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Notes

Effects of tetracycline on the 2 h urinary and salivary excretion of metronidazole

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Summary

The effect of co-administration of tetracycline on the 2 h urinary and salivary levels of metronidazole (MZ) and its acetic acid metabolite (MAM) has been investigated. A modified high-performance liquid chromatography method has been employed for the determination of both metronidazole and its acetic acid metabolite content. Tetracycline was found to have effects on the 2 h levels of the drug in urine and saliva. Similar results were observed for the metabolite content in urine.

Metronidazole is the drug of choice for treatment of common endemic protozoal diseases in Sudan, e.g., giardiasis and amoebic dysentry (Gordeeva, 1965). The drug is misused, often being taken for the treatment of gastrointestinal disorders, involving diarrhea, without proper diagnosis. Moreover, it is often prescribed in conjunction with other drugs, e.g., tetracycline, for treating diarrhea of unknown etiology, which is quite common in Sudan.

The effects of tetracycline on metronidazole plasma level and metabolism have thus far not been properly investigated. It is therefore not yet clear whether this drug interacts with or affects the absorption and/or excretion of metronidazole. If interaction does occur, the possibility of changes in the antiprotozoal activity of the drug cannot be ruled out.

In this study the co-administration effects of tetracycline on the 2 h urinary and salivary levels of metronidazole and its metabolite have been studied. 10 healthy volunteers (male and female university students) who did not take any treatment prior to the experiment consented to take part in this study. The volunteers were divided into two groups (I and II), each of five subjects. MZ (400 mg) and MZ (400 mg) plus tetracycline (500 mg) were administered with 150 ml of water to groups I and II, respectively. Urine samples (7–10 ml) and saliva samples (5 ml) were collected from each volunteer prior to and 2 h after administrations. The samples were frozen until analysis. The content of creatinine per ml of urine was determined for each urine sample. The content of metronidazole and its acetic acid

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metabolite in each sample was measured using a modified HPLC technique (Rona and Bela, 1987). The system used comprised an LDC/Milton Roy (U.K.) instrument equipped with a UV detector fixed at wavelength 318 nm, and a C_{18} column (250 × 4.5 mm). The mobile phase used consisted of a 45% methanol/phosphate buffer (0.01 M, pH 5.0 ± 0.5) with tetrabutylammonium iodide (0.0225 M) as an ion-pairing agent. The ion-pairing agent has the advantage of shifting the polar metabolite from the solvent front, thus avoiding interference from other materials which appear in this area.

Calibration curves for both MZ and MAM in both blank urine and saliva were constructed using 1-(2-chloroethyl)-2-methyl-5-nitroimidazole as an internal standard. This compound was synthesized by reacting MZ with thionyl chloride. The curves obtained were linear over the concentration ranges used giving the following results:

MZ in urine $y = 0.564x + 0.037$, $R = 0.9999$

MZ in saliva $y = 1.6x + 0.02$, $R = 0.9981$

MAM in urine $y = 0.194x + 0.065$, $R = 0.9989$

MAM in saliva $y = 0.702x + 0.028$, $R = 0.9999$

Analysis of urine and saliva samples by the adopted method gave good results, as shown in Figs 1 and 2.

Using this method, the contents of MZ and MAM in urine of volunteers of group I, 2 h after taking the drug (400 mg orally) alone, were found to be 1.83(±1.05) and 2.64(±1.10) μg/mg creatinine, respectively (Table 1). The concentrations of MZ and MAM in group II volunteers given a dose of tetracycline (500 mg) together with MZ (400 mg) were found to be 6.46(±2.53) and 15.11(±7.98) μg/mg creatinine, respectively. It is evident from these results that the concomitant intake of tetracycline with MZ (Table 1) resulted in a 3.5-fold increase in the 2 h urinary level of MZ (statistically significant, $t_{calc.} = 4.1935$, $t_{table} = 2.7764$, $P = 0.05$). There was also a 5.7-fold increase in the level of the metabolite (MAM) (statistically significant, $t_{calc.} = 3.7148$, $t_{table} = 2.7764$, $P = 0.05$). The results obtained indicate that some interaction has taken place. The higher urinary content of MZ could be attributed to greater absorption and/or excretion of the drug in the presence of tetracycline. It could also be due to complexation between the drug and tetracycline through hydrogen bonding which could affect tubular reabsorption of MZ. On the other hand, the increase in the level of excreted MAM could be explained by increased metabolism of MZ to MAM or increased excretion of the latter in the presence of tetracycline (normal ratio of MAM/MZ 1.6 vs 2.7 when tetracycline was co-administered).

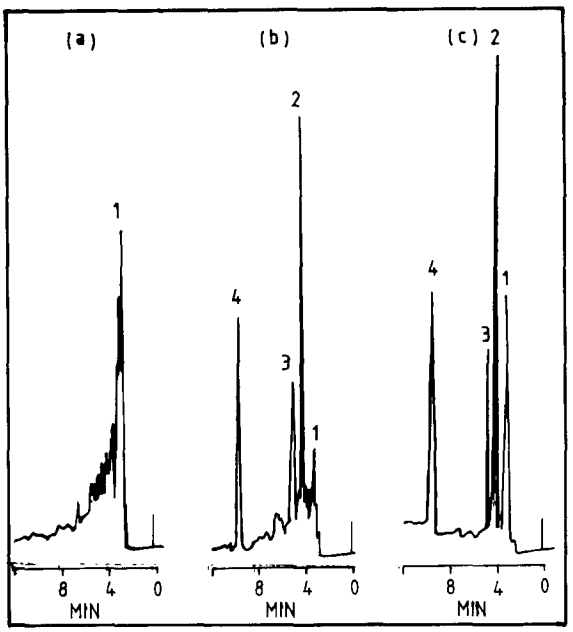

Fig. 1. HPLC chromatograms obtained from urine samples. (A) Blank urine containing no nitroimidazoles; (b) blank urine spiked with MZ, MAM (each 3.75 μg/ml) and internal standard (3.75 μg/ml); (c) volunteer urine obtained 2 h after intake of MZ tablets (400 mg). (1) Solvent front, (2) MZ, (3) MAM, (4) internal standard.

2 h after the administration of MZ (400 mg) to volunteers the salivary level of the drug was found to be 5.70 (±2.39) μg/ml. MAM could not be detected in saliva. The absence of the metabolite in saliva could either be due to the fact that it cannot be excreted in saliva (Kaye et al., 1980) or that it is present in amounts below the lower

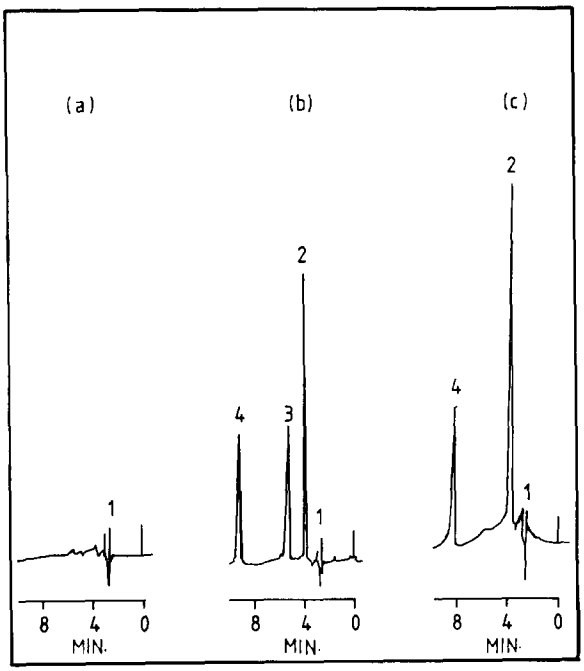

Fig. 2. HPLC chromatograms obtained from saliva samples. (a) Blank saliva containing no nitroimidazoles; (b) blank saliva spiked with MZ, MAM (each 5.0 μg/ml) and internal standard (3.75 μg/ml); (c) volunteer saliva obtained 2 h after intake of MZ tablets (400 mg). (1) Solvent front, (2) MZ, (3) MAM, (4) internal standard.

limits of detection by the adopted HPLC method (5 ng/ml). The results obtained for group II (concomitant intake of tetracycline, 500 mg, and MZ, 400 mg) show an increase of 1.7-fold in the content of salivary MZ (statistically significant, $t_{calc.} = 6.5357$, $t_{table} = 2.7764$, $P = 0.05$). The result parallels that observed in urine and could thus strengthen the idea of an interaction between tetracycline and MZ.

TABLE 1

2 h urinary content (μg / mg creatinine) of MZ and MAM and salivary content (μg per ml) of MZ in volunteers of group I (MZ alone) and group II (MZ plus tetracycline)

Group	Urinary content		Salivary content	
	MZ	MAM	MZ	MAM
I	1.83 ± 1.05	2.64 ± 1.10	5.70 ± 2.39	–
II	6.46 ± 2.53	15.11 ± 7.97	9.77 ± 0.80	–

The results of the present work indicate quite clearly that possible interactions are likely when MZ is taken with tetracycline, a quite common practice in Sudan.

Caution should therefore be exercised when this occurs as it might affect the activity of MZ as an antiprotozoal drug. A more comprehensive investigation of the problem is still required. This should involve proper randomised pharmacokinetic studies of the interactions. Such studies are now being carried out in the authors' laboratories.

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