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Autobacteriographic Studies of Clarithromycin and Erythromycin in Mice

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The antimicrobial activity of clarithromycin was compared with that of erythromycin in experimentally infected mice by whole-body autobacteriography. In mice with systemic staphylococcal infections, the number of vital microbes in the body was relatively low in the early period after oral administration of erythromycin, but increased thereafter to the levels found in nonmedicated control mice. On the other hand, with clarithromycin treatment, a significantly smaller number of microbes was evident throughout the body. The microbes were scarcely seen in the parenchyma of any organs during the examination period. This potent antimicrobial activity of clarithromycin compared with that of erythromycin was further demonstrated in mice with respiratory infections. On the other hand, to examine the distribution properties of both antibiotics in the whole body, an autoradiographic study was carried out with [*N-methyl*-^{14}C]clarithromycin and [*N-methyl*-^{14}C]erythromycin. Both labeled antibiotics were distributed widely throughout the body after oral administration in both uninfected control mice and mice with systemic infections. However, the radioactivity was more marked and persistent for [^{14}C]clarithromycin than it was for [^{14}C]erythromycin, particulary in the lungs. The observations described above indicate the superior in vivo antimicrobial activity of clarithromycin compared with that of erythromycin and suggest that the superiority of clarithromycin is largely attributed to its favorable distribution properties. The advantages of whole-body autobacteriography, coupled with whole-body autoradiography, are discussed.

Clarithromycin is a new macrolide antibiotic which differs from erythromycin chemically in that it has an *O*-methyl substitution at position 6 of the lactone ring (12). It has been previously reported that clarithromycin has an antibacterial spectrum similar to that of erythromycin and that its in vitro antibacterial activity is equal to or slightly superior to that of erythromycin against various species of organisms (4–7, 19). However, when administered orally in a mouse protection test, clarithromycin was 2 to 10 times more potent than erythromycin, as determined by the 50% effective dose, against *Haemophilus influenzae*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*, suggesting improved pharmacokinetic properties after oral dosing (6).

To evaluate the potency of antimicrobial agents against experimentally infected animals, Sakuma et al. (16–18) and Tani et al. (20) proposed the unique method of whole-body autobacteriography and demonstrated its advantages after the administration of various antimicrobial agents. By this method, one can semiquantitatively follow the distribution and localization of pathogenic microbes on the whole-body section of host animals and can visualize it in a single photograph. This method can be used for quicker and more reliable evaluations compared with the conventional bacteriological method.

The present study was undertaken to compare the antimicrobial activity of clarithromycin with that of erythromycin by means of whole-body autobacteriography in mice with staphylococcal infections. Furthermore, the tissue distribution properties of each antibiotic were examined by means of whole-body autoradiography by using ^{14}C-labeled antibiotics.

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MATERIALS AND METHODS

Materials. Clarithromycin (6-*O*-methylerythromycin) was synthesized in our research center (11). Erythromycin was obtained from Abbott Laboratories (North Chicago, Ill.). In the autoradiographic study, [*N-methyl*-^{14}C]clarithromycin, which was prepared in our research center, and [*N-methyl*-^{14}C]erythromycin, which was obtained from Dupont, NEN Research Products (Boston, Mass.), were used. The specific radioactivities of [^{14}C]clarithromycin and [^{14}C]erythromycin were 8.00 and 35.21 μCi/mg, respectively. Both labeled antibiotics were >97% pure, as determined by thin-layer chromatography. For dosage preparation, the radioactivity of each antibiotic was adjusted to the same specific activity with a corresponding nonlabeled antibiotic. Clarithromycin and erythromycin were each suspended in a solution of 5% gum arabic for oral administration. The experimental animals used were male ICR mice (weight, 20 to 25 g) obtained from Shizuoka Laboratory Animal Center. The bacterial strain used for the infection model was *Staphylococcus aureus* Smith 4, which is held in our culture collection.

Infection models. *S. aureus* was grown on brain heart infusion agar (Difco Laboratories, Detroit, Mich.) at 37°C for 18 h, and the colonies were suspended in physiological saline or 5% gastric mucin for the inoculum. The experimental infection models used in this study were systemic and respiratory infections in mice. Systemic infection was induced by intraperitoneal inoculation of *S. aureus* (2.2×10^7 CFU/0.5 ml of 5% mucin per mouse). Respiratory infection was induced by intratracheal inoculation of *S. aureus* (2.2×10^8 CFU/0.05 ml of saline per mouse) by a previously reported method (8). Clarithromycin or erythromycin was administered orally to both types of infected mice 1 h after inoculation at a dose of 20 mg/kg, and the animals were subjected to whole-body autobacteriography. Infected mice, which received the antibiotic-free vehicle 1 h after inocula-

tion, were also used as controls. A group of nine animals was used for each treatment. In the systemic infection experiments, an additional four mice in each treatment group were used to observe the survival rate during a 24-h period. In the whole-body autoradiographic study, [^{14}C]clarithromycin or [^{14}C]erythromycin was administered (20 mg/kg) to both uninfected and systemically infected mice 1 h after inoculation. Six animals, which were fasted overnight before dosing, were used for each treatment. For the autobacteriographic and autoradiographic preparations, three and two mice, respectively, were sacrificed at 2, 4, and 8 h after antibiotic administration under ether anesthesia by immersion in a mixture of dry ice-hexane (−70°C).

Whole-body autobacteriography. After the infected mice were frozen, they were placed into metal cases and were embedded in a gel of 5% carboxymethyl cellulose. The metal cases were put into the dry ice-hexane mixture and left there until the carboxymethyl cellulose was completely frozen. The frozen block was trimmed in a cryomicrotome (type 450; PMV Co., Stockholm, Sweden) at −18°C until a whole-body section surface of interest appeared. The exposed tissue surface was covered with a thin layer of sterilized cotton just large enough to cover it. Then, cellulose tape (Scotch tape no. 810; Minnesota Mining and Manufacturing Co., St. Paul, Minn.) was placed over the entire cut surface, which allowed careful cutting of thin saggital sections (thickness, 45 μm). The sections were then freeze-dried overnight.

The medium conditions used in the original method for autobacteriography (16, 17) were modified as follows, for the oral antibiotics used in this study, to avoid antibiotic diffusion from the gastrointestinal tract in whole-body sections (Y. Kohno, K. Ohta, T. Suwa, and T. Suga, J. Antimicrob. Chemother., in press). Selective medium was prepared with brain heart infusion broth containing 2% agar and 7.5% NaCl, and the medium was adjusted to pH 5.5 with 1 N HCl. For vital staining of the organism, triphenyl tetrazolium chloride was added to the medium at a concentration of 1 mg/ml. The whole-body sections obtained as described above, which adhered to the cotton layer, were transferred onto a single layer of the medium, and then the cooled medium was layered over the sections at 4°C. After 48 h of incubation at 37°C, the distribution of red colonies (vital microbes) on the whole-body section was observed.

Whole-body autoradiography. Freeze-dried sections of mice, which received radiolabeled antibiotics, were obtained by the method described by Ullberg (21). These sections were placed in apposition to X-ray film (no. 150; Fuji Photo Film Co.) at 4°C in a light-tight container. After exposure for 2 weeks, the developing and fixing of the films was done under standardized conditions.

RESULTS

Whole-body autobacteriography. (i) Systemic infection. The distributions of vital microbes in the bodies of systemically infected mice treated with the vehicle (control), erythromycin, or clarithromycin 1 h after inoculation are shown in Fig. 1, 2, and 3, respectively. In the control mice, pathogenic microbes were detected in most of the organs and tissues in the body from the early period after inoculation. Thereafter, the extensive propagation of microbes was evident, and numerous microbes were observed in almost all the organs and tissues. In particular, the locations showing the most intense propagation of microbes were the external surfaces of the organs. As for the survival rate, all four mice died within 12 h after inoculation. In erythromycin-treated mice,

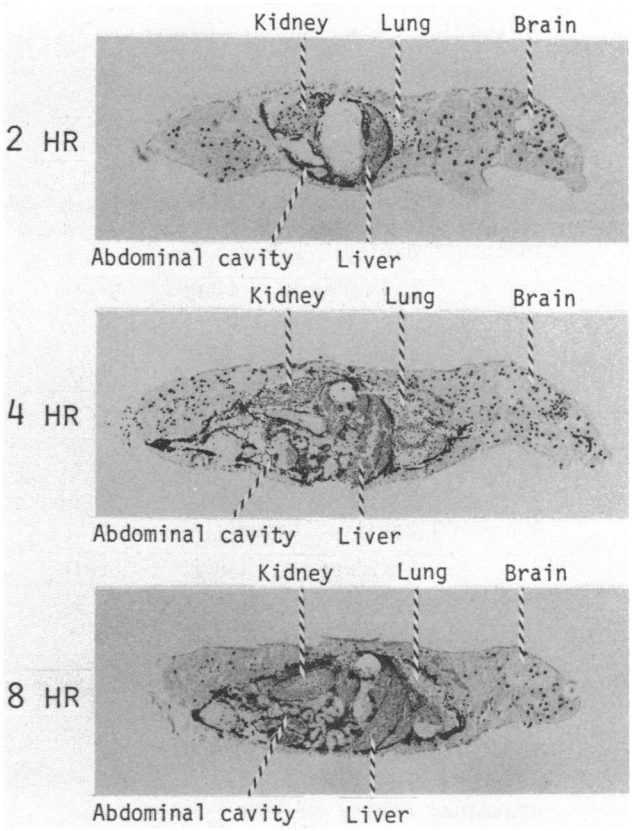

FIG. 1. Whole-body autobacteriographs showing the distribution of vital microbes in control mice. The mice were inoculated intraperitoneally with *S. aureus* 1 h before vehicle administration.

the microbes decreased in number compared with those in controls. These microbes were mainly detected in the abdominal cavity and in such organs as the liver, kidney, and spleen 2 to 4 h after administration. At 8 h after administration, however, the microbes increased in number significantly and were distributed widely throughout the body, as was observed for the control mice. Only one of the four mice was alive 24 h after inoculation. On the other hand, in mice treated with clarithromycin, the distribution pattern of microbes at 2 h after administration was essentially similar to that in mice treated with erythromycin. Then, the microbes gradually disappeared, so that only a relatively small number of microbes were detected in the abdominal cavity and on the surface of the liver 4 h after administration. The microbes were scarcely seen in the parenchyma of any of the organs. At 8 h after administration, a very few microbes were detected only around the abdominal cavity. All four mice were alive 24 h after inoculation.

(ii) Respiratory infection. Figure 4 shows the distribution of vital microbes in the lungs of control and antibiotic-treated mice. In the control mice, numerous microbes were observed to be distributed in the lungs. The density of microbes was not apparently changed throughout the examination period. No microbes were detected in other body organs. In the lungs of erythromycin-treated mice, the distribution pattern of the microbes appeared to be similar to that in the lungs of control mice. At all time intervals, erythromycin was shown to be ineffective in lowering the density of microbes, and numerous microbes were evident. On the other hand, in the lungs of clarithromycin-treated

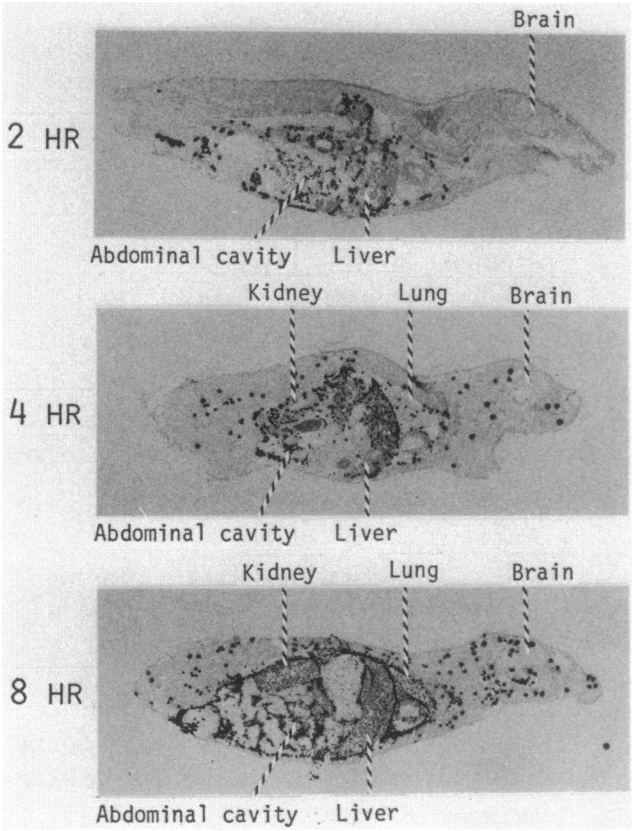

FIG. 2. Whole-body autobacteriographs showing the distribution of vital microbes in erythromycin-treated mice. The mice were intraperitoneally inoculated with *S. aureus* 1 h before antibiotic administration.

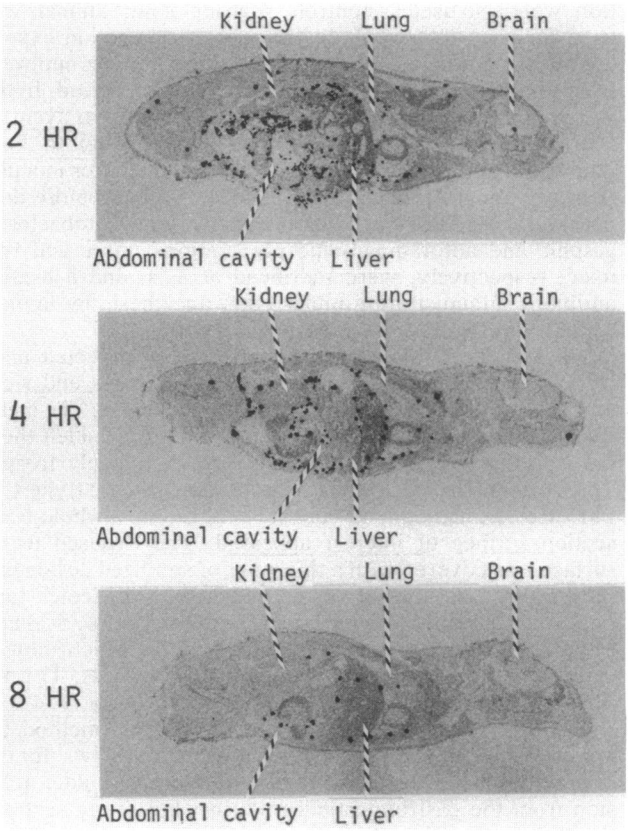

FIG. 3. Whole-body autobacteriographs showing the distribution of vital microbes in clarithromycin-treated mice. The mice were inoculated intraperitoneally with *S. aureus* 1 h before antibiotic administration.

mice, microbial density was considerably lower than those in both control and erythromycin-treated mice as early as 2 h after drug administration. The number of microbes further decreased with time, and only a few microbes were detected in the lungs 8 h after administration.

Whole-body autoradiography. [^{14}C]clarithromycin or [^{14}C]erythromycin (each at 20 mg/kg) was administered orally to both uninfected and systemically infected mice that were inoculated with *S. aureus* 1 h before drug administration. As shown in Fig. 5, in uninfected mice the radioactivities of both labeled antibiotics, which attained their peaks at 2 h, were distributed widely in the body. The radioactivity in almost organs was higher than that in the blood. A high level of radioactivity of [^{14}C]clarithromycin was observed in the gastrointestinal tract, liver, lung, kidney, and spleen. As for [^{14}C]erythromycin, the radioactivities in organs and tissues were generally lower than those for [^{14}C]clarithromycin; this was especially true in the lung. At 8 h after [^{14}C]clarithromycin administration, relatively high radioactivity was still observed in almost all organs and tissues. In infected mice, the distribution pattern of radioactivity of both labeled antibiotics was similar to that observed in uninfected mice (Fig. 6), except that the radioactivity in the body peaked 4 h after administration.

DISCUSSION

As an experimental method for the study of infective processes, Bonventre et al. (1–3) demonstrated autoradiographically the distribution and localization of pathogenic bacteria in mice after the injection of radioisotope-labeled pathogens, including *S. aureus*. This method is useful and can be most effectively used during the early stages of infection. However, the autoradiograph could not reveal the extensive staphylococcal multiplication, because of the dilution of the isotope and the appearance of radioactive metabolites during bacterial division. On the other hand, the whole-body autobacteriography used in the present study was able to satisfactorily evaluate the distribution and localization of *S. aureus* throughout the infection process. Moreover, our modified medium conditions for oral antibiotics effectively prevented the growth inhibitory effect on the vital microbes in tissues surrounding the stomach, which is caused by antibiotic diffusion from the gastrointestinal tract during the incubation process.

The autobacteriographic images of nonmedicated mice with systemic staphylococcal infections coincided well with the images provided in previous reports (16, 17). In these mice, a high density of microbes was arranged in a clumplike fashion on the external surfaces of organs. This clumping in vivo has been described previously and was found to be a property associated with the virulence of *S. aureus* (F. A. Kapral, Bacteriol. Proc. 71, 1965). Comparative autobacteriography after oral administration of clarithromycin and erythromycin to systemically infected mice revealed that the antimicrobial activity of clarithromycin was superior to that of erythromycin. Two hours after treatment, apparent antimicrobial activity was observed for both antibiotics. It

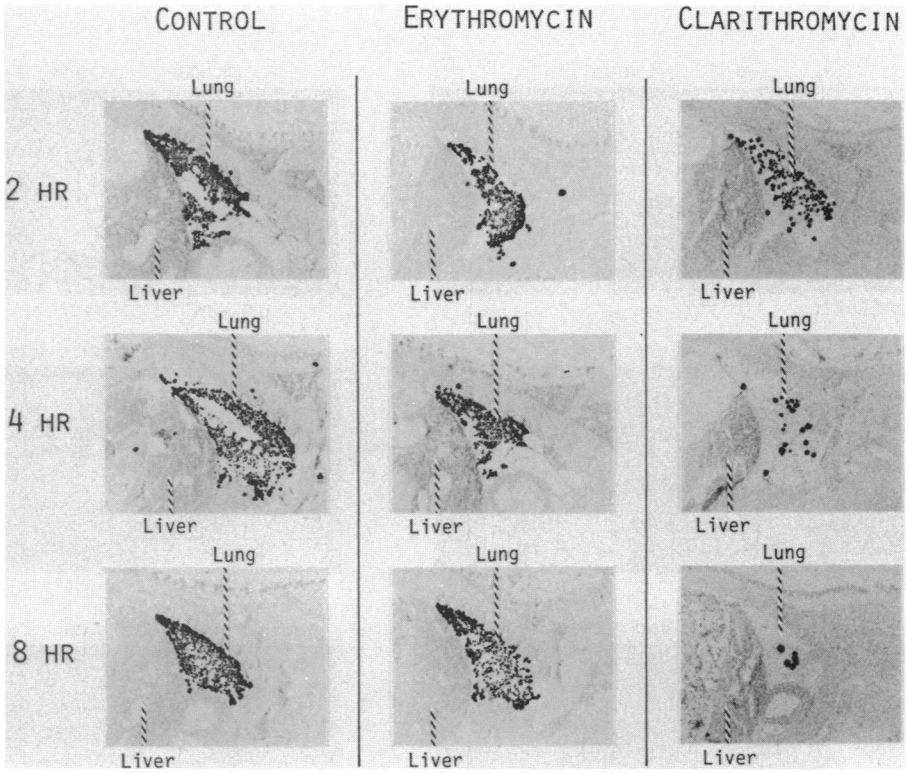

FIG. 4. Autobacteriographs showing the distribution of vital microbes in the lungs of control (vehicle-treated), erythromycin-treated, and clarithromycin-treated mice. The mice were inoculated intratracheally with *S. aureus* 1 h before administration.

should be noted, however, that numerous microbes were present in the liver, kidney, and spleen 4 h after erythromycin treatment, whereas the microbes were scarcely seen in the parenchyma of these organs after clarithromycin treatment; a small number of microbes was detected only on the external surface of the liver and in the abdominal cavity. It seems that in this case, autobacteriography provided information which would not have been readily obtainable by conventional bacteriological methods. If viable microbes had been enumerated by plating methods, the microbes present on the surface of the liver would have been counted. As a consequence, an erroneous conclusion that the microbes were actually present within the organ might have been made. The most dramatic difference of microbial distribution between both antibiotics was found at 8 h: the microbial distribution and density in the whole bodies of erythromycin-treated mice showed a pattern similar to that in bodies of nonmedicated mice, while the microbes in clarithromycin-treated mice were almost completely diminished. These results indicate the more potent and persistent efficacy of clarithromycin in vivo. Nagate et al. (14) previously examined the potency of clarithromycin relative to that of erythromycin against systemic infections in mice under experimental conditions similar to those of the present study: both antibiotics were administered orally 1 h after inoculation of *S. aureus* Smith 4 (10^7 CFU per mouse). They reported that clarithromycin was six times more potent than erythromycin in terms of the 50% effective dose. This may well correspond to our results of autobacteriography, including the reference data of a higher survival rate for clarithromycin-treated mice.

The prominent antimicrobial activity of clarithromycin was further elucidated against respiratory infections in mice. In nonmedicated mice, numerous microbes were confined to the lungs throughout the examination period. In this infection model, the efficacy of erythromycin was scarcely detected on the autobacteriograph. It appears that the infection conditions used were too severe for erythromycin to show its antimicrobial efficacy on the autobacteriograph. Even under these conditions, however, the density of microbes in clarithromycin-treated mice was reduced to a very low level, and superior antimicrobial activity in the lungs was demonstrated.

Instability in gastric juices is one of the limitations of erythromycin, and its pharmacokinetics are not completely satisfactory (10, 11). On the contrary, clarithromycin is highly stable under acidic conditions. Fernandes et al. (6) reported that when clarithromycin and erythromycin were administered orally to mice, peaks levels of bioactivity in serum were 0.3 and 0.1 μg/ml, respectively, when administered at the same oral dose (25 mg/kg). Moreover, the half-life of clarithromycin in serum was approximately twice that of erythromycin after administration by both the oral and subcutaneous routes. This suggests that, in addition to having high stability in gastric acid, clarithromycin is less easily inactivated in the body. In the present study, the distributions of [^{14}C]clarithromycin and [^{14}C]erythromycin were examined in both uninfected and systemically infected mice by means of whole-body autoradiography. This method provides obvious advantages, since it provides a comprehensive picture of the distribution of radiolabeled antibiotics by using a similar whole-body section, as was used in the autobacteriographic study. In this situation, we can simultaneously compare the in vivo antimicrobial activities and distribution properities of antibiotics by their whole-body images. As a result, significantly higher and persistent radio-

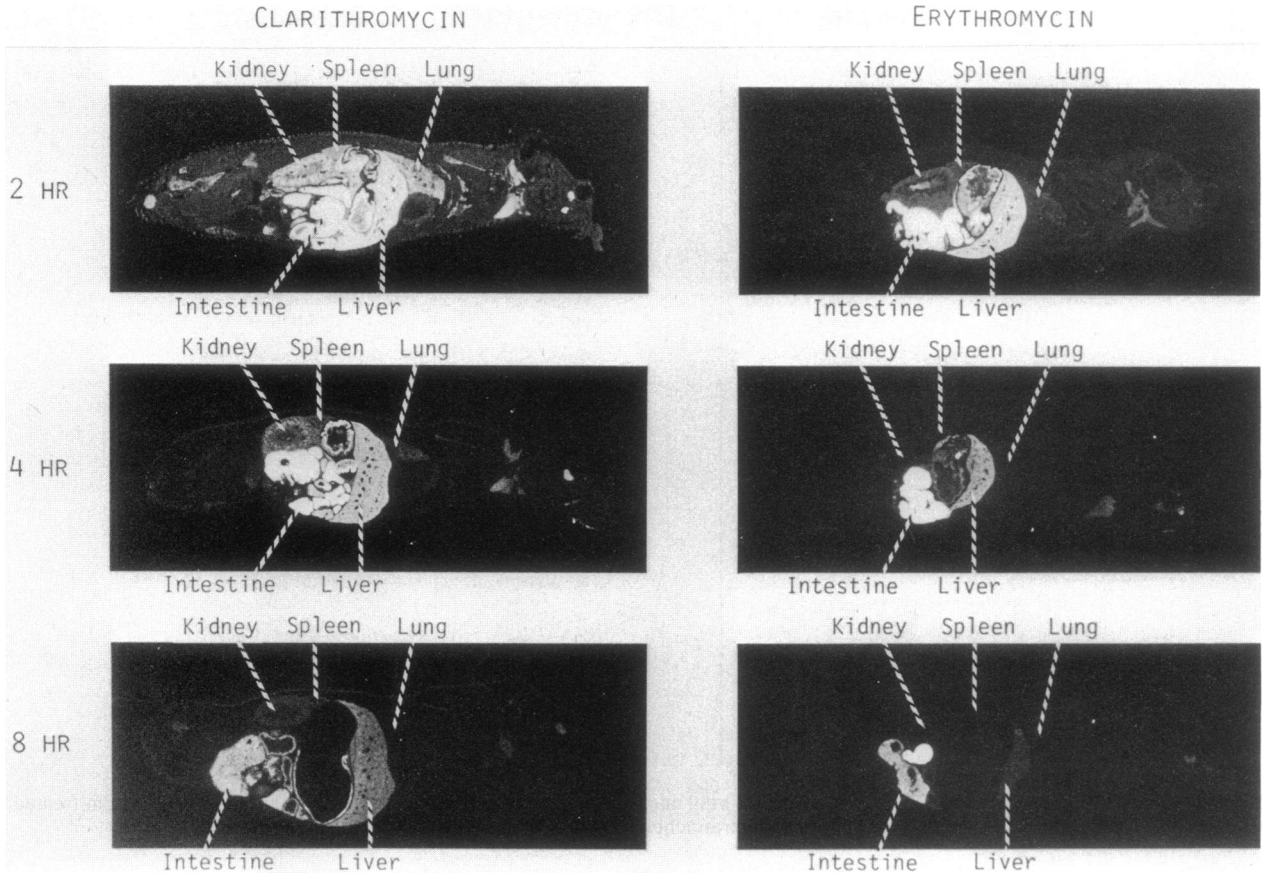

FIG. 5. Whole-body autoradiographs showing the distribution of radioactivity after oral administration of [^{14}C]clarithromycin and [^{14}C]erythromycin to uninfected mice.

activities for [^{14}C]clarithromycin were observed throughout the bodies of both uninfected and infected mice, particularly in the lungs, when compared with those for [^{14}C]erythromycin. These observations are well in agreement with the results of our previous study on the pharmacokinetics of [^{14}C]clarithromycin and [^{14}C]erythromycin in rats; peak levels of [^{14}C]clarithromycin in plasma and lungs were double and 15 times those of [^{14}C]erythromycin, respectively, at the same oral dose (9). In that study, the differences in radioactivity between both antibiotics were considered to be due to the rapid metabolism of [^{14}C]erythromycin in the liver and subsequent biliary excretion at a higher rate and to a higher tissue affinity of intact clarithromycin, especially to the lungs. The radioactive absorption of [^{14}C]erythromycin has been reported to be substantially complete (13).

The favorable tissue distribution of clarithromycin, even in infected mice, corresponded well to the disappearance of microbes, as visualized by the whole-body autobacteriography described above; few microbes were seen within any organs following clarithromycin treatment. In the meantime, the MIC and MBC of clarithromycin and erythromycin against *S. aureus* Smith (1.0×10^4 CFU/ml) were reported to be 0.20 and 0.20 μg/ml and 0.20 and 0.39 μg/ml, respectively (15). This indicates that clarithromycin has antimicrobial activity in vitro similar to that of erythromycin. Thus, observations made by whole-body autoradiography, coupled with autobacteriography, may provide a direct explanation

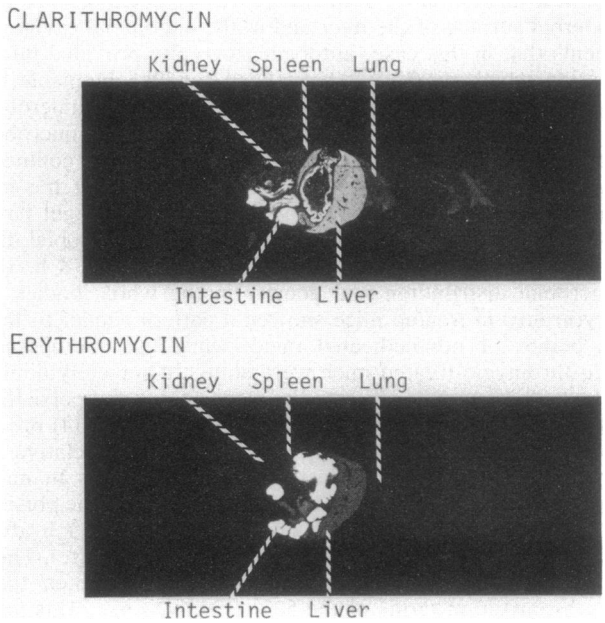

FIG. 6. Whole-body autoradiographs showing the distribution of radioactivity 4 h after oral administration of [^{14}C]clarithromycin and [^{14}C]erythromycin to infected mice. The mice were inoculated intraperitoneally with *Staphylococcus aureus* 1 h before administration.

of the superior antimicrobial effect of clarithromycin in infected mice.

We conclude that whole-body autobacteriography demonstrates the superior antimicrobial activity of the new macrolide antibiotic clarithromycin compared with that of erythromycin in mice with systemic or respiratory infections and that the superiority of clarithromycin may be largely attributed to its favorable distribution properties, as revealed by whole-body autoradiography.

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