Trimethoprim enhances the antibacterial activity of nalidixic and oxolinic acids and delays the emergence of resistance

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Summary. Trimethoprim enhances the in vitro activity of nalidixic and oxolinic acids against some representative pathogenic microorganisms, including those which are most frequently responsible for urinary tract infections, and delays the emergence of resistance in many of them.

Nalidixic and oxolinic acids are widely employed in the treatment of urinary tract infections, because of their antibacterial spectrum and pharmacokinetic characteristics. But relapses and failures are frequent, mainly due to the emergence of bacterial resistance[1-4]: it was therefore suggested that nalidixic acid should always be used in combination with another antimicrobial drug[5]. However, relatively few reports about such combinations have appeared and they had no particular rationale[6-11].

There is general agreement that an antimicrobial combination will be effective if the combined drugs act on sequential steps in the pathway of an essential enzymatic reaction[12]. The bactericidal effect of nalidixic and oxolinic acids is mainly due to the inhibition of bacterial deoxyribonucleic acid synthesis[13-17]. On the other hand, trimethoprim selectively inhibits the bacterial dihydrofolatoreductases[18,19] thus blocking, among other things, the synthesis of purines and pyrimidines, that is of basic constituents of deoxyribonucleic acid. We therefore thought that the combination of nalidixic or oxolinic acids and trimethoprim would cause a typical sequential blockade in a vital metabolic pathway and thus produce a synergistic effect and delay the emergence of resistance. The study carried out along those lines is briefly described here.

Methods. Stock solutions of nalidixic (NA) and oxolinic (OxA) acids were made in 0.03 N NaOH and contained 1000 µg/ml. Final concentrations in broth or agar ranged from 0.1 to 512 µg/ml. Trimethoprim (T) was dissolved in broth and the final concentrations ranged from 1 to 100 µg/ml when used alone; in combination tests it was used at the fixed concentration of 1 µg/ml. The bacterial strains employed are listed in table 1 and were purchased from the Istituto Sieroterapico Milanese (Milan, Italy).

Minimal inhibitory concentrations (MIC) (the lowest concentrations that inhibited development of visible growth) were determined by the standard serial 2-fold dilution method 24 h after the inoculation of microorganisms (0.3 ml of a 12-h culture into 9.7 ml of liquid medium). Clarks-Lubs liquid medium [peptocomplex (Biolife, Milan) 5 g, glucose (Merck, Darmstadt) 5 g, phosphate buffer (pH 7.1) to 1000 ml] was used throughout and the cultures were incubated at 37 °C.

In the combination tests, the effect of a fixed concentration of trimethoprim (1 µg/ml) on the antibacterial activity of NA and OxA was evaluated by determining the FIC index[20].

The rate of development of resistance to NA and OxA, alone or in combination with a fixed concentration of trimethoprim (1 µg/ml), was determined in Clarks-Lubs solid medium (Clarks-Lubs liquid medium + agar 1.5%) by the subculture technique: the culture which grew in the highest drug concentration of serially diluted Clarks-Lubs agar plates was used as inoculum for the subsequent sensitivity determination. The bacterial strains employed are listed in table 2.

Results. As shown in table 1, trimethoprim, at the fixed concentration of 1 µg/ml, enhances the activity of NA and OxA against all the microorganisms tested: the FIC

Table 1. FIC indexes of the combinations of trimethoprim (T) and nalidixic (NA) or oxolinic (OxA) acids. The FIC index is the sum of the fractional inhibitory concentrations (FIC) of the 2 drugs, calculated by dividing the concentration of the drug in the minimum inhibitory concentration (MIC) of the combination by its MIC when acting alone (according to Elion et al.[20]). Clarks-Lubs liquid medium

Microorganism	Strain	FIC index NA+T	FIC index OxA+T
Enterobacter aerogenes	ISM 67/11	0.21	0.51
Escherichia coli	ISM 65/75	0.26	0.26
Escherichia coli	ATCC 13762	0.26	0.30
Klebsiella pneumoniae	ATCC 10031	0.52	0.41
Proteus morganii	ISM 66/68	0.30	0.41
Proteus vulgaris	ATCC 6380	0.52	0.51
Proteus vulgaris	ISM 66/24	0.22	0.21
Pseudomonas aeruginosa	ISM 72/1	0.11	0.51
Pseudomonas aeruginosa	ISM 630412	0.21	0.21
Salmonella typhi	ISM 67/25	0.12	0.30
Salmonella paratyphi A	ISM 68/18	0.51	0.51
Salmonella paratyphi B	ISM 66/19	0.22	0.12
Salmonella typhimurium	ISM 66/25	0.51	0.21
Shigella dysenteriae	ISM 66/15	0.27	0.90
Staphylococcus aureus	ISM 77/3	0.41	0.41
Streptococcus faecalis	ATCC 8043	0.51	0.41

Table 2. Development of resistance to nalidixic (NA) and oxolinic (OxA) acids, alone or in combination with trimethoprim (T) (at the fixed concentration of 1 µg/ml). Minimal inhibitory concentrations (MIC, µg/ml) of NA and OxA. Clarks-Lubs agar

Microorganism	MIC in the first test				MIC after 4–10 transfers (No. of transfers in parenthesis)			
	NA	NA+T	OxA	OxA+T	NA	NA+T	OxA	OxA+T
Escherichia coli ISM 65/77	–	–	2.5	0.25			250	10 (4)
Escherichia coli K12 ATCC 13762	8	1	–	–	128	2 (6)	–	–
Enterobacter aerogenes ISM 67/11	32	4	–	–	>512	16 (4)	–	–
Klebsiella pneumoniae ATCC 10031	4	0.5	–	–	>512	16 (10)	–	–
Proteus morganii ISM 0171	4	2	0.25	0.1	512	256 (4)	25	2.5 (10)
Proteus rettgeri ISM 02121	2	1	–	–	256	64 (9)	–	–
Pseudomonas aeruginosa ISM 72/1	–	–	50	25	–	–	>250	>250 (4)
Pseudomonas aeruginosa ISM 630412	–	–	25	5	–	–	>250	>250 (4)
Salmonella paratyphi A ISM 68/18	4	2	–	–	128	64 (4)	–	–

indexes are always below 1.0. On the other hand, the rate of emergence of resistance is not always reduced by the presence of trimethoprim (table 2).

Discussion. Our speculation, based on the mechanisms of action, that the combination of nalidixic or oxolinic acids and trimethoprim must lead to the strengthening of the antibacterial activity and delay the emergence of bacterial resistance, has been substantially confirmed by the experimental results. Bearing in mind the pharmacokinetics of these drugs[21-24], it seems to us that such combinations deserve clinical evaluation of their potential usefulness in the treatment of urinary tract infections.

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Renal polycystosis in the rat induced by prednisolone tertiary butyl acetate

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Summary. A single i.m. injection of 66 mg/kg prednisolone tertiary butyl acetate given on the 1st day of life produced glomerular degeneration and collecting duct and proximal tubule cysts in rat kidneys. There was evidence of delayed nephrogenesis leading to persistance of the neogenic zone.

Ignorance about the pathogenesis of human polycystic kidney disease has prompted a number of attempts to find suitable animal models[2]. Prednisolone tertiary butyl acetate (PTBA) has been shown to produce collecting duct cysts in rabbits[3] and collecting duct and distal tubular cysts in rats[4]. We report here histological studies of its effects on the development of the newborn rat kidney which extend the previous observations.

Materials and methods. 40 litters of Sprague-Dawley derived rats were used. Each litter was culled to 10 animals on the day of birth (=day 1) and 7 were given an i.m. injection of Codelcortone TBA (Merck, Sharp, and Dohme) equivalent to 66 mg PTBA/kg b.w. The 3 remaining animals in each litter received an equivalent volume of isotonic saline.

Animals were killed with chloroform on days 4, 8, 10, 12, 14 and 16 and the kidneys were either fixed in Bouin's fluid for paraffin processing, or in 10% phosphate buffered formalin for embedding in methyl methacrylate resin[5]. Paraffin sections were cut at 7 µm and stained with Masson's trichrome, Harris' haematoxylin and eosin, or PAS. Methacrylate sections were cut at 2µ and stained with Delafield's haematoxylin and phloxine or PAS.

Results. The parts of the kidney most affected by the treatment were the glomeruli, the proximal tubules, and the collecting ducts. The terminal parts and ampullary regions of some collecting ducts were found to be slightly dilated at day 4 and dilation was more obvious at older ages (figure 1). From day 8 onwards variable numbers of medullary collecting ducts also had become cystic.

Signs of glomerular degeneration were observed on day 4 but this was more obvious from day 8 onwards. The glomerular tufts of some Malpighian bodies were degenerating and Bowman's space slightly or extensively dilated (figure 2). The glomeruli effected were at the periphery, the juxtamedullary ones were normal.

Occasional proximal tubules were mildly cystic in the outer cortex at days 4 and 8 but dilatation was more obvious at later stages (figure 2). From day 12 the cells in the walls of these cysts often became vacuolated and small spheres of cytoplasm or whole cells were seen being shed into the lumen. The PAS technique revealed sloughing-off the brush border and apices of the cells into the lumen of the larger cysts, all this material apparently contributing to PAS-positive casts found lower down in the collecting ducts. At days 14 and 16 there was often evidence of marked fibrosis around the cysts. None of the collecting duct or proximal tubule cysts exhibited obvious hyperplasia of their wall. The neogenic zone in control animals had disappeared by day 8 but it persisted in patches in many PTBA treated kidneys until day 16.

Discussion. An earlier study[3] of the effects of PTBA on newborn rats concluded that in the first 8 days post-partum renal cysts were distal tubular or collecting duct in origin and that the glomeruli were not affected. We have observed evidence of glomerular degeneration as early as day 4 and this was very marked at older ages. In addition proximal tubule dilatation was seen from day 8 onwards. It appears from our studies that the first parts of the kidney to be affected by PTBA are selected ampullary regions of the