The Antibiotic Micrococcin Is a Potent Inhibitor of Growth and Protein Synthesis in the Malaria Parasite

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The antibiotic micrococcin is a potent growth inhibitor of the human malaria parasite Plasmodium falciparum, with a 50% inhibitory concentration of 35 nM. This is comparable to or less than the corresponding levels of commonly used antimalarial drugs. Micrococcin, like thiostrepton, putatively targets protein synthesis in the plastid-like organelle of the parasite.

Antibiotics of the thiocillin-thiazolyl class, which are collectively known as thiopeptides (16), are highly modified peptides whose site of action lies within eubacterial large subunit (LSU) rRNA (1). The most familiar member of this class of antibiotics is thiostrepton, which is produced by Streptomyces thiostrophiae. It binds tightly to a small region of eubacterial LSU rRNA (18) and inhibits the GTPase reaction catalyzed by ribosomes in the presence of EF-G (10). Although eukaryotic ribosomes are insensitive to thiostrepton (19), we found that thiostrepton inhibits growth of blood-stage cultures of the malaria parasite Plasmodium falciparum, probably by inhibiting protein synthesis in the unusual plastid-like organelle of the parasite (7). Thiostrepton also interacts directly with an RNA fragment derived from the plastid-encoded rRNA, and not with nucleus-encoded RNA fragments (13). Since in Escherichia coli thiostrepton binding is correlated with inhibition of protein synthesis (18), the target for thiostrepton in P. falciparum is presumably plastid-encoded protein synthesis. The plastid genome was recently characterized in detail (20) and is, perhaps, a general feature of the phylum Apicomplexa (6, 8). Antibiotics that target the plastid may, therefore, be of considerable interest as novel chemotherapeutic agents against these parasites which are responsible for important human and animal diseases. In particular, the spread of chloroquine and antifolate resistance in P. falciparum suggests that modification of existing drugs may not circumvent the problem of multidrug-resistant parasites (12).

Like thiostrepton, micrococcin is a thiopeptide, although it is produced by Bacillus and Micrococcus spp. (2). Both compounds inhibit binding of aminoacyl-tRNA to the ribosomal A site as well as other functions linked to eubacterial ribosomal GTP hydrolysis (summarized in reference 1). However, subtle differences in the modes of action of the two drugs, as described below, prompted us to test the effects of micrococcin on parasite growth and protein synthesis, which were correlated with the uptake of [3H]hypoxanthine and [3H]leucine, respectively, in blood stage cultures (5). The results were calculated as the means of triplicate determinations (Fig. 1) and revealed effects of micrococcin on both growth (incorporation of [3H]hypoxanthine) and protein synthesis (incorporation of [3H]leucine) that are statistically significant. While the effects of thiostrepton were similar to those seen previously (7), comparable inhibition with micrococcin occurred at about 100-fold lower drug concentrations. For micrococcin, the estimated 50% inhibitory concentration (IC50) for growth was 35 ± 7.9 nM, while that of thiostrepton was 3.2 ± 0.9 M. Inhibition of
protein synthesis also required higher drug concentrations; the IC_{50} for micrococcin was 90 ± 22 nM and that for thiostrepton 15 ± 4 M. The greater sensitivity of growth as opposed to protein synthesis was consistent with selective inhibition of a minor component of total protein synthesis, as expected if these drugs targeted plastid-encoded rRNA.

It is intriguing why micrococcin is much more potent than thiostrepton in inhibition of the growth of P. falciparum, since both antibiotics interact in similar ways with eubacterial LSU rRNA (14). However, there are subtle differences in the ways in which the two drugs act. For example, micrococcin stimulates ribosome-dependent GTP hydrolysis in the presence of EF-G, whereas thiostrepton completely inhibits this process (2). Whether the mechanistic difference between the drugs is related to the 100-fold discrepancy in their potencies against *P. falciparum* is unclear, since the effectiveness of micrococcin might result from fortuitously concentrating in the plastid-like organelle. This is similar to accumulation of chloroquine in the food vacuole of the parasite (17), with resistance genetically linked with the chloroquine-resistant phenotype. J. Biol. Chem. 270:2046–2049.

Thiostrepton in inhibition of the growth of *P. berghei* was 90 nM and that for thiostrepton 22 nM, whereas thiostrepton completely inhibits this process (2). Whether the mechanistic difference between the drugs is related to the 100-fold discrepancy in their potencies against *P. falciparum* is unclear, since the effectiveness of micrococcin might result from fortuitously concentrating in the plastid-like organelle. This is similar to accumulation of chloroquine in the food vacuole of the parasite (17), with resistance genetically linked with the chloroquine-resistant phenotype. J. Biol. Chem. 270:2046–2049.

The growth-inhibitory effects of micrococcin compare favorably with those of proven antimalarial drugs tested in vitro against sensitive strains of the parasite (Table 1). Antimalarials such as pyrimethamine, chloroquine, and mefloquine act rapidly and have in vitro IC_{50}s that are in the nanomolar range for sensitive strains and can be as high as 100 nM for resistant strains. Antibiotics are generally poorly effective as antimalarial agents, since they are slow acting, although they are used in part because of their nontoxic effects (11). Since the IC_{50} of tetracycline derivatives for in vitro cultures of *P. falciparum* is 26 to 39 M (3), the 1,000-fold greater effectiveness of micrococcin prompts investigation of this perhaps forgotten antibiotic as a potential chemotherapeutic against *Apicomplexa* in general. Also, the potencies of these antibiotics against the mouse malaria parasite *Plasmodium berghei*, including chloroquine-resistant strains, are worth investigating.

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**REFERENCES**


