Combined Action of 6-Mercaptopurine and Antibiotics on Gram-Negative Bacteria *in Vitro*[1] (36495)

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(Introduced by Anthony V. Pisciotta)

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Many drugs which are used for the treatment of neoplastic diseases possess some degree of antibacterial activity (1–3). One of these agents, 6-mercaptopurine (6-MP), is administered to patients for extended periods of time and occasionally is given in high doses intravenously (600 mg/m^2/day) (4). Concentrations of 1–5 μg of 6-MP/ml of serum occur in individuals receiving therapeutic doses of the drug (4–6). It is known that 6-MP can suppress the metabolic activity of some bacteria *in vitro* (1, 2), and combined actions between 6-MP and antibiotics against a few bacterial isolates have been noted previously (2, 3). The studies described here were designed to evaluate systematically the degree of antimicrobial activity of 6-MP and the effect of 6-MP in combination with various antibiotics on some common bacterial pathogens *in vitro*. These studies have demonstrated synergism between 6-MP and aminoglycoside antibiotics against gram-negative bacteria and weak antagonism of the antimicrobial activity of penicillins by 6-MP.

Materials and Methods. Media and bacteria. All studies were performed with a chemically defined minimal growth medium (pH 7.0) prepared as follows (7): Distilled H_2O, 1000.0 ml; K_2HPO_4, 7.0 g; KH_2PO_4, 3.0 g; Na_3 citrate–3 H_2O, 0.5 g; $MgSO_4$–7 H_2O, 0.1 g; $(NH_4)_2SO_4$, 1.0 g; glucose, 2.0 g. A twofold concentration of this solution was prepared for use in antibiotic susceptibility tests. In some experiments fetal bovine serum (Microbiological Associates) was added to the minimal media. *Escherichia coli,* klebsiella, enterobacter, and pseudomonas were isolated from clinical specimens and grown overnight in minimal growth media before use in susceptibility tests.

Antibiotics and purines. Antibiotics were dissolved in 0.0012 *M* phosphate buffer (pH 7.0) and sterilized by filtration for each experiment. 6-Mercaptopurine (kindly supplied as the dry powder by Burroughs Wellcome and Company) or xanthine, hypoxanthine or adenine (obtained in anhydrous form from P–L Biochemicals Inc.) were dissolved in 0.1 *N* NaOH, neutralized with an equal volume of 0.1 *N* HCl, and sterilized by filtration. Dilutions were prepared with sterile 0.0012 *M* phosphate (pH 7.0).

Antibiotic susceptibility assays. Tests of antibiotic susceptibility were carried out by a modified twofold tube dilution technique using microtiter "U" plates and pipettes. Overnight bacterial cultures in minimal media were diluted 1:10,000 in twofold concentrated minimal media and 0.05 ml of the inoculum was added to appropriate microtiter wells. Antibiotics and/or 6-MP were added in volumes of 0.025 ml each and phosphate buffer was added to bring the final volume in each well to 0.1 ml. This provided a single concentration of minimal media in each well during the subsequent incubation period. Controls containing no antibiotic or 6-MP were included for each organism. The plates were sealed and incubated at 37° for 24 hr. The lowest concentration of the antibiotic that prevented visible turbidity represented

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TABLE I. Effect of 6-Mercaptopurine (6-MP) on the Minimum Inhibitory Concentration (MIC) of Antibiotics for Gram-Negative Bacteria.

Organism	Antibiotic	No. of strains	MIC (μg/ml)[a]	
			6-MP (5 μg/ml)	No 6-MP
E. coli	Kanamycin	40	1.10[b] (0.2 – 1.6)[c]	4.25 (1.6 –12.5)
	Gentamicin	40	0.25 (0.02– 0.4)	1.10 (0.4 – 1.6)
	Streptomycin	33	0.82 (0.2 – 3.1)	3.12 (0.4 –12.5)
	Ampicillin	31	1.02 (0.4 – 3.1)	0.49 (0.1 – 1.6)
	Cephalothin	40	3.40 (0.8 –12.5)	2.10 (0.4 – 6.2)
	Tetracycline	11	0.80 (0.4 – 1.6)	0.80 (0.4 – 1.6)
	Chloramphenicol	11	3.40 (1.6 – 3.1)	3.92 (1.6 – 6.2)
	Polymyxin B	24	0.42 (0.05– 1.6)	0.49 (0.05– 1.6)
Klebsiella	Kanamycin	17	0.79 (0.4 – 1.6)	1.40 (0.8 – 3.1)
	Gentamicin	20	0.29 (0.2 – 0.4)	0.72 (0.4 – 3.1)
	Cephalothin	20	2.82 (0.8 – 6.2)	1.82 (0.8 – 3.1)
Enterobacter	Kanamycin	17	0.56 (0.4 – 1.6)	1.30 (0.8 – 3.1)
	Gentamicin	20	0.39 (0.1 – 0.8)	0.84 (0.4 – 3.1)
Pseudomonas	Gentamicin	10	0.54 (0.4 – 1.6)	1.06 (0.8 – 3.1)
	Carbenicillin	10	30.2 (25–50)	25.1 (12.5–50)
	Polymyxin B	10	0.58 (0.4 – 0.8)	0.59 (0.2 – 1.6)

[a] MIC determined by a microtiter adaptation of the tube dilution method for determination of antibiotic susceptibility. A chemically defined growth medium and an inoculum of about 10^5 organisms/ml were used. The cultures were incubated for 24 hr at 37°.

[b] Geometric mean MIC.

[c] Range of MIC observed for individual strains.

its minimum inhibitory concentration (MIC).

Bacterial growth studies were performed with tubes containing approximately 10^5 viable organisms/ml with added antibiotics and/or 6-MP. Before incubation and at specified intervals thereafter the contents of the tubes were mixed by repeated pipetting and 0.1 ml of serial tenfold dilutions were streaked evenly onto Mueller-Hinton agar plates and incubated overnight for colony counts.

Results. Antibacterial activity of 6-MP. Initially, 30 isolates each of *E. coli*, klebsiella, enterobacter and pseudomonas were studied for susceptibility to 6-MP. The development of visible turbidity in cultures containing 6-MP (1–5 μg/ml) was delayed about 6 hr compared with control cultures for all the isolates. A concentration of 100 μg of 6-MP/ml was required to suppress visible growth of 50% of 40 isolates of *E. coli* during a 24-hr period of incubation. Subcultures of tubes containing no visible turbidity grew *E. coli* in all instances indicating that 6-MP (100 μg/ml) was not bactericidal for any of the isolates. Studies of the rate of bacterial growth carried out with 10 isolates of *E. coli* indicated that 6-MP (5 μg/ml) slowed the rate of bacterial growth during the first 9 hr of incubation but by 24 hr the number of viable bacteria in cultures containing 6-MP was only slightly smaller than in the control cultures.

Combined action of 6-MP and antibiotics. The effect of 6-MP (5 μg/ml) on the susceptibility of various bacteria to a variety of antibiotics is depicted in Table I. The mean minimum inhibitory concentration (MIC) of aminoglycoside antibiotics for *E. coli* was decreased 4-fold in the presence of 6-MP (5 μg/ml). The mean MIC of aminoglycoside antibiotics for klebsiella, enterobacter, and pseudomonas species were decreased 2-fold when 6-MP was present. This apparent synergism was observed for virtually all isolates. Organisms which were relatively resistant to aminoglycoside antibiotics displayed the same relative increase in susceptibility to the combination as organisms which were inher-

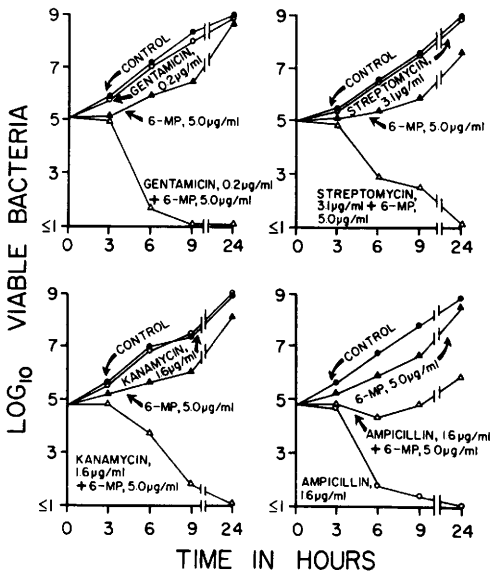

FIG. 1. Bacterial growth curves depicting the combined action of 6-mercaptopurine (6-MP) and antibiotics on a single isolates of *E. coli*.

ently very sensitive to these antibiotics.

The susceptibility of strains of *E. coli* and klebsiella to ampicillin and/or cephalothin was slightly decreased by the addition of 6-MP while the activity of tetracycline, chloramphenicol or polymyxin B was not affected by 6-MP.

Figure 1 illustrates the dynamics of the combined action of 6-MP and antibiotics on individual isolates of *E. coli*. Concentrations of aminoglycoside antibiotics having little or no activity alone against the test organisms were selected. After a several hour lag period the combination of 6-MP and aminoglycoside antibiotics resulted in progressive killing of organisms which did not occur with either drug separately. Most of this killing occurred during the period when 6-MP exerted its greatest effect, *i.e.*, during the first 9 hr of incubation. Conversely, the lethal effect of ampicillin on *E. coli* was antagonized by 6-MP. These results indicate that synergism or antagonism between 6-MP and antibiotics occurs in terms of bacterial killing as well as bacteriostasis.

Effect of serum on combined actions of 6-MP and antibiotics. The geometric mean MIC of kanamycin or gentamicin for 10 strains of *E. coli* were decreased 4-fold in the presence of 6-MP (5 µg/ml) in minimal media with added fetal bovine serum (50%). The geometric mean MIC for ampicillin was increased 2-fold in the presence of 6-MP (5 µg/ml) in this media. Fetal bovine serum (50%) alone had no bacteriostatic activity.

Effect of purines on combined action of 6-MP and kanamycin. The specificity of the effect of 6-MP on the susceptibility of 10 strains of *E. coli* to kanamycin was determined by addition of related purines to the assay system. Hypoxanthine (20 µg/ml) reversed completely the synergism between 6-MP (5 µg/ml) and kanamycin. Xanthine (20 µg/ml) produced a 50% reversal of the synergism. Adenine had antibacterial activity in a concentration of 20 µg/ml but appeared to eliminate the synergism between 6-MP and kanamycin for *E. coli*.

Discussion. The present studies indicate that 6-MP has only weak antibacterial activity against a variety of gram-negative organisms *in vitro*. However, 6-MP displays a marked synergism in combination with aminoglycoside antibiotics against *E. coli* and to a lesser degree against klebsiella, enterobacter and pseudomonas isolates. This effect appears specific since it is readily reversed by an excess of the naturally occurring parent compound, hypoxanthine.

The presence of serum, even in a concentration of 50%, did not reverse the combined action of 6-MP and kanamycin against *E. coli*, indicating that the trace amounts of purines normally present in serum do not interfere with the observed synergism between 6-MP and aminoglycoside antibiotics *in vitro*. However, the possible clinical significance of these drug interactions remains speculative. Pharmacokinetic data indicate that serum levels of 6-MP comparable to the concentrations employed in these studies occur in patients undergoing therapy with 6-MP, especially when the drug is administered intravenously (4–6). Since such patients frequently have impaired host defenses, bactericidal effects of antimicrobial agents should be extremely important for eradication of infections. Controlled clinical trials are needed to

determine whether patients receiving 6-MP who develop infections with gram-negative organisms could be more effectively treated with aminoglycoside antibiotics than with other antibiotics.

The exact mechanism of the synergism between 6-MP and aminoglycoside antibiotics cannot be determined from the present data. Aminoglycosides exert a direct action on the ribosome with subsequent interference with protein synthesis, and they decrease the fidelity of translation of the genetic code (8). 6-Mercaptopurine inhibits utilization of hypoxanthine and other essential nucleic acid precursors, suppresses *de novo* synthesis of purines, and may also be incorporated to produce fraudulent DNA (6, 9). Thus, both 6-MP and aminoglycoside antibiotics exert effects on nucleic acid metabolism, and the combined effects of these agents on nucleic acid metabolism could account for their synergistic activity. The observation that 6-MP produces little effect in combination with antibiotics such as chloramphenicol, tetracycline, or polymyxin B which do not affect nucleic acid synthesis is consistent with this possibility. The mechanism by which 6-MP reduced the activity of penicillin-type antibiotics is not certain. However, these antibiotics require actively growing bacteria to exert their effects and the weak bacteriostatic activity of 6-MP could account for the observed antagonism.

Summary. The antibacterial activity of a commonly used antimetabolite, 6-mercaptopurine (6-MP), was studied *in vitro* using chemically defined media. 6-Mercaptopurine (1–5 μg/ml) had weak bacteriostatic activity against *Escherichia coli,* klebsiella, enterobacter and pseudomonas. Synergism occurred between 6-MP and aminoglycoside antibiotics whereas 6-MP was weakly antagonistic to the action of penicillin and cephalosporin antibiotics. These combined actions were not reversed by fetal bovine serum (50%) but were readily reversed by an excess of hypoxanthine or adenine. No interactions occurred between 6-MP and tetracycline, chloramphenicol or polymyxin B.

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