Antibacterial activity of nifuratel in urine and serum

J. McGEACHIE, G. ROBINSON, AND D. BLACK

From the University Department of Bacteriology, Glasgow Royal Infirmary, Glasgow

SYNOPSIS The minimum inhibitory concentrations of nifuratel for 205 randomly selected isolates from urinary tract infections were tested by tube dilution. Of these, 177 (86.3%) were resistant to more than 6 $\mu\text{g/ml}$ and 140 of 141 (99.3%) strains of *Escherichia coli* were resistant to more than 3 $\mu\text{g/ml}$. Urine levels of nifuratel were examined in two groups: one group had 400 mg given once and the other group had 2 g given over 24 hours. In both groups samples of urine were collected every hour for seven hours after the last dose. After one 400-mg dose the maximum urine level achieved by any subject was 2.0 $\mu\text{g/ml}$ and the mean maximum level was 0.75 $\mu\text{g/ml}$. With the 2 g total dose, the maximum level noted was 4 $\mu\text{g/ml}$ and the mean maximum level was 1.8 $\mu\text{g/ml}$. No measurable inhibition was noted in any of the blood samples removed at one and a half to two hours after the last dose.

Nifuratel (Magma) is a furan derivative, the chemical description of which is N-(5-nitro-2-furylidene)-3-amino-5-methylmercaptomethyl-2-oxadolidinone. It has been used mainly in the treatment of vulvo-vaginal infections, but has also been recommended for the treatment of urinary infections (Coppi and Bertagnoli, 1965; Tynan, Macis, and Ward-McQuaid, 1969). In view of the unfortunate controversy over another 5-nitrofuran, namely, Furaldone, it was considered essential to find if the oral administration could achieve significant inhibitory levels in the urine. The minimum inhibitory concentrations of nifuratel for a sample of strains isolated from proven cases of urinary tract infections would be used to indicate the significance of the level in the urine.

Materials and Methods

ORGANISMS

The minimum inhibitory concentration (MIC) of nifuratel was tested with 205 strains which were isolated from patients with pyuria and bacteriuria. There were 141 strains of *Escherichia coli*, 29 strains of *Proteus mirabilis*, 20 strains of *Klebsiella/Aerobacter* species, seven of coagulase-negative *Staphylococcus albus*, four of *Streptococcus faecalis*, and two strains each of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

NIFURATEL SOLUTIONS

The stock concentration of nifuratel was prepared

Received for publication 21 October 1971.

by dissolving 0.1 g in 10 ml dimethylformamide (Analar); this was kept in the dark and at 4°C for not more than five days then discarded. The working standards were prepared daily by diluting with quarter-strength Ringer's solution (Oxoid) to a concentration of 50 $\mu\text{g/ml}$. The tube sensitivities for the MIC used the strongest concentration of 25 $\mu\text{g/ml}$ with doubling dilutions to 0.1 $\mu\text{g/ml}$.

ASSAY OF NIFURATEL IN URINE

Two groups were studied. In the first group there were 11 normal male subjects each of whom received 400 mg of nifuratel once after breakfast. In the second group of eight normal male subjects, each received 400 mg at four-hourly intervals on the first day followed by 400 mg after breakfast on the second day, a total of 2 g nifuratel in 24 hours.

In the first group a specimen of urine was taken before administration of the drug to act as a baseline. In both groups urine was collected every hour for seven hours after the administration of the last dose of the drug. The fluid intake in both groups was unrestricted. In subjects in the first group, blood samples were removed for assay before and one and a half hours after ingestion of the drug. The assays were performed on the serum by tube dilution.

As a control of the methods, a similar series of assays was made following a single dose of 250 mg ampicillin.

METHOD OF ASSAY

In separate series of experiments it had been found that Seitz filtration of nifuratel solution in urine

resulted in a significant decrease in measurable inhibition of the indicator organism. On the other hand exposure to 60°C in a water bath up to one hour did not affect the potency significantly. As a routine all specimens of urine were treated by exposure to 60°C for 30 minutes to sterilize them. Doubling dilutions of urine in quarter-strength Ringer's solution (Oxoid) were made from a neat aliquot in the first tube to a dilution of 256. The indicator organism was a coagulase-negative *Staphylococcus albus* with a MIC of 0.5 µg/ml; one drop of 1:100 dilution of an overnight culture was added to each tube. Sterility controls of the urine both before and after sterilization were included. As an indication of the bactericidal effect, the last three tubes to show

inhibition of the indicator organisms were sub-cultured on to nutrient agar.

Results

SENSITIVITY OF URINARY ISOLATES TO NIFURATEL

The MIC of the 205 strains are shown in Table I and Figure 1. The strains of *Escherichia coli* were all resistant to 1.5 µg/ml; indeed 140 of the 141 (99.3%) were resistant to 3 µg/ml or more. Only the strains of coagulase-negative *Staphylococcus albus* showed a marked sensitivity to nifuratel; five of the seven isolates were sensitive to less than 3 µg/ml. The collective MIC of all the strains is shown in Fig. 1; 177 of the 205 strains (86.3%) were resistant to more than 6 µg/ml.

CONCENTRATIONS OF NIFURATEL IN THE URINE

After one dose of 400 mg

The maximum concentration found was 2.0 µg/ml, which occurred in three subjects within the first three hours (Table II). The maximum mean concentration was 0.75 µg/ml which occurred at approximately two hours after the start of the experiment (Figure 2).

Strain	MIC (µg/ml)						
	25	25	12	6	3	1.5	0.75
<i>Escherichia coli</i>	42	3	75	20	1	—	141
<i>Proteus mirabilis</i>	24	1	4	—	—	—	29
<i>Klebsiella Aerobacterspp.</i>	16	2	2	—	—	—	20
<i>Strep. faecalis</i>	2	1	1	—	—	—	4
<i>Staph. albus</i>	—	—	1	1	1	3	7
<i>Staph. aureus</i>	—	1	—	—	1	—	2
<i>Ps. aeruginosa</i>	2	—	—	—	—	—	2
Total	86	8	83	21	3	3	205

Table I MIC of 205 strains

Fig. 1 Minimum inhibitory concentration of nifuratel in 205 isolates.

Hours after Last Dose	400 mg		2 g	
	Range (µg/ml)	Mean	Range (µg/ml)	Mean
1	0.0-2.0	0.50	0.5-4.0	1.80
2	0.0-2.0	0.75	0.0-4.0	1.80
3	0.0-2.0	0.40	0.5-2.0	1.10
4	0.0-1.0	0.30	0.5-2.0	0.80
5	0.1-1.0	0.10	0.0-2.0	0.50
6	0.0-0.5	0.20	0.0-1.0	0.30
7	0.0-1.0	0.40	0.0-2.0	0.40

Table II Concentrations of nifuratel in urine

Fig. 2 Concentration of nifuratel in urine.

After 2 g in 24 hours

The maximum concentration was found to be 4 µg/ml which occurred in two subjects within the first two hours after the last dose. The maximum mean concentration was 1.8 µg/ml at one to two hours after the last dose.

Bacteriostatic and bactericidal levels

In those specimens which showed inhibition, approximately 70% showed a difference of one tube dilution between the bacteriostatic concentration and that giving a bactericidal effect; the rest had identical activity.

Comparison of the concentrations of nifuratel and ampicillin in the urine

The levels of nifuratel and ampicillin are shown in Table III. The comparisons were made over a six-hour period.

Hours after Last Dose	400 mg Nifuratel	250 mg Ampicillin
1	0.50	76.8
2	0.75	307.2
3	0.40	307.2
4	0.30	153.6
5	0.10	76.8
6	0.20	38.4

Table III Comparison of the concentrations (µg/ml) of nifuratel and ampicillin in urine

Blood levels of nifuratel

Only one postadministration assay on blood samples was prepared from each subject. None of the specimens removed between one and a half and two hours after ingestion of 400 mg nifuratel revealed any inhibition of the indicator. A similar assay of the blood from the subject on ampicillin gave an inhibition level of 2.4 µg/ml serum.

Discussion

Nitrofurans have been used for the treatment of urinary tract infections for nearly two decades, since Sweetser and Harrison (1951) used a dilute solution

of nitrofurazone to irrigate the bladder. Nitrofurantoin has achieved a greater impact on the treatment of urinary infections and has been widely used since the reports of Norfleet, Beamer, and Carpenter (1952) and of Mintzer, Kadison, Shlaes, and Felsenfeld (1953). Another 5-nitrofurans which was considered to be of use in several clinical situations was furaltadone. However, subsequent clinical and laboratory evaluation of the compound revealed it to be of doubtful efficacy (McCarthy and Finland, 1961; *Lancet*, 1961).

In our study of the effect *in vitro* of nifuratel on urinary pathogens we have found that most had an MIC of more than 3 µg/ml as measured by tube culture inhibition. This level of nifuratel was not obtained by any of the subjects who received a single dose of 400 mg nifuratel. The mean maximum level was less than 1 µg/ml, and only three subjects achieved a level of 2.0 µg/ml. After 2 g nifuratel in a 24-hour period the maximum level produced by two subjects was 4 µg/ml but the mean maximum level was below 2 µg/ml (1.8 µg/ml).

In none of the subjects was a measurable level of inhibition achieved in serum. A comparable assay performed on the serum from the subject who had received 250 mg of ampicillin gave a level of 2.4 µg/ml.

We wish to thank Messrs Calmic Ltd, Crewe, for the supply of nifuratel (Magmilor) as tablets and as powder for standard controls. We are also indebted to Mr T. Parker for the photographs.

References

- Coppi, F., and Bertagnolli, V. (1965). Esperienze cliniche in urologia con il metilmercaptopurina chemioterapico furanico. *Urologia (Treviso)*, 32, 678-683.
- Lancet* (1961). Annotation. Furaltadone. *Lancet*, 2, 357.
- McCarthy, C. G., and Finland, M. (1961). Furaltadone: antibacterial activity *in vitro* and in serum of patients during treatment. *Arch. intern. Med.*, 107, 863-871.
- Mintzer, S., Kadison, E. R., Shlaes, W. H., and Felsenfeld, O. (1953). Treatment of urinary tract infection with a new antibacterial nitrofurans. *Antibiot. and Chemother.*, 3, 151-157.
- Norfleet, C. M., Jr., Beamer, P. R., and Carpenter, H. M. (1952). Furalant in infections of the genito-urinary tract. *Trans. S.-east Sect. Amer. urol. Ass.*, 26-34.
- Sweetser, T. H., Jr., and Harrison, C. H. Jr. (1951). Nitrofurazone in the treatment of urethro-trigonitis. *J. Urol.*, 65, 684-687.
- Tynan, A. P., Macis, F. R., and Ward-McQuaid, J. N. (1969). Nifuratel in urinary infections. *Brit. J. Urol.*, 41, 271-279.