ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1974, p. 538-540

Vol. 5, No. 5
Printed in U.S.A.

Mutant of *Staphylococcus aureus* with Lincomycin- and Carbomycin-Inducible Resistance to Erythromycin

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Received for publication 21 January 1974

A mutant of *Staphylococcus aureus* was isolated in which lincomycin and carbomycin (but not erythromycin) induced resistance to erythromycin. This pattern displayed a reversal of the usual specificity of induction seen in the erythromycin-inducible parent strain from which the mutant was selected.

Erythromycin can induce resistance in certain strains of *Staphylococcus aureus* to three classes of antibiotics that act on the 50*S* ribosomal subunit—the macrolides, lincosamides, and streptogramin B-type (MLS) antibiotics (6). The process of induction is highly specific in that it only involves three (out of at least eight) classes of antibiotics known to act on the 50*S* subunit. Examples of the 50*S* subunit inhibitors to which erythromycin does not induce resistance include chloramphenicol, puromycin, amicetin, thiostrepton, and the streptogramin A-type antibiotics. Associated with this resistant phenotype, it has been found that the 50*S* subunit is unable to bind erythromycin or lincomycin (4, 7), and the appearance of N^6,N^6-dimethyl adenine (m_2^6A) in 23*S* rRNA has been described as the structural change presumed to be responsible for altered function (2). Experimental support for this conclusion is based on ribosome reconstitution studies (3) in which it was shown that the resistant phenotype could be reconstituted if one took 23*S* ribosomal ribonucleic acid (rRNA) from induced or constitutively resistant cells and 5*S* rRNA plus ribosomal proteins from susceptible cells. m_2^6A is produced by post-transcriptional modification of the rRNA and is derived from the sequence AAAG (1).

Induction was studied with the use of solid media and was manifested by a characteristic distortion of the inhibitory zone surrounding antibiotic-impregnated filter paper disks which contain MLS antibiotics placed on a lawn of inducible cells at a distance of 1 cm from a test disk which contains 15 μg of erythromycin (Fig. 1a). Erythromycin was found to act as inducer in all cases of inducible natural isolates. The explanation of the distortion of the inhibitory zone is as follows. As erythromycin diffuses from the disk, the subinhibitory levels in the agar which surrounds the disk induce the cells in accordance with the requirements for induction previously described (7). When an inhibitory level of erythromycin is finally attained ($>10^{-6}$ M), cells have already been induced by the earlier subinhibitory concentrations (optimum of 10^{-8} to 10^{-7} M). We assume that in the course of erythromycin concentration build-up, the concentration must pass through the inducing range of concentrations before attaining an inhibitory level. Therefore, bacterial growth surrounding the erythromycin disk consists of cells which have been induced in situ by the diffusion of erythromycin from the test disk. Cells growing within this zone then remain fully induced as a consequence of continued exposure to otherwise inhibitory erythromycin concentrations. Erythromycin thus provides a protective "umbrella" of induction in a zone surrounding the test disk approximately 2 cm in diameter. When this circular zone of induced cells intersects the zone of potential inhibition by a noninducing MLS antibiotic, some of the cells in the intersection, having become induced by erythromycin, can then grow in the presence of both erythromycin and the noninducing test MLS antibiotic. This is demonstrated by the fact that cells induced with erythromycin (10^{-7} M) can then grow in 10^{-4} M erythromycin plus 5×10^{-5} M lincomycin but not in 5×10^{-5} M lincomycin alone. In this intersection zone, erythromycin continues to provide the necessary stimulus for induction. From biochemical studies, we conclude that within a radius of at least 1 cm surrounding the erythromycin disk, specific adenine residues in 23*S* rRNA are methylated to form m_2^6A. The relationship between erythromycin and the MLS antibiotics with respect to relative inducing activity is

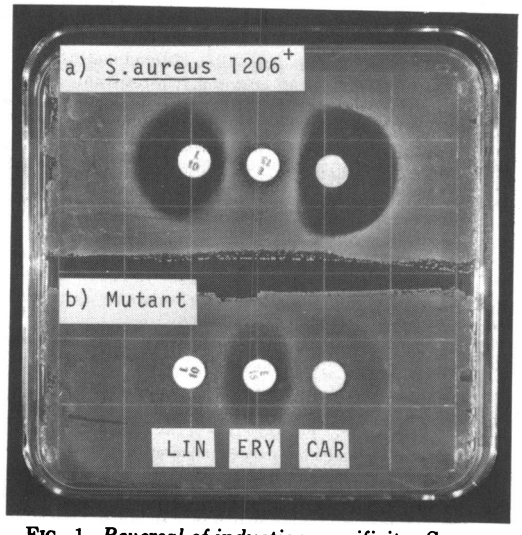

FIG. 1. *Reversal of induction specificity. S. aureus cells grown in broth were smeared onto nutrient agar; three antibiotic disks were placed on the inoculated plate 1 cm apart, and the plates were incubated at 37 C for 18 h. (a) S. aureus 1206⁺ (parent strain); (b) S. aureus mutant selected on carbomycin medium.*

apparently reversed in the mutant strain which we describe here.

Saito et al. (4, 5) have isolated mutants of *S. aureus* inducible by heat treatment (42 C) or by exposure to subinhibitory levels of macrolides other than erythromycin, such as oleandomycin or leucomycin. It would be of interest to know whether induction by antibiotics is strictly a property of macrolides or whether lincosamides could also function in such a capacity. The finding of a lincomycin-inducible strain would be pertinent to this question.

To isolate mutants, portions of individual 2-day-old colonies of the parent inducible strain (1206⁺) were picked up with a loop and smeared onto 1 cm^2 of solid medium containing 10 μg of carbomycin per ml; the minimal inhibitory concentration of carbomycin for susceptible strains of *S. aureus* is less than 10 μg/ml. After 2 days of incubation at 37 C, mutant colonies appeared on the carbomycin medium. One mutant colony was picked from each inoculum. Many of the mutants selected in this manner are constitutively resistant to all of the MLS antibiotics tested, including erythromycin, as described previously (7), and from studies of 23*S* rRNA, N^6-dimethylation of adenine takes place in the absence of erythromycin (2). From studies of resistance patterns with the aid of test disks, the constitutive phenotypes that can be isolated fall into two main classes: (i) "generalized constitutives," in which co-resistance is seen to all members of a test battery of antibiotics consisting of MLS antibiotics; and (ii) "partial constitutives," in which resistance is seen only to some but not all members of the test battery of MLS antibiotics (7). Erythromycin continues to serve as inducer of resistance to those MLS antibiotics to which resistance is not expressed constitutively. One mutant, isolated in the present studies and shown in Fig. 1b, grew on solid medium containing 10 μg of carbomycin per ml and was found to be susceptible to erythromycin but inducible by lincomycin and carbomycin instead. Apparently, this mutant was inducible by carbomycin present in the solid medium during the initial selection. The existence of this type of mutant phenotype suggests that both macrolides and lincosamides can potentially act as inducers but that they differ quantitatively in such a way that in the parental strain (1206⁺) erythromycin is maximally active as an inducer at concentrations lower than those at which it is maximally active as an inhibitor of ribosome function, whereas the reverse is true of lincomycin, streptogramin B-type antibiotics, and noninducing macrolides.

Cells of *S. aureus* 1206⁺ induced by growth in 10^{-7} M erythromycin will not grow when transferred to medium supplemented with 5×10^{-5} M lincomycin alone but will grow in this concentration of lincomycin when the medium is also supplemented with 10^{-4} M erythromycin. This suggests that lincomycin alone cannot maintain the induced state, as can erythromycin, and is therefore inactive as an inducer. Since carbomycin is also inactive as an inducer, the same presumably applies to this antibiotic as well.

The existence of a lincomycin-inducible mutant suggests that macrolide and lincosamide antibiotics have qualitatively similar specificities for both inhibition of ribosome function and induction of resistance, and that at least one functional difference between erythromycin and other noninducing MLS antibiotics is their relative potencies for induction of 23*S* rRNA methylation on one hand and inhibition of ribosome function on the other. In the mutant described above, the relative potencies for these two processes have apparently been reversed by mutation, presumably by making the induction process much more susceptible to lincomycin in such a way that induction occurs at concentrations lower than those at which lincomycin is active as inhibitor of ribosome function. Of particular interest in these studies is the fact that the mutants reported have lost part of their

capacity for induction by erythromycin while acquiring capacity for induction by carbomycin and lincomycin. This property was not characteristic of the oleandomycin- and leucomycin-inducible mutants reported by Saito et al. (5), in which erythromycin could also function as an inducer of resistance, unlike the strain described here.

From these studies, we conclude that erythromycin and lincomycin not only bind to interacting (if not overlapping) sites on the 50*S* ribosome subunit, but that they can also act reciprocally as inducers of resistance to each other in appropriate mutant strains.

This investigation was supported by National Science Foundation grant GB-17108 and by awards from the Upjohn Co. and the Eli Lilly Co.

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