A New Group of Rifamycin Derivatives Displaying Activity Against Rifampicin-Resistant Mutants of *Staphylococcus aureus*

Rifampicin, which is one of the most highly active antibiotics from the group of rifamycin derivatives[1], offers the following advantages from the microbiological standpoint[2,3]: (1) A bactericidal effect on proliferating bacteria which is of rapid onset. (2) A very high specific activity against gram-positive organisms. [The minimum inhibitory concentration (MIC) for 50 staphylococci freshly isolated from hospital sources is in the region of 0.001 to 0.03 μg/ml.] (3) Good activity against gram-negative bacteria, i.e. activity of roughly the same order of magnitude as that of chloramphenicol and the tetracyclines. Particularly interesting is the fact that rifampicin also inhibits growth of *Pseudomonas aeruginosa* (the MIC for 20 hospital strains lying in the region of 7–15 μg/ml). (4) A mechanism of action which, compared with that of other antibiotics, is of a new type and is based on inhibition of DNA-dependent RNA-polymerase[4]. This latter enzyme, as produced by bacteria, is specifically inhibited, whereas no influence is exerted on RNA-polymerase from mammalian cells[5–7].

From the microbiological aspect, however, it must be noted that, compared with other antibiotics, the resistance rate with rifampicin is relatively high (approx. 1×10^{-9}) and that the resistance is of a type involving a one-step mutation[2].

Resistance to rifampicin – which can develop in bacterial populations within 24 h, even in the presence of very high selection concentrations – appears to be due to an alteration in DNA-dependent RNA-polymerase. As a result of this alteration, polymerase from resistant bacteria is no longer inhibited by rifampicin[8].

The purpose of the experiments to be described here was to determine whether one of the semi-synthetic derivatives of rifamycin SV prepared in our laboratories has the ability to inhibit rifampicin-resistant mutants of *Staph. aureus*.

Material and methods. Rifampicin-resistant mutants of *Staph. aureus* SG 511 and of freshly isolated hospital strains of *Staph. aureus* were isolated from 18-h cultures by plating out appropriate dilutions against a 250 μg/ml selection concentration of rifampicin. These resistant clones were kept in slant cultures (agar + 250 μg rifampicin/ml) at 4 °C. The various derivatives were assayed against sensitive and resistant strains of *Staph. aureus* in serial dilution tests, the final concentration of the bacteria after inoculation amounting to 10^4 per ml. The results obtained with *Escherichia coli* originate from gradient plate streak assays carried out with bacterial suspensions of 5×10^9 per ml[2].

The rate at which resistant mutants occurred was determined by the methods of LURIA and DELBRÜCK[9] and NEWCOMBE[10].

The experiments with DNA-dependent RNA-polymerase were carried out using the method described by WEHRLI et al.[11].

Results. Of a large number of rifamycin derivatives[12] screened in an initial series of tests, all – with only one exception – showed complete cross-resistance with respect to mutants which were resistant to rifampicin or rifamycin SV. The single exception was 3-piperidino-rifamycin SV [Figure and Table II, No. (**1**)], which inhibited growth of rifampicin-resistant *Staph. aureus* mutants in concentrations of the order of 16 μg/ml. This finding was subsequently confirmed in tests on 10 separately isolated rifampicin-resistant clones of *Staph. aureus* SG 511 as well as 10 freshly isolated hospital strains of *Staph. aureus*, from which the rifampicin-resistant mutants had been isolated by the method already described.

Against rifampicin-resistant gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, the compound displayed no activity.

We determined the resistance rate in the case of the piperidino derivative for a rifampicin-sensitive strain of *Staph. aureus*, for a rifampicin-resistant strain of *Staph. aureus*, and also for rifampicin-sensitive and rifampicin-resistant strains of *B. subtilis*.

The results are presented in Table I, in which the resistance rates to rifampicin have also been included for purposes of comparison.

These findings prompted us to extend our investigations to include about 40 further rifamycin derivatives substituted with amines in Position 3. Given in Table II are a few typical examples indicating the relationship between the structure of the substituents and the biological activity of the respective derivatives. The values listed in this table are also representative of other types of bacteria from the group of gram-positive and gram-negative organisms.

From this tabular review it can be seen that the introduction of simple monalkylamino and dialkylamino groups in Position 3 of the rifamycin chromophore [compounds Nos. (**2**) and (**3**)] produces, at the most, only slight activity against resistant mutants of *Staph. aureus*. The pyrrolidino derivative No. (**4**), too, has only a minimal effect. On the other hand, the homopiperidino

Table I. Resistance rate of rifampicin and 3-piperidino-rifamycin SV

	Concentration (in μg/ml) used for sclection	Sensitive *Staph. aureus* SG 511	Rifampicin-resistant *Staph. aureus* SG 511	Sensitive *B. subtilis*	Resistant *B. subtilis*
Rifampicin	100	7×10^{-9}		1×10^{-9}	
3-piperidino-rifamycin SV	100	$<5 \times 10^{-11}$	$<5 \times 10^{-11}$	$<5 \times 10^{-11}$	$<5 \times 10^{-11}$

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[12] For a survey, see H. BICKEL, F. KNÜSEL, W. KUMP and L. NEIPP, *Antimicrobial Agents and Chemotherapy* (Brown Bromfield, Ann Arbor, Mich., USA 1966), p. 352.

derivative No. (**5**) as well as alkyl-substituted piperidino derivatives [e.g. No. (**6**)] and di-alkyl-substituted pyrrolidino derivatives display activity which is just as good as, or even better than, that of the piperidino derivative No. (**1**). There seems to be evidence to suggest a relationship between structure and activity inasmuch as, to obtain the desired effect, it is necessary to introduce into rifamycin at Position 3 secondary cyclic amines, especially those which occupy at least as much space as the piperidine. This requirement is met by alkyl or aryl-substituted piperidines or pyrrolidines, whereas substituents with polar groups or hetero-atoms appear to result in diminished activity, as exemplified by Nos. (**7**) and (**8**).

Some of the derivatives effective against rifampicin-resistant *Staph. aureus* mutants have already been found to display also comparable activity against rifampicin-resistant strains of *M. tuberculosis* (F. KRADOLFER, personal communication).

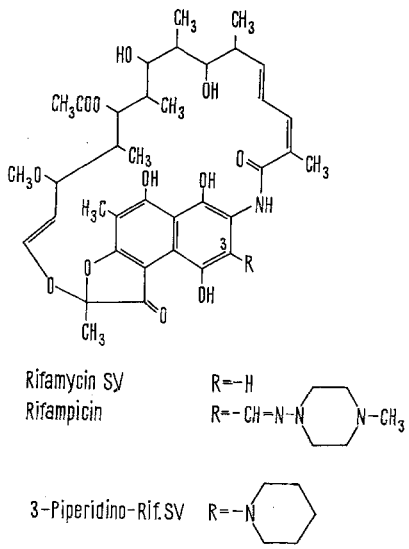

Table II

Structure R	MIC (μg/ml) in serial dilution test		MIC (μg/ml) in gradient plate streak assay
	Rifampicin-sensitive *Staph. aureus* SG 511	Rifampicin-resistant *Staph. aureus* SG 511	Rifampicin-sensitive *E. coli*
(**1**) —N⟨ ⟩	0.002	16–32	25
(**2**) —NHC_2H_5	0.001	125–250	4
(**3**) —N(CH_2CH_3)(CH_2CH_3)	0.03	64–125	>100
(**4**) —N⟨ ⟩	0.0004	64–125	5
(**5**) —N⟨ ⟩	0.004	4–8	>100
(**6**) —N⟨ ⟩-CH(CH_3)(CH_3)	0.009	1–2	>100
(**7**) —N⟨ ⟩—OH	0.002	>250	4
(**8**) —N⟨ O ⟩	0.002	>250	7.5

In concentrations of up to 200 μg/ml, the piperidino derivative No. (**1**) has no influence on DNA-dependent RNA-polymerase from resistant cells of *Staph. aureus*. The experiments in which this finding was obtained were carried out on enzyme preparations from cells of *Staph. aureus* which were resistant to rifampicin but sensitive to derivative No. (**1**) at concentrations of 10 μg/ml (W. WEHRLI and M. STAEHELIN, personal communication).

Polymerase from rifampicin-sensitive cells of *Staph. aureus* is inhibited by over 90% in the presence of concentrations of rifampicin as low as 0.06 μg/ml[5].

Discussion. In the light of the preliminary results briefly outlined above, it may be stated that: Among the many synthetically prepared rifamycin derivatives there exists a group of compounds – derived from the parent substance rifamycin SV by substitution with cyclic secondary amines in Position 3 – whose antibacterial properties differ appreciably from those of rifampicin, which is at present one of the most highly active rifamycin derivatives.

Firstly, the rate at which resistant mutants occur among gram-positive organisms – irrespective of whether the latter are resistant or sensitive to rifampicin – is at least 100 times lower in the case of this group of derivatives than it is with rifampicin, although these derivatives are also highly active against sensitive strains. Secondly, such derivatives display weaker activity than rifampicin against gram-negative bacteria. Thirdly, they are capable of inhibiting rifampicin-resistant mutants of *Staph. aureus* in concentrations of 1–10 μg/ml. In concentrations of up to 200 μg/ml, however, derivatives from this group have no influence on polymerase from resistant cells of *Staph. aureus*.

Since, in the case of the piperidino rifamycins, it is impossible to demonstrate in rifampicin-resistant bacteria the occurrence of binding between the enzyme and the antibiotic, which is responsible for the antibacterial activity of rifampicin, it is conceivable that these derivatives may exert an additional, as yet unknown, effect on the metabolism of gram-positive bacteria, an effect which is masked in sensitive cells by the rapid onset of polymerase inhibition, and which only becomes apparent when the cells are resistant ones.

Experiments designed to shed further light on these findings are now in progress.

Zusammenfassung. Unter einer grossen Anzahl semisynthetischer Rifamycinderivate wurde eine Gruppe strukturell verwandter Verbindungen gefunden, die neben ihrer hohen Wirksamkeit (MIC ~ 0,002–0,01 μg/ml) gegenüber rifampicinsensiblen, grampositiven Keimen in vitro auch eine gute Wirkung (MIC ~ 1–10 μg/ml) gegenüber rifampicinresistenten, grampositiven Keimen aufweisen. Die Resistenzrate grampositiver rifampicinresistenter und -sensibler Keime ist bezüglich dieser Verbindungen mindestens 100mal kleiner als bei Rifampicin. Die Wirkung gegenüber resistenten Keimen scheint nicht auf einer Beeinflussung der DNA-abhängigen RNA-Polymerase zu beruhen. Eine Struktur-Wirkungs-Beziehung wird angegeben.

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