RESISTANCE OF *ESCHERICHIA COLI* TO RIFAMPICIN AND SORANGICIN A—A COMPARISON

GÜNTER RÖMMELE, GABRIELA WIRZ, RENATE SOLF, KLAUS VOSBECK, JOHANNES GRUNER and WALTER WEHRLI

Research Department, Pharmaceuticals Division, Ciba-Geigy Limited, CH-4002 Basel, Switzerland

(Received for publication August 26, 1989)

Sorangicin A, a macrolide polyether antibiotic and the ansamycin antibiotic rifampicin inhibit DNA-dependent RNA polymerase to a similar extent. Resistance to sorangicin A is due to a mutation in the RNA polymerase which renders the enzyme less sensitive. Parallel investigations with rifampicin revealed partial cross-resistance, which was more marked in sorangicin A-resistant mutants than in rifampicin-resistant mutants.

Sorangicin A is a new type of macrolide polyether antibiotic isolated from the gliding bacterium *Sorangium cellulosum*. Its antimicrobial properties are similar to those of the ansamycin antibiotic rifampicin. Sorangicin A is highly active against Gram-positive bacteria (MIC $<0.01 \sim 2\,\mu g/ml$) and less so against Gram-negative bacteria (MIC 2 $\sim > 32\,\mu g/ml$). The drug also resembles rifampicin in its mechanism of action, since it specifically affects bacterial DNA-dependent RNA polymerase[1~3]. The chemical structures of the two drugs are, however, quite different.

The aim of the present work was to analyze the mechanism of resistance of *Escherichia coli* to sorangicin A and to compare it to the resistance against rifampicin.

Materials and Methods

Materials

[^{3}H]CTP was purchased from Radiochemical Centre, Amersham, and the nucleotides from SERVA (Heidelberg, Germany). Sorangicin A was obtained from Prof. H. REICHENBACH, Braunschweig, BRD.

Bacterial Strains

The sorangicin A and rifampicin-sensitive *E. coli* strains B-205, B-662, B-1375, B-1385 and B-1478 were isolated from human faecal samples.

Selection of *E. coli* Strains Resistant to Sorangicin A or to Rifampicin

Individual culture tubes containing 5 ml each of Brain Heart Infusion (BHI) broth were inoculated with 10^4 *E. coli* B-1375 and incubated overnight at 37°C. From each overnight culture, 10^9 organisms were spread on two BHI agar plates containing $500\,\mu g/ml$ of sorangicin A or rifampicin. After overnight incubation at 37°C, single colonies of resistant organisms were picked, passaged over antibiotic-free BHI agar and again over BHI agar containing $500\,\mu g/ml$ of sorangicin A or rifampicin. The biochemical profiles of the resistant mutants obtained in this manner were compared with those of the respective parent strains using the API-20 system and were found to be very similar in each case.

Determination of MICs

MIC of sorangicin A and rifampicin were determined by the 2-fold agar dilution method. The bacteria were grown overnight in BHI broth. A 1:100 dilution of the overnight culture (*ca.* 10^6 bacteria/ml) was applied to BHI agar plates containing graded concentrations of the test antibiotic using a STEER's replicator

yielding approximately 10^4 organisms per spot. The plates were incubated overnight at 37°C. The MIC was considered to be the lowest concentration of antibiotic producing inhibition of visible growth in relation to the control culture.

Measurement of DNA-dependent RNA Polymerase Activity

DNA-Dependent RNA synthesis was measured using *E. coli* cells made permeable by treatment with diethyl ether[4,5]. *E. coli* cells harvested in mid-long phase were centrifuged off at 0°C, washed once and resuspended in buffer A (Tris-HCl (pH 7.9) 80 mM, KCl 160 mM, $MgCl_2$ 14 mM, EGTA 4 mM, spermidine·HCl 0.8 mM (pH 7.9), sucrose 0.5 M; 10^{10} cells/ml). This suspension was shaken gently with an equal volume of cold diethyl ether for 90 seconds at 0°C. The ether phase was then removed and the cells centrifuged off and washed twice in buffer A. For storage at −20°C the cells were resuspended in storage buffer (K-phosphate (pH 7.4) 20 mM, NaCl 122 mM, KCl 3 mM, $MgCl_2$ 2 mM) containing 60% glycerol (*ca.* 5×10^{10} cells/ml). DNA-Dependent transcription in ether-treated *E. coli* cells was measured as described earlier[6].

Results

Mutants Resistant against Sorangicin A or Rifampicin and Cross-resistance

E. coli B-1375 was inhibited by both sorangicin A and rifampicin, with an MIC of 8 μg/ml. Mutants resistant to sorangicin A could be obtained as easily as mutants resistant to rifampicin. Approximately one hundred independent mutants each were selected against 500 μg/ml of sorangicin A or against 500 μg/ml of rifampicin. The analysis of the pattern of cross-resistance between the two antibiotics within this set of mutants of *E. coli* B-1375 yielded interesting results (Table 1): Half the mutants selected against sorangicin A (sor^R) were also highly resistant to rifampicin ($rif^R sor^R$), whereas the other half were only partially cross-resistant. We were unable to isolate mutants highly resistant to sorangicin A that retained normal sensitivity to rifampicin ($sor^R rif^S$). By contrast, half the mutants selected against rifampicin (rif^R) were normally sensitive to sorangicin A ($rif^R sor^S$); another 20% exhibited partial cross-resistance to sorangicin A, and only 30% were fully cross-resistant ($rif^R sor^R$). Thus, cross-resistance of sor^R mutants to rifampicin is more marked than that of rif^R mutants to sorangicin A.

Inhibition of RNA Synthesis

The mechanism of resistance to sorangicin A was analyzed by measuring the inhibition of DNA-dependent RNA polymerase by sorangicin A and rifampicin and comparing it with the corresponding MICs. We used 5 different *E. coli* strains and a series of mutants derived from them exhibiting different patterns of cross-resistance against sorangicin A and rifampicin. The results are shown in Table 2 and can be summarized as follows:

1) RNA synthesis of the wild-type isolates was inhibited at similar, low concentrations of sorangicin A and rifampicin, and the MICs of both antibiotics were 8 ~ 16 μg/ml.

2) In the resistant mutants, an increase in the MIC was always paralleled by a decrease in the sensitivity of the enzyme to the respective drug. Thus, resistance to sorangicin A appears to be due to a mutation in the RNA polymerase, rendering the enzyme less sensitive, as is the case with

Table 1. Pattern of resistance of *Escherichia coli* B-1375 to sorangicin A and rifampicin.

	No. of mutants	MIC (μg/ml)	
		Sorangicin A	Rifampicin
1) Wild-type	—	8	8
2) Mutants selected	38	>500	>500
against 500 μg/ml	42	>500	62 ~ 125
sorangicin A			
3) Mutants selected	31	>500	>500
against 500 μg/ml	20	31 ~ 62	>500
rifampicin	51	8 ~ 16	>500

Table 2. Comparison between antibacterial activity (MIC) and inhibition of RNA polymerase by sorangicin A and rifampicin.

Escherichia coli strains		Sorangicin A		Rifampicin	
		MIC (μg/ml)	IC_{50} (μg/ml)[a]	MIC (μg/ml)	IC_{50} (μg/ml)[a]
E. coli B-1375	[b]	8	0.02	8	0.01
E. coli B-662	[b]	8	0.02	8	0.02
E. coli B-1478	[b]	8	0.02	8	0.01
E. coli B-1385	[b]	16	0.03	16	0.02
E. coli B-205	[b]	16	0.02	16	0.01
E. coli B-1375 sor^R1	[c]	>500	4.5	125	0.1
E. coli B-1375 sor^R2	[c]	>500	>10	125	0.3
E. coli B-1375 sor^R3	[c]	>500	>10	>500	>10
E. coli B-1375 rif^R	[d]	8	0.02	>500	>10
E. coli B-662 rif^R	[d]	16	0.01	>500	>10
E. coli B-1478 rif^R	[d]	16	0.01	>500	>10
E. coli B-1385 rif^R	[d]	>500	6	>500	>10
E. coli B-205 rif^R	[d]	>500	>10	>500	>10

[a] Concentration of sorangicin A or rifampicin needed to induce 50% inhibition of RNA synthesis.
[b] Wild-type strains.
[c] Strains selected against 500 μg/ml sorangicin A.
[d] Strains selected against 500 μg/ml rifampicin.

rifampicin.

3) Three mutants highly resistant to rifampicin, but normally sensitive to sorangicin A also had RNA polymerases that were highly resistant to rifampicin, but normally sensitive to sorangicin A. As expected from the MICs, an enzyme with the reverse properties, namely high resistance to sorangicin A, but normal sensitivity to rifampicin, has not been found.

Discussion

The analysis of *E. coli* mutants resistant to sorangicin A clearly shows that they contain an RNA polymerase less sensitive to the drug than that of the wild-type. Thus the mechanism of resistance to sorangicin A is due to a mutation in the enzyme and is closely related to that of rifampicin[3].

Rifampicin is known to bind very strongly to RNA polymerase[3,7]. The binding site is located on the β subunit[8,9] and has been partially analyzed by various groups[10~13]. The chemical structures of rifampicin and sorangicin A are quite different. The data on cross-resistance between the drugs indicate, however, that the corresponding binding sites overlap to a large extent. The existence of mutants with an RNA polymerase highly resistant to rifampicin, but sensitive to sorangicin A indicates that some binding regions are essential for rifampicin binding, but not for sorangicin A binding. On the other hand, the absence of mutants with high resistance to sorangicin A and normal sensitivity to rifampicin could be interpreted as an indication that most of the sorangicin A binding site is closely related to that of rifampicin.

Acknowledgments

We thank Prof. H. REICHENBACH, Gesellschaft für Biotechnologische Forschung, Braunschweig, BRD, for providing us with sorangicin A.

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