Synergistic Antimicrobial Activities of Folic Acid Antagonists and Nucleoside Analogs

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The alarming increase in the numbers of antibiotic-resistant bacterial pathogens is one of the world’s most pressing public health problems and points to the need for new anti-infective therapies (7). Folic acid antagonists such as trimethoprim-sulfamethoxazole (SXT) are well-established antibiotics in prophylaxis and for the therapy of a variety of infections (22). Moreover, SXT is a valuable antibiotic combination for infections caused by multidrug-resistant pathogens such as methicillin-resistant Staphylococcus aureus, Burkholderia cepacia, and Stenotrophomonas maltophilia (1, 11, 22). Sulfamethoxazole acts as an inhibitor of bacterial dihydropteroate synthase, inhibiting the synthesis of dihydropteroate from dihydropteroic acid and p-aminobenzoate (34). Trimethoprim inhibits dihydrofolate reductase, which generates tetrahydrofolic acid from dihydrofolic acid (16). Thus, trimethoprim and sulfamethoxazole inhibit different enzymatic steps of the bacterial synthesis of methylenetetrahydrofolate, a necessary cofactor of thymidylate synthase in the generation of dTMP from dUMP.

However, the effects of these antibiotics can be antagonized by the bacterial utilization of extracellular thymidine (2, 13, 18, 33, 36, 39). Various bacteria have the ability to use an alternative pathway by the uptake of extracellular thymidine and the subsequent intracellular phosphorylation to dTMP by thymidine kinase (19, 39). There have been several reports of unsuccessful treatment with folic acid antagonists, supposedly due to elevated thymidine concentrations in human tissues containing necrotic cells such as tumors, pus, and airway secretions of cystic fibrosis (CF) patients (20, 21, 31). It is believed that DNA released from human necrotic cells is catalyzed via dTMP to thymidine, which in turn antagonizes the antimicrobial activity of folic acid antagonists (31). In this context it is of note that for several bacterial species, including S. aureus, S. maltophilia, enterococci, pneumococci, Escherichia coli, and Proteus mirabilis, SXT therapy has been shown to be associated with the emergence of SXT-resistant thymidine-dependent small-colony variants in diverse human specimens (3, 9, 12, 14, 15, 30, 35).

Interestingly, some nucleoside analogs are known to impair thymidine transport into bacteria (28). However, for purposes of antimicrobial chemotherapy, the combined activities of folic acid antagonists and nucleoside analogs have not yet been evaluated.

This study was aimed at (i) screening for nucleoside analogs that impair the bacterial utilization of extracellular thymidine, (ii) analyzing the combined antimicrobial activities of SXT and selected nucleoside analogs against S. aureus, and (iii) evaluating the antimicrobial spectrum of SXT combined with the nucleoside analog 5-ido-2′-deoxyuridine.

MATERIALS AND METHODS

Microbroth assays. Ninety-five microliters of cation-adjusted Mueller-Hinton broth (10 to 12.5 mg/liter Mg2+, 20 to 25 mg/liter Ca2+) (Becton, Dickinson, and Company, Sparks, MD) supplemented with 40 mg/liter of trimethoprim-sulfamethoxazole (SXT) at a ratio of 1:19, i.e., 2 mg/liter (6.9 μmol/liter) of trimethoprim and 38 mg/liter (150.2 μmol/liter) of sulfamethoxazole (both from Sigma-Aldrich, Munich, Germany), and various thymidine concentrations ranging from 0 to 200 μg/liter (0 to 0.826 μmol/liter) (Sigma-Aldrich) was added to each well of a 96-well microtiter plate (Greiner). Five microliters of a bacterial suspension of S. aureus ATCC 25923 (exponential growth phase) was added to each well to yield a final concentration of about 5 × 105 cells/ml. After 0 and 24 h of incubation without shaking at 37°C, 100-μl samples from successive dilutions (range of dilutions, 1:103 to 1:107) were plated onto sheep blood agar (Heipha, Eppelheim, Germany) for CFU enumerations. The thymidine concentration in primary Mueller-Hinton broth was proven to be negligible (thymidine concentration of <10 μg/liter) by a bioassay (data not shown).

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Macrobath assays. The antimicrobial activities of SXT in combination with nucleoside analogs were determined by time-kill methods, as described previously (26, 27), according to guidelines set by the CLSI (formerly NCCLS) (6, 23). Therefore, 10 ml of cation-adjusted Mueller-Hinton broth supplemented with 40 mg/liter of trimethoprim-sulfamethoxazole at a ratio of 1:19, nucleoside analogs, and thymidine was added to glass test tubes at the concentrations indicated below. The thymidine concentrations used were chosen in accordance with thymidine concentrations found in CF sputum (median, 163 µg/liter) as described previously (4). One-hundred-microliter samples of a bacterial suspension (exponential growth phase) were added to each glass test tube to yield a final concentration of about 5 × 10^9 cells/ml. The bacterial suspensions were then incubated at 37°C without shaking for different time intervals. When incubated for a period of 24 h, the bacterial suspensions were vortexed once after 20 h and reincubated again before sampling, as recommended by the NCCLS (23). The antibiotic activities of SXT in combination with the nucleoside analogs were analyzed by CFU enumerations on sheep blood agar. Therefore, 100-µl samples of the different bacterial dilutions (range, 1:10^1 to 1:10^8) were plated onto sheep blood agar and incubated for 24 h. For each experiment, control experiments with and without SXT were performed in the presence of thymidine. The activity of SXT was verified by MIC determinations against reference strain S. aureus ATCC 25923. Experiments were performed in triplicate, if not indicated otherwise. Details of the different experiments are indicated below.

(i) Screening for nucleoside analogs that impair bacterial utilization of extracellular thymidine. The antimicrobial activities of SXT in combination with different nucleoside analogs at equimolar concentrations against S. aureus ATCC 25923 were analyzed at a constant thymidine concentration after 24 h of incubation. The concentrations used were as follows: 40 mg/liter of SXT, 200 µg/liter of thymidine, and 100 µg/liter of nucleoside analogs. The following nucleoside analogs were analyzed: adenosine, cytidine, guanosine, inosine, uridine, 2’-deoxyadenosine, 2’-deoxyctydine, 2’-deoxyguanosine, 2’-deoxyinosine, 2’-deoxyuridine, 5-bromo-2’-deoxyuridine, 5-chloro-2’-deoxyuridine, 5-fluoro-2’-deoxyuridine, and 5-iodo-2’-deoxyuridine (all from Sigma-Aldrich).

(ii) Analysis of the combined antimicrobial activities of SXT and selected nucleoside analogs against S. aureus in the presence of thymidine. The antimicrobial activities of SXT in combination with a nucleoside analog (uridine, 2’-deoxyuridine, or 5-iodo-2’-deoxyuridine) against S. aureus ATCC 25923 were analyzed at a constant thymidine concentration. The concentrations used were as follows: 40 mg/liter of SXT, 200 µg/liter of thymidine, and nucleoside analogs at a range of 0 to 10 µm/liter. Bacterial suspensions were incubated for 0, 2, 4, 6, and 24 h. They synergy between SXT and 5-iodo-2’-deoxyuridine was evaluated. These experiments were performed in triplicate.

Moreover, the antimicrobial activity of SXT (0.1 mg/liter, 1 mg/liter, 10 mg/liter, and 100 µg/liter) in combination with 5-iodo-2’-deoxyuridine (0.1 µg/ml, 1 µm/ml, 10 µm/ml, and 100 µm/ml) in the absence and presence of thymidine was analyzed at a constant thymidine concentration. The concentrations used were as follows: 40 mg/liter of SXT, 200 µg/liter of thymidine, and 100 µm/liter 5-iodo-2’-deoxyuridine. All strains used in the study were susceptible to SXT as determined by broth macrodilution for S. aureus ATCC 25923 (MIC of SXT, 2 mg/liter), Staphylococcus epidermidis ATCC 14990 (MIC of SXT, 2 mg/liter), Streptococcus pyogenes ATCC 12351 (MIC of SXT, 1 mg/liter), Enterococcus faecalis ATCC 29212 (MIC of SXT, 0.5 mg/liter), Escherichia coli ATCC 25922 (MIC of SXT, 2 mg/liter), Klebsiella pneumoniae ATCC 13883 (MIC of SXT, 8 µg/liter), and Stenotrophomonas maltophilia V7/24102 (a clinical blood culture isolate) (MIC of SXT, 8 µg/liter). The MICs refer to both components of the combination, i.e., trimethoprim and sulfamethoxazole.

Exclusion of drug carryover. Drug carryover was excluded in accordance with CLSI (formerly NCCLS) guidelines (23). One-hundred-microliter samples of the different bacterial test broths with and without the different antimicrobial agents in the highest concentrations used were added in the center of dried sheep blood agar plates, allowing 20 min for absorption of the antibiotics into the agar. The inoculum was then streaked over the surface of the plate. After 24 h of incubation at 37°C, the CFU of the plates with and without antibiotics were compared. No reduction of CFU on plates with antibiotics was found, indicating that drug carryover was negligible in the present study.

Definitions and statistics. Synergy in our study was defined as a ≥2-log, decrease in CFU/ml between the combination and its most active constituent

TABLE 1. Antimicrobial activities of diverse nucleoside analogs with and without SXT against Staphylococcus aureus in the presence of thymidine

<table>
<thead>
<tr>
<th>Nucleoside analog</th>
<th>Avg antimicrobial activity</th>
<th>Without SXT</th>
<th>With SXT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>3.08 ± 0.589</td>
<td>1.48 ± 0.434</td>
<td></td>
</tr>
<tr>
<td>Cytidine</td>
<td>3.17 ± 0.484</td>
<td>2.17 ± 0.365</td>
<td></td>
</tr>
<tr>
<td>Guanosine</td>
<td>3.12 ± 0.548</td>
<td>1.67 ± 0.425^ (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Inosine</td>
<td>3.02 ± 0.748</td>
<td>1.03 ± 0.469^ (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>Uridine</td>
<td>3.00 ± 0.558</td>
<td>2.19 ± 0.161</td>
<td></td>
</tr>
<tr>
<td>2’-Deoxyadenosine</td>
<td>2.91 ± 0.070</td>
<td>1.66 ± 0.164</td>
<td></td>
</tr>
<tr>
<td>2’-Deoxycytidine</td>
<td>2.91 ± 0.163</td>
<td>2.12 ± 0.149</td>
<td></td>
</tr>
<tr>
<td>2’-Deoxyguanosine</td>
<td>3.22 ± 0.270</td>
<td>1.21 ± 0.723^ (&lt;0.05)^</td>
<td></td>
</tr>
<tr>
<td>2’-Deoxyinosine</td>
<td>3.18 ± 0.053</td>
<td>0.31 ± 0.069^ (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>2’-Deoxyuridine</td>
<td>2.92 ± 0.257</td>
<td>−2.43 ± 0.447^ (&lt;0.01)</td>
<td></td>
</tr>
<tr>
<td>5-Bromo-2’-deoxyuridine</td>
<td>2.82 ± 0.326</td>
<td>−&lt;3.5^ (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>5-Chloro-2’-deoxyuridine</td>
<td>2.84 ± 0.147</td>
<td>−&lt;3.5^ (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>5-Fluoro-2’-deoxyuridine</td>
<td>−3.5</td>
<td>−&lt;3.5^ (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>5-Iodo-2’-deoxyuridine</td>
<td>2.88 ± 0.441</td>
<td>−&lt;3.5^ (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Control (SXT + thymidine)</td>
<td>1.81 ± 0.165</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (SXT)</td>
<td>−3.10 ± 0.323</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (thymidine)</td>
<td>3.04 ± 0.303</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a Indicates that the antimicrobial activity of the nucleoside analog in combination with SXT is significantly higher than the antimicrobial activity of SXT alone in the presence of thymidine.

^b P > 0.05 by Bonferroni-Holm test.

^c SXT alone in the presence of thymidine.

^d SXT alone in the absence of thymidine.

^e Growth control without SXT but with supplementation of thymidine.

^f Concentrations of analogs were 100 µmol/liter; the concentration of SXT was 40 mg/liter against Staphylococcus aureus. Averages ± standard deviations on a log scale are shown.

Impact of thymidine on the antimicrobial activity of SXT against S. aureus. The influence of different thymidine concentrations on the antimicrobial activity of SXT against S. aureus ATCC 25923 was determined by microbroth assays (Fig. 1). The antimicrobial activity of SXT was inversely related to the concentrations on the antimicrobial activity of SXT against S. aureus ATCC 25923 was determined by microbroth assays (Fig. 1). The antimicrobial activity of SXT was inversely related to the concentrations with the less active component being tested at an ineffective concentration (23). Bacteriostatic activity was defined as a 0- to <3-log killing, and bactericidal activity was defined as a ≥3-log killing after 24 h.

Statistical analysis of the logarithmic values was performed with the unpaired Student t test. The Student one-sample t test was performed in cases when the values were below the limit of detection [log_{10}(CFU/mL) < -3.5]. P values of <0.05 were considered to be statistically significant. Experiments were performed in triplicate. As parametrical tests assume a normal distribution, we performed the corresponding Kolmogorov-Smirnov test in order to rule out a major deviation of this assumption. Such a major deviation from the assumption of a normal distribution was not found for any data set. For each part of the analysis (Tables 1 and 2 and see Fig. 3), we performed alpha-adjustment according to the Bonferroni-Holm test. All tests remained significant unless indicated otherwise. However, keep in mind that a statistical analysis based on three values should be interpreted critically.

RESULTS

SYNERGISM OF SXT AND NUCLEOSIDE ANALOGS: 1227

Impact of thymidine on the antimicrobial activity of SXT against S. aureus. The influence of different thymidine concentrations on the antimicrobial activity of SXT against S. aureus ATCC 25923 was determined by microbroth assays (Fig. 1). The antimicrobial activity of SXT was inversely related to the thymidine concentration. In the absence of thymidine, SXT showed bactericidal activity. Between thymidine concentrations of ≥25 and ≥150 µg/liter, only bacteriostatic activity could be observed for this agent. Thymidine concentrations of ≥200 µg/liter even allowed bacterial growth in the presence of SXT.

Screening for nucleoside analogs that impair bacterial utilization of extracellular thymidine. The effects of different nucleoside analogs alone or in combination with SXT against

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TABLE 2. Antimicrobial activities of 5-iodo-2'-deoxyuridine, SXT, and 5-iodo-2'-deoxyuridine with SXT against different bacterial species in the presence of thymidine.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Avg antimicrobial activity [log10(CFU24 h /CFU0 h)] ± SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With IdUrda, With SXT, With IdUrd and SXT</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>3.09 ± 0.036, 1.75 ± 0.051, 1.75 ± 0.051</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>3.29 ± 0.108, 1.97 ± 0.140, 1.97 ± 0.140</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>0.98 ± 0.392, 0.71 ± 0.018, 0.71 ± 0.018</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>3.15 ± 0.172, 1.34 ± 0.488, 1.34 ± 0.488</td>
</tr>
<tr>
<td>E. coli</td>
<td>3.20 ± 0.108, 3.46 ± 0.083, 3.46 ± 0.083</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>3.07 ± 0.153, 1.63 ± 0.571, 1.63 ± 0.571</td>
</tr>
<tr>
<td>S. maltophilia</td>
<td>2.33 ± 0.036, 1.77 ± 0.173, 1.77 ± 0.173</td>
</tr>
</tbody>
</table>

a IdUrda, 5-iodo-2'-deoxyuridine.

b Indicates that the antimicrobial activity of 5-iodo-2'-deoxyuridine in combination with SXT is significantly higher than the antimicrobial activity of SXT alone against the same bacterial species in the presence of thymidine.

c The concentration of 5-iodo-2'-deoxyuridine was 100 μmol/liter, and the concentration of SXT was 40 mg/liter. Averages ± standard deviations on a log scale are shown.

S. aureus ATCC 25923 were evaluated in the presence of thymidine (Table 1). Controls revealed that in the absence of thymidine, SXT alone showed high antimicrobial activity, with log10(CFU24 h /CFU0 h) values of −3.10 ± 0.332. However, in the presence of thymidine, SXT showed only weak antimicrobial activity, with log10(CFU24 h /CFU0 h) values of 1.81 ± 0.165. Thymidine-supplemented cation-adjusted Mueller-Hinton broth without SXT showed log10(CFU24 h /CFU0 h) values of 3.04 ± 0.303. Nucleosides and 2'-deoxynucleosides at concentrations of 100 μmol/liter exhibited, at best, bacteriostatic activity, with log10(CFU24 h /CFU0 h) values of ≥−2.43 (i.e., 2.43-log killing at 24 h or less) when combined with SXT in the presence of thymidine. As 2'-deoxyuridine in combination with SXT showed the highest antimicrobial activity of these combinations in the presence of thymidine, halogenated derivatives of 2'-deoxyuridine were further evaluated. 5-Bromo-2'-deoxyuridine, 5-chloro-2'-deoxyuridine, 5-fluoro-2'-deoxyuridine, and 5-iodo-2'-deoxyuridine in combination with SXT showed high antimicrobial activities in the presence of thymidine. With log10(CFU24 h /CFU0 h) values of <−3.5, these antimicrobial combinations were bactericidal. Further experiments with the halogenated nucleoside analogs at lower concentrations revealed that 5-iodo-2'-deoxyuridine was the most potent nucleoside analog (data not shown). All nucleoside analogs except for 5-fluoro-2'-deoxyuridine exhibited no intrinsic antimicrobial activity, showing log10(CFU24 h /CFU0 h) values of ≥2.82. 5-Fluoro-2'-deoxyuridine showed intrinsic antimicrobial activity, with log10(CFU24 h /CFU0 h) values of <−3.5, possibly because this compound inhibits thymidylate synthase in addition to thymidine utilization (37).

Analysis of the combined antimicrobial activities of SXT and selected nucleoside analogs against S. aureus in the presence of thymidine. The time-kill curves of a nucleoside (uridine), a deoxynucleoside (2'-deoxyuridine), and a halogenated nucleoside analog (5-iodo-2'-deoxyuridine) in combination with SXT in the presence of thymidine are shown in Fig. 2. A total of 10 μmol/liter of 5-iodo-2'-deoxyuridine in combination with SXT showed bactericidal activity, with a log10(CFU24 h / CFU0 h) value of −3.33 ± 0.612. In contrast, uridine and 2'-deoxyuridine at this concentration allowed bacterial growth when combined with SXT.

The bactericidal or bacteriostatic activities of different concentrations of 5-iodo-2'-deoxyuridine in combination with SXT in the presence of thymidine were further evaluated (Fig. 3). S. aureus without the addition of any antibiotics showed a 2.76-log growth at 24 h. Likewise, S. aureus with the addition of 5-iodo-2'-deoxyuridine alone revealed similar growth kinetics, indicating that this nucleoside analog lacks intrinsic antimicrobial activity. SXT alone impaired bacterial growth. Nonetheless, a 1.74-log growth at 24 h was still observed. In contrast, SXT in combination with 5-iodo-2'-deoxyuridine at the concentrations indicated in Fig. 3 exhibited higher antimicrobial activity.

FIG. 1. Influence of various thymidine concentrations on the antimicrobial activity of SXT against S. aureus ATCC 25923. CFU were determined after 0 and 24 h of incubation at 37°C with constant SXT (40 mg/liter) supplementation. Mean values from three different experiments are shown. Error bars indicate standard deviations.

FIG. 2. Combined antimicrobial activities of SXT (40 mg/liter) and nucleoside analogs (10 μmol/liter) at a constant thymidine concentration of 200 μg/liter against S. aureus ATCC 25923. Mean values from three different experiments are shown. Error bars indicate standard deviations.
Mean values from three different experiments are shown. Error bars indicate standard deviations.

activities even with bacteriostatic or bactericidal activity after 24 h of incubation. Differences in activity between SXT alone and SXT in combination with 5-iodo-2'-deoxyuridine at concentrations of ≥0.25 μmol/liter after 24 h of incubation were significant (P < 0.05). Synergistic activity existed for 5-iodo-2'-deoxyuridine at concentrations of ≥1 μmol/liter in combination with SXT against S. aureus ATCC 25923 in the presence of thymidine.

Moreover, because of the variability of thymidine concentrations in vivo, determinations of time-kill kinetics were performed at three different thymidine concentrations. Growth controls and 5-iodo-2'-deoxyuridine alone in the presence of thymidine (200 μg/liter, 400 μg/liter, and 800 μg/liter) showed strong bacterial growth (range, 10^5 to 10^9 CFU/ml) after 24 h of incubation (data not shown). SXT alone at concentrations of 10 mg/liter and 100 mg/liter, concentrations that are above the MIC of SXT (2 mg/liter), showed only a weak growth reduction (range, 10^5 to 10^6 CFU/ml) in the presence of thymidine after 24 h of incubation (Fig. 4). SXT at concentrations below the MIC showed no antimicrobial activity (range, 10^5 to 10^9 CFU/ml) after 24 h of incubation. The combination of 5-iodo-2'-deoxyuridine and SXT showed synergistic antimicrobial activity if SXT was used at concentrations above the MIC and if 5-iodo-2'-deoxyuridine was used at concentrations of ≥1 μmol/liter at a thymidine concentration of 200 μg/liter or at concentrations of ≥10 μmol/liter at a thymidine concentration of ≥400 μg/liter.

Evaluation of the antimicrobial spectrum of SXT combined with the nucleoside analog 5-iodo-2'-deoxyuridine in the presence of thymidine. SXT, 5-iodo-2'-deoxyuridine, and SXT in combination with 5-iodo-2'-deoxyuridine were tested against various bacterial species, including S. aureus, S. epidermidis, S. pyogenes, E. faecalis, E. coli, K. pneumoniae, and S. maltophilia (Table 2). The addition of 5-iodo-2'-deoxyuridine to SXT led to significantly higher antimicrobial activities in the presence of thymidine, with bactericidal effects against S. aureus, S. epidermidis, and K. pneumoniae. Significantly higher antimicrobial activities with bacteriostatic effects were found for E. faecalis, S. maltophilia, and S. pyogenes. In contrast, no improvement of antimicrobial activity was found for E. coli, as SXT alone already showed bactericidal activity at this thymidine concentration. 5-iodo-2'-deoxyuridine at a concentration of 100 μmol/liter did not show intrinsic antimicrobial activity against any bacterial species tested (data not shown). These experiments showed that in the presence of thymidine, the addition of 5-iodo-2'-deoxyuridine to SXT leads to significantly higher antimicrobial activities against relevant human pathogens.

**DISCUSSION**

It was previously suggested that thymidine is present in human body fluids and that some bacterial species use this extracellular thymidine to bypass the antimicrobial activities of folic acid antagonists (31). Greko and colleagues previously showed that tissue infections in secluded infection sites of calves were refractory to treatment with trimethoprim-sulfadoxine due to elevated thymidine concentrations (13). Thymidine concentrations were previously reported to be high in the blood of several animals (24). In human serum and plasma, thymidine concentrations range from <12 μg/liter to 145 μg/liter (8, 17, 24, 32). Higher levels of thymidine, however, are likely to occur in damaged tissue (20, 25, 29, 33). Consistent with these observations, we recently demonstrated that elevated concentrations of thymidine and its metabolite dTMP are present in various human specimens such as CF sputum, pus, and urine (4). Moreover, in human pooled uninfected dialysate, SXT showed only bacteriostatic activity against S. epidermidis, whereas previous thymidine depletion by the addition of thymidine phosphorylase restored bactericidal activity (25).

The present data show that thymidine concentrations of ≥25 μg/liter impair the antimicrobial activity of SXT against S. aureus and that thymidine concentrations of ≥200 μg/liter even allow bacterial growth in the presence of SXT. These data are consistent with data from a previous study by Tokunaga et al., who reported that thymidine at concentrations of ≥100 μg/liter antagonized the antimicrobial activity of trimethoprim in E. coli (37). Moreover, data from a mouse infection model revealed that the activity of folic acid antagonists is much higher against thymidine kinase-deficient bacteria than against wild-type bacteria (37). Thymidine kinase-deficient bacteria are able to take up extracellular thymidine, which is abundant in mice (24); however, they are not able to convert thymidine to dTMP intracellularly.

In the present study, we screened for nucleoside analogs that impair the bacterial utilization of extracellular thymidine and enhance the antibiotic activity of SXT. Therefore, in addition to substances that are known to impair the function of the nucleoside transporters NupC and NupG in E. coli (28), we evaluated nucleoside analogs that potentially interfere with bacterial thymidine uptake or intracellular thymidine phosphorylation. We identified several nucleoside analogs that enhance SXT activity in the presence of thymidine. Because of the high
antimicrobial activity against *S. aureus*, the broad antimicrobial spectrum, and the existing clinical data, 5-iodo-2′-deoxyuridine was further evaluated.

5-Iodo-2′-deoxyuridine, a thymidine kinase inhibitor (38), has been approved in clinical trials. An unacceptable cytotoxicity of this agent in the therapy of herpes simplex virus encephalitis at a dose of about 400 μmol/kg of body weight/day for 5 days was previously reported (5). In a phase I trial of the sequential administration of ralitrexed, an inhibitor of human thymidylate synthase, and 5-iodo-2′-deoxyuridine, dose-limiting cytotoxicity for 5-iodo-2′-deoxyuridine was determined at a steady-state plasma concentration of about 100 μmol/liter (10).

In the present study, we determined that in vitro, a concentration of 8 μmol/liter of this nucleoside analog combined with SXT exhibits bactericidal activity against *S. aureus* ATCC 25923 at thymidine concentrations of 200, 400, and 800 μg/liter. Moreover, in the presence of thymidine, significantly higher antimicrobial activities against various relevant human pathogens were found for this combination than for SXT alone. Future studies at higher total inocula and over a longer time course are required to assess the prevention of resistance for this most promising combination against relevant human pathogens. Although we tested only selected bacterial strains under *in vitro* conditions, it is tempting to speculate that the combination of SXT and 5-iodo-2′-deoxyuridine at nontoxic concentrations may reach levels in human tissues that result in a substantially

FIG. 4. Time-kill curves for strain *S. aureus* ATCC 25923 at different thymidine concentrations (200, 400, and 800 μg/liter) following exposure to SXT (0.1, 1, 10, and 100 mg/liter) and 5-iodo-2′-deoxyuridine (0.1, 1, 10, and 100 μmol/liter). All experiments were performed once.
higher antimicrobial activity against relevant human pathogens in the presence of elevated extracellular thymidine concentrations.

In view of the growing problem of antibiotic-resistant bacteria, the supply of new antibiotics and the optimization of established ones are crucial for the maintenance of effective therapeutic options against infections (7). This study provides evidence that in vitro, 5-ido-2'-deoxyuridine enhances the extent of killing of SXT against bacterial pathogens in the presence of elevated thymidine concentrations. Further studies are required to evaluate the clinical potential of this combined anti-infective agent.

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