indole-*O*-methyl transferase activity (Ritta & Cardinali 1980), and since noradrenaline released PGs from bovine pineal explants (Cardinali et al 1979), it was suggested by Cardinali et al (1982) that the PGs may be involved in the pre- and/or postsynaptic events in the pineal gland, leading to MT synthesis. It was also suggested that the structural similarities between MT and indomethacin may interfere with pineal binding of MT. However, ibuprofen does not structurally resemble MT and therefore the latter suggestion is not a probable explanation. The fact that, in man, both ibuprofen and indomethacin administration produce a reduction in plasma MT concentration supports the theory that the PGs may be involved in the biochemical events leading to human MT synthesis.

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Metabolic *N*-oxidation of metronidazole

E. E. Essien, J. I. Ogonor, H. A. B. Coker*, M. M. Bamisile, *Department of Pharmaceutical Chemistry, School of Pharmacy, College of Medicine, University of Lagos, Lagos, Nigeria*

Metronidazole when treated at the *N*-3 nitrogen with a mixture of hydrogen peroxide and acetic acid, or liver homogenate preparations, yields the *N*-3 oxide as identified by thin-layer chromatographic analysis on silica gel G, R_F 0·62 in ethanol–chloroform–ammonia (50 : 49 : 1), by chemical reduction with sulphur dioxide, and by ultra-violet spectrophotometry and nuclear magnetic resonance spectroscopy. Incubation of metronidazole at 37 °C with rat liver 10 000*g* supernatant fortified with cofactors gave a product with identical chromatographic and UV spectral data suggesting that metronidazole like other tertiary amine drugs undergoes microsomal *N*-oxidation.

The exact mechanism of the anti-protozoal action of metronidazole is not clear. Apparently the drug's biological activities are related to the reduction of its nitro group. Mutagenic activity towards a variety of bacteria has been reported by Rosenkranz & Speck (1975) who also showed that the mutagenicity was detectable in a strain of the Ames histidine auxotrophs of *S. typhimurium* that lacked nitroreductase activity. Similarly a rare strain of *Bacteroide fragilis,* relatively resistant to metronidazole, was found to have greatly diminished nitroreductase activity (Tally et al 1979).

The nature of the metabolites remains a matter of controversy. Either of the two side chains can be oxidized in the liver to give methyl alcohol or 'acid' metabolites (Connor et al 1977), but only the alcohol metabolite was detected in the serum of a subject with normal renal function (Wheela et al 1978). The alcohol metabolite has also been implicated as a potent mutagen (Connor et al 1977).

Some tertiary amines have been shown to be metabolized via *N*-oxidation (Beckett et al 1971; Dagne & Castagnoli 1972). Metronidazole, with two tertiary nitrogen centres, has the possibility of *N*-oxidation by microsomal enzymes so this has been investigated.

* Correspondence.

Materials and methods

Metronidazole was a gift from May and Baker Nig. Ltd. Hydrogen peroxide, acetic acid, chloroform, ethanol and methanol were all of analytical reagent grade (BDH). Nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide, glucose-6-phosphate (G-6-P), potassium dihydrogen orthophosphate, sodium hydroxide and magnesium chloride were all reagents from BDH and were used as received. Dragendorff's reagent was freshly prepared, according to the BP.

White Sprague-Dawley rats were obtained from the animal house of the College of Medicine, University of Lagos and extracts of liver, homogenized in an Ultra Turrax homogenizer, were made isotonic with potassium chloride (1·15%) solution and centrifuged at 10 000*g* using a Sor Vall ultracentrifuge (RC2B model) for 30 min and the supernatant used.

Synthesis of N-oxide derivative of metronidazole. About 1 g of pure metronidazole was dissolved in 50 mL of ethanol and a 1 : 1 v/v mixture of glacial acetic acid and hydrogen peroxide 30% (60 mL) added. The mixture was kept in the dark for 24 h at room temperature (25 °C) and stirred magnetically. After treatment with manganese dioxide and filtration, the resulting yellow solution was concentrated under vacuum at room temperature and taken up in saturated aqueous Na_2CO_3 solution then extracted with 5 × 20 mL of chloroform–ethanol (80 : 20) v/v mixture. The concentrate chromatographed on silica gel G254, with ethanol–chloroform–ammonia (50 : 49 : 1) v/v as solvent gave the *N*-oxide as a yellowish white crystalline solid recrystallized from methanol–chloroform mixture (m.p. 140–145 °C uncorrected). Table 1 gives NMR details.

Biological Studies. To 2 mL of the ice-cold live supernatant was added 1 mL of cofactor solution [nicotinamide (0·6 M) 1 mL, magnesium chloride (0·01 M solution) 2 mL, glucose-6-phosphate 60 mg, nicotinamide adenine dinucleotide phosphate 34 mg, water to 10 mL and 2 mL phosphate buffer (pH 7·4)] together with 1 mg metronidazole in a 25 mL conical flask. This was replicated 15 times and divided into two sets which were incubated in a rocking water bath at 37 °C for 30 and 120 min, respectively, after which they were immediately placed in an ice-cooled bath. The contents were then extracted with chloroform–ethanol, concentrated and chromatographed using the TLC system described. Metronidazole *N*-oxide was obtained by preparative TLC and characterized using UV and NMR procedures.

Results and discussion

TLC examination of the products of peracid oxidation revealed the presence of one major spot at R_F 0·62 which, on purification, gave the *N*-oxide. The UV spectrum in ethanol (95%) showed a bathochromic shift, λ_{max} 340 nm relative to metronidazole, λ_{max} 310 nm. The NMR spectra of the drug and *N*-oxide were similar suggesting the most probable point of oxidation as the *N*-3 centre which is more basic than the *N*-1 centre and also suffers less from steric hindrance. To establish further the authenticity of the *N*-oxide, it was reduced chemically to metronidazole with sulphur dioxide (Becket et al 1972, 1975).

Table 1. NMR spectra of metronidazole and metronidazole-*N*-oxide.

Metronidazole	δ = 2·45 ppm (3H, 5, $-CH_3$); δ = 3·78 (2H, t, $N-CH_2$); δ = 4·40 (2H, t, $O-CH_2$); δ = 7·90 (1H, s, C-H).
N-oxide	δ = 2·50 ppm (3H, s, $-CH_3$); δ = 3·80 (2H, t, $N-CH_2$); δ = 4·40 (2H, t, $O-CH_2$); δ = 7·99 (1H, s, C-H).

Solvent—DMSO-d_6, internal standard-TMS.

UV spectra of metronidazole, metronidazole *N*-oxide and reduced *N*-oxide (solvent-methanol). Metronidazole (λ_{max} = 310 nm); synthesized metronidazole *N*-oxide (λ_{max} = 340 nm); metabolic *N*-oxide (λ_{max} = 340 nm); reduced *N*-oxide (max = 310 nm).

The UV absorption spectra of the synthesized and metabolically produced *N*-oxides were identical and both products underwent the same chemical reduction. The principles involved in the metabolic *N*-oxidation of tertiary amine drugs have been fully described by Beckett & Belanger (1974). From the results it seems reasonable to infer that when administered to man, metronidazole may be *N*-oxidized.

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