15 patients studied were given 500 mg orally every 12 h for a minimum of five doses to achieve steady-state concentrations. The mean concentrations of clarithromycin and 14-(*R*)-hydroxy-clarithromycin in plasma just prior to the final dose were 1.38 and 0.67 μg/ml, respectively, and those 4 h after the final dose (at the time of lung resection) were 1.89 and 0.80 μg/mL, respectively. The concentrations of the parent and metabolite in lung tissue at the time of lung resection averaged 54.3 and 5.12 μg/g, respectively, with a mean calculated ratio of concentrations of the parent to metabolite being 11.3 in lung tissue and 2.4 in plasma. Clarithromycin and its active metabolite are extensively distributed into human lung tissue.

Clarithromycin is a new macrolide antibiotic with a broad in vitro antimicrobial spectrum of activity (13). Clarithromycin differs from erythromycin in the methyl substitution at the 6-hydroxy position of the 14-membered ring. This minor structural change results in greater acid stability, improved pharmacokinetics, better gastrointestinal tolerance, and a broader spectrum of activity compared with those of erythromycin (1, 9, 10, 12, 13). Clarithromycin undergoes hepatic metabolism following oral dosing, the principal metabolite being 14-(*R*)-hydroxy-clarithromycin (14-OH-clarithromycin). This metabolite has been demonstrated to have appreciable antimicrobial activity and has in fact been reported to be more active than the parent drug against many strains of *Haemophilus influenzae* (8). The combination of clarithromycin and 14-OH-clarithromycin in a 2:1 or 4:1 ratio has also been shown to have enhanced in vitro activity against *H. influenzae* compared with the activity of either compound alone (6, 8).

Because macrolide antibiotics are often used in the treatment of community-acquired respiratory tract infections due to pathogens such as *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *H. influenzae*, and *Legionella pneumophila*, adequate penetration of clarithromycin into lung tissues is of importance. The penetration of 14-OH-clarithromycin is also an important consideration because of enhanced activity of clarithromycin against certain pathogens in the presence of this metabolite. Clarithromycin has previously been demonstrated to have extensive penetration into lung tissues (4). However, the microbiological assay used in this earlier study did not distinguish between the parent drug and metabolite and therefore did not quantitate the relative concentration of each compound. The objective of the present study was to examine by the analytical technique of high-performance liquid chromatography (HPLC) the penetration of clarithromycin and 14-OH-clarithromycin into lung tissues.

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This was a phase IV open-label study of clarithromycin (Abbott Laboratories, Abbott Park, Ill.) and its principal metabolite, 14-OH-clarithromycin. Adult patients between the ages of 18 and 65 who were undergoing elective thoracic surgery and lung resection were considered eligible for this study. Exclusion criteria included pregnancy or child-bearing potential if female, history of hypersensitivity to any macrolide antibiotic, evidence of significant renal or hepatic dysfunction, treatment with any experimental agent within 4 weeks of study entry, and concomitant treatment with drugs which might interact with clarithromycin (e.g., theophylline, warfarin, carbamazepine, or digitalis). The study was approved by the Institutional Review Board of the institutions where surgeries were performed, and written informed consent was obtained from each patient prior to study entry.

Patients enrolled into the study received 500 mg of clarithromycin orally every 12 h, each tablet administered with 180 ml of water. Complete medical histories were obtained from each enrolled patient, and complete physical examinations and laboratory review of serum chemistries and hematology were performed prior to receiving study medication. Clarithromycin was administered beginning at least 2 days prior to scheduled surgery, the last dose being given the morning prior to the procedure. Each patient was required to receive a minimum of five doses to achieve steady-state drug concentrations prior to surgery. On the morning of surgery, a 5-ml blood sample was obtained for clarithromycin assay, after which the last dose of clarithromycin was administered under fasting conditions. Lung resection was then performed under general anesthesia, during which a single healthy lung tissue sample and concomitant 5-ml blood sample were obtained. After surgery, patients were free to receive any medical or antimicrobial therapy, according to standard medical practice and initiated at the discretion of the primary care physicians.

Blood samples were collected in heparinized tubes and promptly centrifuged. Plasma was then transferred to labelled

TABLE 1. Concentrations of clarithromycin and 14-OH-clarithromycin in plasma and lung tissues

Drug	Concn ± SD in:			Ratio ± SD (lung/intraoperative plasma drug concn)
	Plasma (μg/ml)		Lung tissue (μg/g)	
	Minimum	Intraoperative		
Clarithromycin	1.38 ± 0.99	1.89 ± 0.66	54.32 ± 33.82	29.6 ± 15.3
14-OH-clarithromycin	0.67 ± 0.41	0.80 ± 0.29	5.12 ± 3.18	6.5 ± 3.4
Ratio (clarithromycin/14-OH-clarithromycin)	1.98 ± 0.96	2.42 ± 0.74	11.25 ± 3.80	NA[a]

[a] NA, not applicable.

polyethylene vials and frozen at −20°C until assayed. Tissue samples were stored in airtight containers and frozen at −20°C until the time of assay.

Lung tissue samples were thawed and blotted dry with clean absorbent paper. Accurately weighed tissue samples were homogenized in normal saline to contain approximately 0.1 mg of tissue per ml of homogenate. Aliquots of the homogenate were extracted in the same manner as the plasma samples were (2). In brief, plasma or lung homogenate samples were mixed with an internal standard (erythromycin A 9-*O*-methyloxime; Abbott 41036), alkalinized with 200 μl of 0.10 M sodium carbonate solution, and extracted with 3.0 ml of ethyl acetate-hexane (1:1, vol/vol). The organic solution was evaporated to dryness, the residues were dissolved in an aqueous acetonitrile solution (1:1, vol/vol), and 20 to 80 μl of the solution was analyzed on the chromatography system.

The plasma and lung parent drug and metabolite concentrations were determined by HPLC with electrochemical detection using a previously published procedure (2). The concentrations in tissue were not corrected for the contamination of blood. For this study, the inter- and intraday coefficients of variation over the standard curve concentration ranges (0.04 to 4.0 μg/ml for clarithromycin and 0.04 to 2.5 μg/ml for 14-OH-clarithromycin) were ≤7% for both plasma and lung samples. Samples with concentrations greater than the upper limit of the curve were reassayed at a reduced volume. Coefficients of determination (r^2) for the curves of both clarithromycin and 14-OH-clarithromycin were in the range of 0.998 to 1.000 for the entire study. The lower limit of detection was 0.04 μg/ml for both compounds.

Nineteen hospitalized patients scheduled to undergo elective lung resection were enrolled in this study. Fifteen patients (seven males and eight females) completed all phases of the study and are included in this report. The mean (± standard deviation) age, weight, and height of these patients were 45.7 ± 11.9 years (range, 25 to 66 years), 72.0 ± 12.6 kg (range, 47.7 to 91.8 kg), and 167.1 ± 10.9 cm (range, 140.3 to 182.9 cm), respectively. Renal and hepatic function tests were within the normal range in all patients. Lung resection was performed secondary to infection in seven patients (coccidioidomycosis in all cases), malignancy in five patients, and other causes (hamartoma, fibroma, and obstruction) in three patients. The drug was well tolerated, and no adverse effects were reported.

All patients received a minimum of five total doses of clarithromycin. Preoperative blood samples were obtained at a mean of 12.4 ± 1.0 h following the previous dose of clarithromycin and within 15 min prior to the final dose of study drug. Final preoperative doses of clarithromycin were administered at a mean of 2.6 ± 1.0 h prior to the start of the procedure. Lung tissue and concomitant blood samples were obtained at a mean of 4.3 ± 0.7 h after the preoperative dose.

Steady-state concentrations of clarithromycin and 14-OH-clarithromycin obtained by HPLC are shown in Table 1. Clarithromycin was highly concentrated in lung tissues, with a mean concentration of 54.32 ± 33.82 μg/g (range, 17.87 to 134.68 μg/g). The ratio of lung to intraoperative plasma clarithromycin concentrations ranged from 8.3 to 60.4 (mean, 29.6 ± 15.3). The lung 14-OH-clarithromycin concentrations were 2.4- to 13-fold higher than concomitant concentrations in plasma (mean ratio, 6.5 ± 3.4). The ratio of clarithromycin to 14-OH-clarithromycin was approximately 2:1 in both preoperative and intraoperative plasma samples. The parent-to-metabolite ratio in lung tissues was much higher, however, with a mean ratio of 11.25 ± 3.80 (range, 6.77 to 20.23).

Clarithromycin penetrates into both alveolar macrophages and polymorphonuclear leukocytes; this uptake results in extremely high ratios (range, 12:1 to >30:1) of intracellular to extracellular concentrations (7, 11). The high concentrations in lung tissue observed in our study can probably be explained by the intracellular accumulation of clarithromycin. These intracellular concentrations of clarithromycin contribute to the eradication of lower respiratory tract infections caused by intracellular pathogens such as *L. pneumophila.*

The exact reason for the difference in lung penetration between clarithromycin and its metabolite, 14-OH-clarithromycin, is unknown. One hypothesis is that the parent drug is in a more un-ionized state at physiological pH than is the metabolite. The parent compound could continue to penetrate the cell membrane and lead to intracellular accumulation. This is supported, in part, by data which demonstrate that the uptake of clarithromycin by human polymorphonuclear leukocytes is increased as pHs are increased (7). Information considering intracellular accumulation of 14-OH-clarithromycin has not been reported.

High intracellular concentrations, however, may not offer an advantage for the treatment of extracellular pathogens. The response of extracellular pathogens may be more reflective of the concentrations in plasma and their relationship to the MIC. The concentrations in plasma observed in our study did exceed the MIC for 90% of common extracellular pathogens causing community-acquired pulmonary infections (3, 5, 13). In addition, clarithromycin and 14-OH-clarithromycin maintained a 2:1 concentration ratio in plasma, which has been demonstrated to have enhanced activity against pathogens such as *H. influenzae* (6, 8).

Results of this study differ from those previously reported regarding the penetration of clarithromycin into lung tissues by microbiological assay (4). The combined concentrations of clarithromycin and 14-OH-clarithromycin as well as the tissue-to-plasma drug concentration ratios in the present study were nearly threefold greater than those previously reported. The exact reasons for these differences are not known, since both studies utilized 2- to 3-day courses of 500 mg of clarithromycin every 12 h, with samples obtained approximately 4 h after the

last dose. Although actual concentrations differ within the two studies, both studies are in agreement as to the extensive lung tissue penetration of clarithromycin.

Finally, a word of caution must be said concerning the clinical interpretation of total concentrations in the lung tissue and the ratios of lung to plasma drug concentrations. Concentrations reported from homogenized tissue samples represent the uptake of drug into the vascular and extravascular compartments of the lungs. The extravascular compartment consists of an extracellular and an intracellular portion. Thus, the actual reported concentration for homogenized tissue samples of the lung is actually an average of the various concentrations within the different compartments. Further studies using bronchial biopsy and bronchoalveolar lavage are needed to provide measurements within actual sites of penetration.

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