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Induction of Erythromycin Resistance in *Staphylococcus aureus* by Erythromycin Derivatives

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The ability of 53 erythromycin analogues to induce resistance to erythromycin in *Staphlococcus aureus* was evaluated. Only derivatives with antibacterial activity induced resistance, although some antibacterial compounds did not induce resistance. No derivatives without antibacterial activity but with ability to induce resistance were found.

A class of erythromycin-resistant strains of *Staphylococcus aureus* remains susceptible to other macrolide antibiotics, and, when these strains are exposed to low levels of erythromycin, resistance to other antibiotics is induced. This type of resistance has been termed erythromycin-inducible resistance and has been studied by a number of authors (1-3, 7, 10, 13-17). Lai and co-workers (4-6) have shown that N^6,N^6-dimethyladenine, which is not normally present in 23S ribosomal ribonucleic acid, is found on induction of resistance by concentrations of erythromycin between 10^{-8} and 10^{-7} M. It has been shown that the induction of erythromycin resistance results in modified 50S ribosomal subunits, which are unable to bind erythromycin or lincomycin (15). The inability to bind erythromycin is apparently due to methylation of a single adenine residue in a tetranucleotide sequence, AAGG, of 23S ribonucleic acid (4-6). Thus far, erythromycin-inducible resistance has only been described in strains isolated from natural sources and has not been produced in erythromycin-susceptible organisms by mutation. The finding of such erythromycin-resistant strains is therefore of clinical significance. In this report we examine a number of erythromycin analogues to determine what structural features of the molecule are involved in the induction process and whether the ability of these derivatives to serve as active antibiotics can be dissociated from their ability to induce resistance to erythromycin. This communication reports the results of this study.

MATERIALS AND METHODS

The structures of the erythromycin analogues 1 to 53 are given in previous reports (8, 9, 12). The activity of erythromycin and erythromycin analogues on bacteria was determined by the agar diffusion cup plate or disk assay. The ability of a compound to induce resistance to lincomycin or clindamycin was determined by minor modifications of the agar disk assay described by Weisblum and Demohn (15) with the use of *S. aureus* 1206, which was kindly supplied by B. Weisblum. Compounds 46 through 53 were purified by thin-layer chromatography on silica gel 60 F-254 (12). Thin-layer chromatography of other compounds indicated that they were contaminated by less than 1% of erythromycin A, if at all. In addition, to confirm the results with the disk assay, bioautograms of ability to induce resistance were produced with a few of the compounds (3, 4, 6, 21, 22, 24, 25, and 26), especially those with low antibacterial activity. The compounds were separated from any erythromycin A contaminant by thin-layer chromatography. The resulting thin-layer chromatograms were placed in contact with Mueller-Hinton agar plates seeded with an overlay of *S. aureus* 1206 for 30 min. The chromatograms were then removed, and the bioautograms were developed with a 0.25-inch (ca. 0.64-cm) strip of Whatman no. 1 filter paper saturated with a solution of clindamycin (100 μg/ml) analogous to the disk assay (15). Approximately 0.01 and 0.04 μmol of each compound were chromatographed. In all cases both levels of analogue produced similar results qualitatively. Plates with developing solvent only and those with erythromycin A served as controls.

RESULTS AND DISCUSSION

The ability of the erythromycin analogues to induce resistance to lincomycin and clindamycin is shown in Table 1. All compounds which induced resistance to lincomycin had antibacterial activity when assayed against a susceptible bacterial strain. Some erythromycin derivatives, which exhibited 1 to 2% of the antibacterial activity of erythromycin, did not induce resistance. These include derivatives **30, 32, 36, 37, 38, 39,** and **40**. In addition, derivatives **31** and **44**, which exhibited 10% of the antibacterial

TABLE 1. *Ability of erythromycin derivatives to induce resistance to lincomycin*[a]

Erythromycin derivative	In vitro activities: Antibacterial activity[b] relative to erythromycin (%)	In vitro activities: Ribosome binding[c] concn (μM) for 50% inhibition of binding of 1.2 μM [^{14}C]erythromycin	"Induces" resistance to lincomycin and clindamycin[d]
Erythromycin	100	1.3, 0.9	+
1	10	38.0	+
2	2	44.7	+
3	1	170	+
4	5	60.2	+
5	75	7.9	+
6	0	>3,000	−
7	75	4.0	+
8	50	1.1	+
9	100	1.2	+
10	100	1.3	+
11	10–20	3.4	+
12	0	200	−
13	40	3.2	+
14	0	372	−
15	0	214	−
16	0	>10,000	−
17	0	>1,000	−
18	0	>3,000	−
19	0	>3,000	−
20	0	3,550	−
21	2	22.4	+
22	2	37.2	+
23	2	38.0	+
24	2	21.9	+
25	1	63.1	+
26	0	100	−
27	0	501	−
28	0	1,770	−
29	0	200	−
30	1	138	−
31	10	100	−
32	1	380	−
33	0	126	−
34	0	269	−
35	0	83.2	−
36	1	58.9	−
37	2	19.0	−
38	1	79.4	−
39	2	60.3	−
40	1	224	−
41	0	224	−
42	2.5	141	−
43	0	224	−
44	10	20.9	−
45	6	11.0	+
46	75	0.27	+
47	20	0.16	+
48	25	6.3	+
49	15	2.0	+
50	10	31.6	+
51	25	0.71	+
52	10	1.5	+
53	7	25.1	+

[a] The antibacterial activity relative to erythromycin was determined by the agar diffusion assay versus *Bacillus subtilis* or *Sarcenea lutea*. Ability of the derivative to induce resistance to lincomycin and clindamycin was assayed by placing 0.013 μmol of the derivative on a disk and using the assay described by Weisblum and Demohn (15). The value of 0.013 μmol was chosen because it is equivalent to 10 μg of erythromycin A, the amount used in their assay (15).

[b] Taken from the data of Pestka et al. (9) for all compounds except 31, 42, and 46–53, for which antibacterial activity relative to erythromycin was determined by the agar diffusion disk assay.

[c] Taken from the data of Pestka and LeMahieu (8) and Vince et al. (12).

[d] Ratio of 3:1 of erythromycin analogue to lincomycin or clindamycin was used in this in vitro agar diffusion test.

activity of erythromycin A, did not induce resistance. No compounds were found that induced resistance but lacked antibacterial activity. Tests for induction were done by the disk assay (15). Each disk contained 0.013 μmol (equivalent to 10 μg of erythromycin A) of the erythromycin derivative tested. Thus, if induction required higher concentrations, this might not have been detected.

The ability to design analogues that are anti-

bacterial but do not induce resistance would have clinical significance. However, of these derivatives tested, those that did not induce erythromycin resistance had relatively little or no antibacterial activity. Derivatives 31 and 44, which exhibited 10% antibacterial activity relative to erythromycin A but did not induce resistance, were the most active compounds. The ability to dissociate antibacterial activity and induction of resistance indicates that it is possible to produce active erythromycin derivatives that produce no induction of erythromycin resistance. Nevertheless, this may be of limited use, since other macrolides do not induce this type of resistance in strains obtained from natural sources. However, in the laboratory a mutant of *S. aureus* 1206 was isolated in which lincomycin and carbomycin, but not erythromycin, induced resistance to erythromycin (11).

No erythromycin derivatives that lacked antibacterial activity and did not bind to ribosomes, but could induce resistance, were found. In contrast, a number of derivatives (36, 37, 38, 39, 40, 42, and 44) were active antibacterial agents and were bound to ribosomes, but did not induce resistance. This suggests that ribosomal binding is probably not directly related to induction of resistance and further suggests the existence of a site, different from the ribosomal erythromycin-binding site, that is responsible for induction. Nevertheless, the precise nature of the induction and methylation mechanisms remain to be elucidated.

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