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MINIREVIEW

Trimethoprim Resistance

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INTRODUCTION

Trimethoprim (TMP) is a synthetic antibacterial agent that belongs to a group of compounds called diaminopyrimidines. These agents inhibit dihydrofolate reductase (DHFR; EC 1.5.1.3), an enzyme that catalyzes the reduction of dihydrofolate to tetrahydrofolate in microbial and eucaryotic cells (14, 39). The diaminopyrimidines are smaller than and structurally unlike folate, in contrast to aminopterin and methotrexate, which are DHFR inhibitors that are structurally similar to folate. Aminopterin and methotrexate antagonize both mammalian and microbial DHFR, whereas diaminopyrimidines such as TMP are more active against microbial DHFR than against mammalian DHFR (14). The reason for this difference in potency has been eludicated by X-ray crystallography studies. TMP was found to fit well to the substrate-binding site of *Escherichia coli* DHFR but not mammalian DHFR (55).

TMP is active in vitro against most aerobic gram-negative and gram-positive bacteria (16). Bacterial pathogens known to be intrinsically resistant to TMP are fewer than susceptible ones. TMP is also active against certain types of malaria (18, 54) and, in combination with sulfonamides, against *Pneumocystis carinii* (67), although TMP alone has only very weak activity against the DHFR of *P. carinii* (2).

TMP was first used clinically in the treatment of *Proteus* septicemia in combination with polymyxin and sulfonamides in 1962 (62). Synergy found between TMP and sulfonamides led to the clinical use of these drugs in combination in the United Kingdom and the United States in 1968 and worldwide soon after (17). A TMP-sulfonamide combination has been efficacious in the treatment of a variety of different infections (67). Because of side effects caused by sulfonamides and clinical outcome equivalent to that obtained with TMP alone in the treatment of urinary and respiratory tract infections (5, 13, 47, 50, 51), TMP has also been used clinically alone. TMP alone was first used for the prophylaxis of urinary tract infections in Finland in 1972 (47) and in other European countries and the United States in 1979 (50).

With the widespread use of TMP, TMP-resistant bacterial pathogens have emerged as a significant clinical problem. The purpose of this minireview is to review TMP resistance in bacteria, considering mechanisms, spread, and approaches to the suppression of TMP resistance.

MECHANISMS OF TMP RESISTANCE

Mechanisms of bacterial resistance to TMP include cell wall impermeability, alternative metabolic pathways, production of a resistant chromosomal DHFR enzyme, overproduction of a chromosomal enzyme, and production of a plasmid-mediated TMP-resistant DHFR enzyme (1, 15, 27, 31, 35, 73, 76, 82).

Pseudomonas aeruginosa and some other *Pseudomonas* spp. are intrinsically resistant to TMP because of poor penetration through the cell wall (35, 82). Acquired TMP resistance based on cell wall permeability has been reported in *Klebsiella pneumoniae* and *Serratia marcescens* (74, 86). Alterations in outer membrane proteins have been found associated with resistance to multiple antimicrobial agents, including TMP, in *Klebsiella*, *Enterobacter*, and *Serratia* isolates (34).

Mycobacterium tuberculosis and other *Mycobacterium* spp. are resistant to TMP, but the mechanisms of resistance have not been studied (12, 16, 88). *Chlamydia trachomatis* is clinically resistant to TMP. Although TMP does not completely inhibit the growth of chlamydiae in vitro, chlamydial inclusion bodies are abnormally small and reduced in number (37). The antifolate agents appear to be ineffective in the treatment of enterococcal infections, despite the susceptibility in vitro to TMP and the combination of TMP-sulfonamide (20, 32, 36, 93). TMP in combination with sulfonamides is not bactericidal against enterococci, despite the inhibition of growth at low drug concentrations (61).

The reduction of dihydrofolic acid to tetrahydrofolic acid by DHFR in bacteria is necessary for the biosynthesis of several amino acids and nucleotides. Tetrahydrofolic acid is also needed as a cofactor in essential thymidylate synthesis. Thymine-requiring bacteria have lost their ability to synthesize thymidylate and can bypass the need for DHFR by using exogenous thymine or thymidine (8, 35). These strains are highly resistant to TMP (8). TMP also promotes thymine or thymidine uptake in thymine-dependent bacteria (8). Thymine-dependent bacteria occur only rarely among clinical pathogens but have been isolated from different clinical sources, including blood, during TMP-sulfonamide treatment (36, 48, 50). The presence of these strains should be suspected if after normal growth on isolation media no growth on thymine-deficient susceptibility test media is found (8).

Lactobacillus spp. have decreased susceptibility to TMP, whereas *Bacteroides* spp., *Clostridium* spp., *Neisseria* spp., *Branhamella catarrhalis*, and *Nocardia* spp. are TMP resistant because of TMP-insensitive DHFR (82, 83). *Clostridium* spp. have a permeability barrier as well (83).

The chromosomal DHFR gene of *E. coli* has been sequenced by Smith and Calvo (72). TMP resistance results from promoter mutations that lead to the overproduction of DHFR, with a ca. 10- to 20-fold increase in DHFR activity; structural mutations of the chromosomal DHFR gene lead to a lower affinity for TMP (73). A clinical isolate of *E. coli* with chromosomal DHFR activity more than 200-fold above the normal level has also been characterized (26, 27, 80). In addition to changes in the promoter sequence and in the ribosome-binding site (the Shine-Dalgarno sequence), an increased distance between the ribosome-binding site and the initiation codon was found. TMP resistance in this strain

TABLE 1. Bacterial TMP-resistant DHFR enzymes

Designation	Source	50% Inhibitory concn of TMP (μM)[a]	pI	Molecular mass (kDa)	Reference(s)
DHFR Ia	R483 (Tn*7*)	46–300	6.4	35.2 (2 × 17.6)	11, 29, 65, and 69
DHFR Ib	pUK163 (Tn*4132*)	32	ND[b]	24.5	4, 11, and 89
DHFR IIa	R67	20,000–70,000	5.5	33.6 (4 × 8.4)	11, 65, and 77
DHFR IIb	R388	80,000	5.5	33.2 (4 × 8.3)	11 and 94
DHFR IIc	R751 (Tn*402*)	20,000	7.2	34.0 (4 × 8.5)	11, 28, and 68
DHFR III	pAZ1	1.5–2.1	ND	16.9	30 and 46
DHFR IV	pUK1123	0.2	ND	46.7	90
DHFR V	pLM020	10	ND	ND	78 and 81
DHFR SI	pSK1 and pGO1	50	ND	19.7	9, 53, and 92
Chromosomal	*E. coli*	0.007–0.02	4.0–4.4	21.0	11, 65, and 72
Chromosomal	*S. aureus*	0.04	ND	21.6	92

[a] The data vary depending upon the purification and assay conditions used in different laboratories (11).
[b] ND, Not determined.

was also found to be inducible; a sixfold increase in enzyme production was found at a TMP concentration of 100 μg/ml relative to the enzyme activity in drug-free medium (81).

Several years after the use of the TMP-sulfonamide combination in the United Kingdom, transferable TMP resistance was identified in clinical isolates (21, 22, 25, 38). Two plasmids, R483 and R388 (Table 1), were found to produce TMP-resistant DHFR enzymes (7, 71). The production of these two plasmid-mediated DHFR enzymes caused high-level TMP resistance, with TMP MICs of greater than 1,000 μg/ml (21, 22).

PLASMID-MEDIATED TMP-RESISTANT DHFR ENZYMES

As of the spring of 1987, nine different TMP-resistant plasmid-encoded bacterial DHFR enzymes or their genes had been characterized (Table 1). The mechanism of resistance of all of these enzymes appears to be an alteration in the active site (7, 55, 56).

There are two different types of DHFR I: DHFR Ia is mediated by the 13.6-kilobase transposon Tn*7* (10, 29), which also confers resistance to streptomycin, and DHFR Ib is mediated by the 3-kilobase transposon Tn*4132*, which confers resistance only to TMP (89). These two enzymes have been classified in the same group because both are heat labile and because the concentrations of TMP that inhibit DHFR by 50% (50% inhibitory concentrations) are similar (Table 1) (4, 89). DHFR Ia is a dimeric protein with two 17.6-kilodalton (kDa) subunits (total, 35.2 kDa), and DHFR Ib is a monomeric protein with a molecular mass of 24.5 kDa. The nucleotide sequence of the DHFR Ia gene has been published (29, 69). TMP resistance mediated by type I class DHFR is the most prevalent mechanism in bacterial isolates collected from both hospitals and outpatients (15, 43, 44, 49, 58, 64, 66, 76). Although Tn*7* was first identified in plasmids (10, 36), it has subsequently been found integrated into bacterial chromosomes at a specific site (52); several reports suggest that a chromosomal location is an increasingly common finding in clinical isolates (6, 49).

Three different types of DHFR II enzyme have been characterized (Table 1). In each type the enzyme is a tetramer with four 8.3- to 8.5-kDa subunits (11, 75, 77, 79, 94). Recently, DHFR IIa coded by plasmid R67 was suggested to be a dimeric protein (56). Type II enzymes are highly resistant to TMP, the 50% inhibitory concentrations being more than 10^6 times higher than those for the native *E. coli* chromosomal DHFR (Table 1). Unlike type I DHFR enzymes, type II DHFR enzymes are heat stable. Type II DHFR enzymes are very similar. All contain a similar but not identical 78-amino-acid sequence. The plasmid R388-encoded DHFR enzyme differs in 11 amino acids from the plasmid R751-encoded DHFR enzyme; the R388 and R67 enzymes differ in 17 amino acids; and the R67 and R751 enzymes differ in 17 amino acids (28, 79, 94). Although the molecular masses of the DHFR Ia and DHFR II enzymes are similar (Table 1), neither amino acid nor nucleotide sequence homology exists. The epidemiology of DHFR II genes has been studied by several groups (30, 57, 58, 64). However, the DNA probes used have not been in every case specific for DHFR II gene detection (78). The DHFR Ia enzyme tends to be more common than the DHFR II enzymes. The two types may also coexist (15, 58, 64).

Type III DHFR has been reported in a single bacterial species (30), *Salmonella typhimurium*, which caused epidemics in cattle and sporadic human infections in New Zealand. The enzyme is a monomer with a molecular mass of 16.9 kDa and is relatively sensitive to TMP (46).

DHFR IV was identified in a clinical isolate from Southern India (90). The activity of this enzyme increases when induced by TMP to 600 times that of the chromosomal enzyme (90). DHFR IV has the largest molecular mass (46.7 kDa) of the plasmid-mediated enzymes.

DHFR V was recently found to predominate among TMP-resistant strains isolated in Sri Lanka (78, 81). The 50% inhibitory concentration for this enzyme is more than 1,000 times higher than that for the chromosomal enzyme.

DHFR SI was identified in TMP-resistant *Staphylococcus aureus* in Australia and the United States and is encoded by the related plasmids pSK1 and pGO1 (9, 53). The molecular mass of DHFR SI produced by pSK1 is 19.7 kDa, and the enzyme is believed to be a monomer (92). The genes encoding DHFR SI, DHFR Ia, and DHFR II do not hybridize with each other (9). A DHFR enzyme with properties similar to those of DHFR SI has also been isolated from *Staphylococcus epidermidis* strains (53).

High-level resistance to TMP (MIC, >1,000 μg/ml) is characteristic of plasmid-encoded enzymes mediated by type Ia and II DHFR genes (21, 22, 35, 38). Strains containing DHFR III and DHFR IV, however, are only moderately resistant to TMP, with MICs of 64 and 10 μg/ml, respectively (30, 90). In the future, it will be important to monitor for intermediate-level transferable TMP resistance.

ORIGIN OF DIFFERENT DHFR ENZYMES

Although the origin of plasmid-encoded DHFR enzymes is not clear, there is evidence that DHFR Ia and III are distantly related to bacterial chromosomal DHFR enzymes (29, 46, 69). Among the 20 amino-terminal amino acids of the DHFR Ia and III enzymes, 6 and 10 amino acids are similar to those of *E. coli* chromosomal DHFR, respectively (46, 69). It has been postulated that chromosomal DHFR enzymes and DHFR Ia have the same common ancestral DHFR gene (29) and that the DHFR III gene is a chromosomal gene of some unknown, intrinsically moderately TMP-resistant bacterium (46). The biochemical properties of DHFR Ib are very similar to those of DHFR Ia (89), and it has been postulated to be an active fragment of the DHFR Ia enzyme.

DHFR II enzymes lack homology with both bacterial chromosomal DHFR and mammalian DHFR enzymes (56, 77). Although differences in amino acid sequences between DHFR II enzymes exist, for the enzymes encoded by plasmids R388 and R751, amino acids 22 to 78 are identical, and the R67 enzyme differs in only 6 amino acids in this region (28, 77, 79, 94).

Studies of amino acid and nucleic acid sequences (29, 69), in vitro mutagenesis (87), and X-ray crystallography (55, 56) are expected in the future to generate additional information on the origin and evolution of bacterial DHFR genes.

SPREAD OF TMP RESISTANCE

The epidemiology of TMP resistance has been recently reviewed by Goldstein et al. (31). Plasmid-mediated TMP resistance is a significant problem in developing countries, owing to the widespread use of TMP, mostly in combination with sulfonamides (24, 58). Heavy gastrointestinal colonization of TMP-resistant bacteria during TMP or TMP-sulfonamide treatment has been shown to occur during travel for 2 weeks in Mexico (59, 60). Although TMP has also been widely used in Finland, gastrointestinal colonization of TMP-resistant strains was not found after 10 days of treatment of urinary tract infections with TMP or a TMP-sulfonamide combination (42). This difference might be explained by a more extensive, less restricted consumption of TMP in Mexico (42; S. Levy, Letter, N. Engl. J. Med. **307**:61, 1982).

In industrialized countries, such as the United Kingdom and Finland, TMP resistance tends to be a problem in hospitals, particularly in geriatric units (4, 41, 43). In a hospital study in Finland, TMP resistance was found in 38% of urinary tract isolates (*Pseudomonas* spp. excluded) collected from a geriatric hospital but in only 9 to 12% of strains collected from two university central hospitals mainly for acutely ill patients (41). In geriatric units there is a need for indwelling urinary tract catheters and long-term treatment of urinary tract infections, which usually are associated with resistance problems (23, 41, 43).

In outpatients, recent findings have shown different trends in the development of TMP resistance. Some studies indicate that resistance is falling; Maskell described a decrease from 15% in 1982 to 11% in 1984 in the TMP resistance of gram-negative urinary tract pathogens in the Portsmouth area of the United Kingdom (R. Maskell, Letter, Br. Med. J. **290**:156, 1985). In certain geographical areas, resistance has reached a plateau level; during the last 5 years TMP resistance among *E. coli* urinary tract isolates in the Turku and Rovaniemi areas in Finland has been registered at 10 to 12% and 4 to 6%, respectively (44; P. Huovinen and P. Toivanen, Letter, Lancet **ii**:1285–1286, 1986). Nevertheless, there are also areas where TMP resistance is still increasing; Hamilton-Miller and Purves described an increase from 6 to 19% in TMP resistance among *E. coli* urinary tract isolates from outpatients at the Royal Free Hospital in the United Kingdom from 1981 to 1985 (J. M. T. Hamilton-Miller and D. Purves, Letter, J. Antimicrob. Chemother. **18**:643–644, 1986). In the Helsinki area in Finland, an increase in TMP-resistant *E. coli* urinary tract isolates from 3% in 1980 to 15% in 1986 was documented (44; Huovinen and Toivanen, Letter, Lancet **ii**:1285–1286, 1986). Towner and Slack obtained similar findings for community isolates of *E. coli*, with TMP resistance increasing from 0.3% in 1978 to 11 to 14% in 1984 to 1985 (84).

Several investigators have suggested that TMP-resistant strains are spread from animals to humans (4, 19, 35, 85). Although the exchange of TMP-resistant bacterial strains occurs between animals and humans, the use of TMP for the treatment of human infections may be a more significant selection pressure than the use of TMP for animals.

In monitoring the spread of TMP resistance and comparing different studies, attention should be focused on the source of specimens (40) and methodology used (35, 91). Hospital and outpatient populations should be considered separately. In addition, the number of repeat samples and patients with complicated infections, such as patients with indwelling urinary tract catheters, should be indicated to allow meaningful comparison of different studies (40). Attention should be paid to methods and media used in the laboratory and to the criteria used to define resistance (35, 91).

ATTEMPTS TO SUPPRESS DEVELOPMENT AND SPREAD OF RESISTANCE

The use of TMP, either alone or in combination with sulfonamides, appears to be an important factor in the development and spread of TMP resistance (24, 43, 58, 70), particularly in a hospital setting (41, 43). In outpatients, however, a relationship between consumption and the development of resistance is not always evident (44).

When a TMP-sulfonamide combination was introduced in 1968, it was hoped that the combination would protect both components against the development of bacterial resistance (50). TMP resistance has nevertheless spread, particularly in developing countries where the drug combination has been in use (58).

In 1972, TMP and sulfonamide resistance determinants were both found to be carried by the plasmids R388 and R483 (22, 38). Furthermore, in plasmid R483, TMP resistance was located in transposon Tn*7*, which also confers resistance to streptomycin but not to sulfonamides. It seems likely that under the selection pressure caused by TMP alone instead of a TMP-sulfonamide combination, resistance mediated by determinants like Tn*7* appears to spread independently from sulfonamide resistance (84).

TMP in combination with rifampin has been successfully used, particularly in the treatment of staphylococcal infections (12, 33). In one study, however, TMP combined with rifampin failed to prevent the development of drug resistance in vitro (3). Combinations of TMP with other antimicrobial agents are effective when the total level of TMP resistance is low. When TMP-resistant bacteria are already present in a patient population, however, it is unlikely that the use of combinations will prevent the spread of TMP resistance.

Whether TMP alone promotes the development of resistance more than a TMP-sulfonamide combination in clinical settings is not clear. This question may remain unresolved because of the lack of an adequate scientific test method (35).

There is, however, one factor which can be easily monitored. Changes in the frequency of TMP-resistant, sulfonamide-susceptible pathogens should reflect the selection pressure caused by TMP alone (63, 84). One year after the introduction of TMP monotherapy in the United Kingdom in 1979, the occurrence of TMP-resistant, sulfonamide-susceptible enterobacterial strains was documented (84). By 1985, 35% of TMP-resistant strains were susceptible to sulfonamides.

With few exceptions, the use of antimicrobial agents leads to increased levels of bacterial resistance to the drugs used. Even combination with a second agent has not prevented the development of TMP resistance. Currently, there are no new useful candidates among the antifolate antimicrobial agents available that are effective in the treatment of infections caused by TMP-resistant bacteria. The best method for preventing the spread of resistance is the controlled use of TMP and other antimicrobial agents.

Despite the development and spread of resistance, TMP alone and in combination with a sulfonamide is useful in many parts of the world. It is important, however, to be aware of local patterns of resistance in hospitals and in communities and to follow guidelines for the use of TMP with these patterns in mind (45).

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