CHEMICAL AND METABOLIC N – OXIDATION OF MEFLOQUINE

BY

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ABSTRACT

Mefloquine has been shown to undergo N-oxidation during treatment with a mixture of hydrogen peroxide and acetic acid, or liver microsomal homogenate preparations as identified ny thin layer chromatographic analysis on silica gel G(R_f0.6) in methanol – dichloromethane – ammonia – acetonitrile (40:40:20:10:v/v), by chemical reduction with sulphur dioxide; ultraviolet spectroscopy and by high performance liquid chromatography (Rt 2.2 min) using Pye Unicam apparatus equipped with UV detector at 280 nm, column C-18 silica reverse phase (25cm x i.d. 5mm); mobile phase, 0.01M NaH_2PO : methanol (55:45) pH 8 and flow rate was 2ml/min. *In vitro* metabolic N-oxidation was achieved by incubating parent drug, mefloquine, with rat liver homogenates fortified with cofactors at 37°C. The identical physicochemical characteristics of synthetic and biologically produced mefloquine N-oxide strongly suggests that mefloquine undergoes metabolic N-oxidation.

INTRODUCTION

Mefloquine, DL-erythro-2,8-bis (trifluoromethyl)-(2-piperdyl)-4-quinolinemethanol, is a recently introduced antimalaria, developed by the United States Army Antimalarial Drug Programme in response to the proliferation of multi drug-resistant strains of plasmodium falciparum particularly in the tropics (Schwartz et al, 1980). When administered in a single oral dose of 1.5g, it was found to be radically curative in all experimetally induced infections with a strain of plasmodium falciparum resistant to chloroquine, amodiaquine, quinine and pyrimethamine (Trenholme et al, 1975). Mefloquine also provided, suppressive prophylaxis against mosquito-induced infections with plasmodium vivax and plasmodium falciparum.

The exact mechanism of action of mefloquine has not been fully elucidated to date. However, it has been shown to lack the DNA – binding properties of other quinoline and acridine antimalarial drugs (Davidson et al, 1977). Earlier studies have indicated high tissue concentrations of mefloquine, particularly theliver and lungs, for quite some lengths of periods. Of the several metabolites of mefloquine formed, two have been identified as the 2,8-bis-trifluoromethyl-quinoline-4-me thanol and 2,8-bis-trifluoromethyly quinoline-4-carboxylic acid (Jauch et al, 1980).

Mefloquine in its structure has two nitrogen centres, the quinoline tertiary nitrogen centre and the piperidyl secondary amine. The possibility of N-oxidation by microsomal enzymes (in vitro), which hitherto has not been reported, has been investigated.

EXPERIMENTAL

Materials

Mefloquine was a generous gift from Roche Nig. Ltd., Lagos; glacial acetic acid, chloroform, ethanol, methanol were al analytical reagent grade and products of Britich Drug Houses (BDH), England. Nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide, glucose-6-phosphate (G-6-P), potassium dihydrogen orthophosphate, sodium hydroxide, magnessium chloride were all reagents from British Drug Houses and were used as such without further purification. White rats (albino) were obtained from the animal house of the College of Medicine, University of Lagos. Homogenisation was done with an Ultra Turrax homogeniser and ultracentrifugation was performed on a Sorvall RC2B model at 10,000 x g. Ultraviolet absorption spectra were obtained on a Pye Unicam SP3-800 spectrophotometer. High Performance Liquid Chromatography (HPLC) analyses were carried out on a Pye Unicam PY 4010 Pump, PU 4020 U.V. detector (max 280nm) attached to a single pen recorder pm 8251. Column was a C18 silica bonded reverse phase unit 25cm (i.e. 5mm). Mobile phase was made up of 0.01M NaH_2PO_4: Me OH (55:45 v/v), pH 8. Thin Layer Chromatography (tlc) was done on a silica gel G254 plate (0.5mm thick), solvent system was methanol – dichloromethane – Ammonia (25&) – Acetonitrile (40:40:20:10 v/v). Spots were detected by UV light and Drangendorf reagent.

Preparation of Mefloquine N-oxides

A mixture of mefloquine solution in methanol (0.5g/10ml), glacial acetic acid (10ml) and hydrogen peroxide (30%H_2O_2, 10ml) was kept in the dark and stirred magnetically for about 12 hours. Excess hydrogn peroxide was decomposed by the addition of manganese dioxide (250mg) and mixture shaken for about 10 minutes. The reaction mixture was filtered (Whatman filter paper, No.3) and the filtrate

concentrated in vacuo by means of rotary evaporator. The concentrate was taken up in a dilute solution of aqueous Na_2CO_3 and extracted into chloroform/ethanol mixture (80:20 v/v). The extract was analysed by means of thin layer chromatography (tlc); S.G. G_{254}, methanol :dichloro-methane : ammonia : acetonitrile (40:40:20:10 v/v) and by HPLC, C-18 reverse phase bonded silica (i.d. 5mm); mobile phase 0.01M NaH_{PO}: methanol (1:1 v/v) pH 8, flow rate was 2ml/min and detection was by U.V. at 280nm wavelength. The internal standard used was tinidazole. The ethanol solution of the N-oxide products, obtained by preparative tlc, was treated with sulphur dioxide (generated by the addition of conc. HCl on $Na_2S_2O_5$) and the chromatograms (tlc, hplc) of the resulting products recorded.

IN VITRO STUDY

Liver microsomal preparation were made according to the following procedure : to 2ml of ice-cold liver homogenate (10,000g supernatant) was added 1ml solution of substrate (1ml/ml), 2ml phosphate buffer (pH 7.4), 1ml of cofactor solution – nicotinamide (0.6M solution, 1ml), magnesium chloride (0.01M solution, 2ml), glucose-6-phosphate 60mg, NADP 30mg, water to a total volume of 10ml – in a 25ml conical flask. This was replicated 6 times and divided into two sets of 3 conical flasks each. These were incubated in a rocking water bath at 37°C for 30 and 60 min respectively, after which flaskes were immersed in ice-cold water to terminate reaction. The cofactor solution was made according to the method of Essien et al (1987), and this consisted of a mixture of nicotinamide (0.5M solution, 1ml), magnesium chloride (0.01M solution, 2ml) glucose-6-phosphate 50mg, NADP 25mg, water to a total volume of 10ml. The contents of the flask were then extracted with chloroformethanol mixture, concentrate in vacuo and examined on the t.l.c., UV and HPLC systems as described.

RESULTS AND DISCUSSION

N-oxidation of mefloquine by chemical method gave three spots on the tlc system including the parent compound, mefloquine (r_f0.75 for mefloquine; 0.60, 0.55, N-oxidation products). The HPLC system showed 3 characteristic peaks with retention times 12.7 min for mefloquine; 2.2 and 1.8 min for the N-oxidation products. The U.V. spectra (methanol) showed the mefloquine with absorption at 280 and 228nm and the N-oxide with a prominent absorption at 292nm. Mefloquine in its structure has two nitrogen centres, a tertiary nitrogen in the quinoline nucleus and a secondary amine of the piperidinyl ring. The probable N-oxidation products so obtained could be any of the three M1, M2 or M3 shown in scheme 1. The N-oxide showed identical U.V. spectra (i.e. M1 and M2) and a slight shift in absorbance relative to mefloquine. The authentication of M1 was supported by its UV and HPLC characteristics when subjected to the effect of mild and selective reducing agent, sulphur dioxide SO_2 (generated by the addition of HCL or $Na_2S_2O_5$) (Beckett et al, 1972). This caused the disappearance of the characteristic UV and HPLC peaks.

The biologically produced N-oxide showed similar physicochemical characteristics as M1 (R_f0.6, Rt 2.2 min) on the same tlc and HPLC systems and also gave identical U.V. spectral bahaviour. On treatment with SO_2 the UV spectrum and HPLC chromatogram revealed the disappearance of characteristic peaks further establishing the identity of the metabolic. These findings tend to support the fact that mefloquine undergoes metabolic N-oxidation processes in the presence of mixed function oxidased (in vitro). It is also apparent that in mefloquine the tertiary nitrogen centre is first or preferentially attacked by the oxidase enzymes and a simila finding has been reported for amifloxacin N-oxide (Bruck, 1988).

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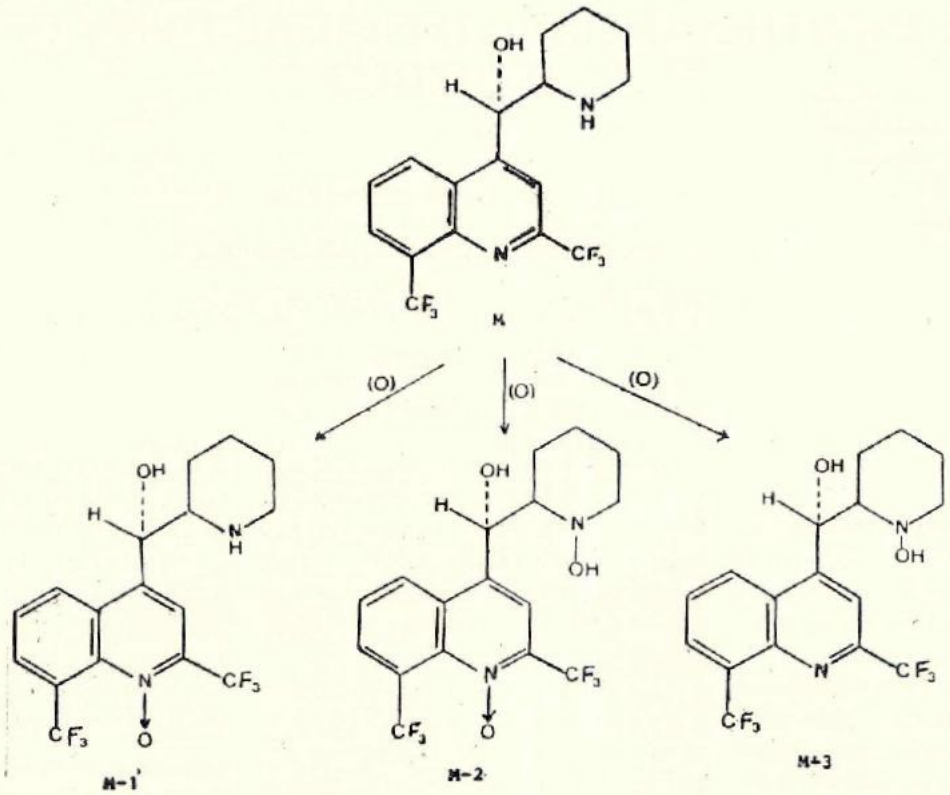

SCHME 1: MEFLOQUINE, M, AND SUSPECTED N-OXIDATION METABOLITES M – 1 and M – 2

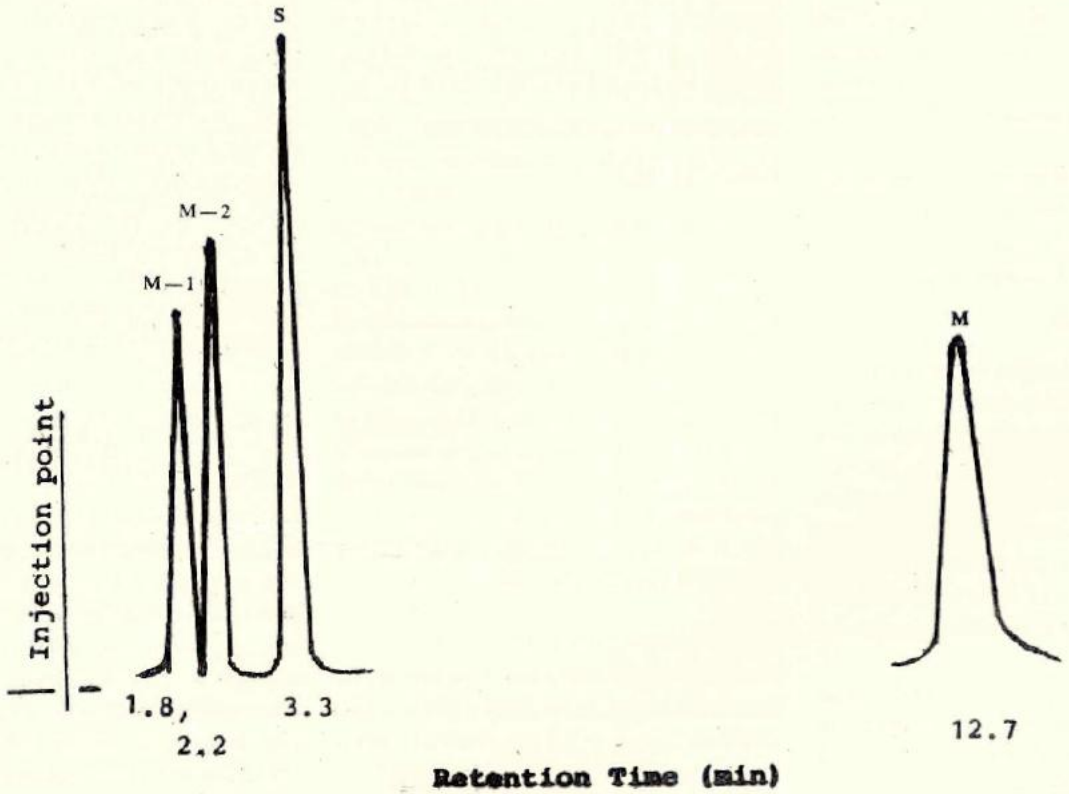

FIGURE 1: HPLC OF MEFLOQUINE, M, MEFLOQUINE N-OXIDATION METABOLITES (M – 2 AND M – 1), INTERNAL STANDARD, S, (TINIDAZOLE).
SYSTEM: C – 18, SPHERISORB, REVERSE PHASE COLUMN (25 cm x 0.5mm i.d.).; 0.01M NaH_2PO_4: MeOH (55 : 45 v/v, pH 8). FLOW RATE 2ml min^{-1}, DETECTION 280nm WAVELENGTH.