STUDIES ON THE METABOLISM OF MEPTAZINOL, A NEW ANALGESIC DRUG

R.A. FRANKLIN & A. ALDRIDGE*

Drug Metabolism Section, Wyeth Laboratories, Maidenhead, Berkshire

C. de B. WHITE**

Department of Clinical Pharmacology, St Bartholomew's Hospital, London EC1A 7BE

- 1 The absorption, metabolism and excretion of the new analgesic meptazinol has been studied in male volunteers following oral and intravenous administration of a mixture of the [$1\text{-}^{14}\text{C}$] and [$7\text{-}^3\text{H}$] labelled compound.
- 2 After oral dosage, absorption from the gastrointestinal tract was rapid as evidenced by the early attainment of peak plasma radioactivity levels and near complete as shown by only small amounts of radioactivity recovered in the faeces.
- 3 Although the absorption of the drug was good, the systemic bioavailability was relatively low. Plasma levels of the unchanged drug remained below the limit of detection (20 ng/ml) after an oral dose of 200 mg. However, after intravenous administration of only 20 mg the peak plasma level was approximately 58 ng/ml. Subsequent elimination was rapid and proceeded in an apparently mono-exponential manner with a half-life of approximately 2 hours.
- 4 Excretion of radioactivity was rapid irrespective of the dosage route and took place chiefly via the urine. Over 60% of the administered radioactivity was recovered in the 0-24 h urine collection. Less than 10% of the administered dose was excreted in the faeces.
- 5 Less than 5% of the drug was excreted unchanged. The major metabolite appeared to be the glucuronide conjugate of the parent drug. No evidence was found for N-demethylation of the compound. A minor metabolite of the drug which accounted for approximately 7% of the recovered radioactivity has been tentatively identified as 6-ethyl - 6 - (3 - hydroxyphenyl) - 1 - methyl - hexahydroazepin - (2H) - 2 - one.

Introduction

Meptazinol [*m* - (3 - ethyl - 1 - methylhexahydro - 1 - H - azepin - 3 - yl phenol) hydrochloride] (Figure 1) has been shown to be a potent analgesic compound in animals, with a potentially low addiction liability (Goode & White, 1971). Recent clinical studies have shown the compound to be effective in relieving severe pain in man (Oosterlinck & De Sy, 1975).

A preliminary account of the metabolic disposition of the orally administered compound has already been reported (Franklin, Aldridge & de B. White, 1975). The present communication describes these studies in more detail together with the results of recent investigations on the pharmacokinetics and metabolism of the compound after intravenous dosage.

Methods

Compounds

[$7\text{-}^3\text{H}$] meptazinol and [$1\text{-}^{14}\text{C}$] meptazinol were prepared by Dr A. White of the Medicinal Chemistry Department, Wyeth Laboratories, U.K. The random tritiation of meptazinol was performed by the T R.I. process at the Radiochemical Centre, Amersham, Bucks. Labile tritium was removed by repeated dissolution in aqueous methanol and subsequent evaporation under reduced pressure. The further purification of the material was then carried out in these laboratories. The biological stability of tritium in the final preparation was demonstrated by the absence of significant quantities of tritiated water in the urine of animals dosed with the compound. Radiochemical purity of the radiocarbon and the tritiated preparations was greater than 95% as shown by thin layer chromatography. For dilution of the radiolabelled material, non-radioactive meptazinol was synthesized by Dr White using methods described in U.K. Patent Specification 1 285 025

Present address

* Department of Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada

** Institute of Psychiatry, De Crespigny Park, London SE5 8AF

Figure 1 Structural formula of meptazinol hydrochloride showing location of radioisotopes.

Measurement of radioactivity

Radioactivity was measured by liquid scintillation counting in a Packard Tricarb spectrometer model 3380 equipped with automatic external standardization. Total radioactivity in urine and plasma was determined following the addition of the sample directly to 'Instagel' (Packard Instruments Ltd, Caversham, Berkshire), or NE 260 (Nuclear Enterprises, Edinburgh) scintillant. Whole blood and faecal homogenates were digested with hydrogen peroxide and perchloric acid by the method of Mahin & Lofberg (1966) prior to the addition of scintillant.

Thin layer chromatography

T.l.c. was performed on Merck precoated silica gel plates in the following solvent systems:

- A. Ethanol/chloroform/heptane/water
20 : 10 : 10 : 5
- B. Chloroform/isopropanol/ammonia
60 : 50 : 1
- C. Chloroform/acetone/acetic acid/water
60 : 20 : 20 : 5
- D. Ethyl acetate/methanol/acetic acid
80 : 10 : 10

Radioactivity was located by the use of a Panax radiochromatogram scanner (Panax Instruments, Mitcham, Surrey) or by autoradiography in which the chromatographic plates were maintained in contact with x-ray film for 24–48 hours.

Isolation and estimation of unchanged drug

Unchanged meptazinol in plasma and urine was determined following extraction with a toluene-based scintillant (8 g butyl PBD/litre toluene) under alkaline conditions. Thus plasma (2–4 ml) or

urine (1 ml) was made alkaline with ammonia solution or bicarbonate buffer pH 9.2, 'spiked' with cold meptazinol (5 µg) to act as carrier and then extracted with scintillant (10 ml) using a vortex mixer. An aliquot of the toluene extract (8 ml) was removed for liquid scintillation counting. This procedure was shown to be quantitative (mean $\pm$ s.e. mean recovery 86.32 ± 2.04) and specific (as shown by t.l.c.) for the unchanged drug.

Studies on the metabolism of the drug

In studying the biotransformation of the compound, urine was applied to columns of Amberlite XAD-2 resin which enabled the removal of endogenous salts and pigments. Drug and metabolites were subsequently eluted with methanol and after reduction to dryness the residue was treated with a solution of β -glucuronidase (Sigma type H-1) 1200 Fishman units/ml urine in acetate buffer at pH 5.0. After incubation for up to 66 h at 37°C the hydrolysate was basified to pH 9.0 and extracted with toluene. This toluene extract was reduced in volume and subjected to comparative t.l.c. in several different solvent systems with reference compounds including meptazinol itself and its potential metabolite 7-oxo-meptazinol.

Since 7-oxo-meptazinol arising from [$7\text{-}^3\text{H}$]-meptazinol would be expected to be deficient in tritium the ratio of $^3\text{H}/^{14}\text{C}$ in the metabolite was determined. The appropriate area of the thin layer plate was scraped into a scintillation vial, the silica deactivated with water (1.0 ml) and the sample counted after the addition of scintillant.

In order to isolate larger quantities of what appeared to be 7-oxo-meptazinol for infra-red spectral analysis, a volunteer ingested a total of 1.2 g as three 8 hourly doses of 400 mg. Urine was collected for up to 24 h after the first dose. The urine collection was 'spiked' with urine obtained from one of the volunteers who had taken the radio-labelled drug. This enabled the efficiency of extraction of the metabolite to be checked and its location on thin layer chromatograms to be determined.

The urine was subjected to β -glucuronidase hydrolysis and then extracted with toluene at pH 5.0. This procedure removed the minor metabolite exclusively. The extract was subsequently chromatographed on several thin layer plates in solvent system B. The metabolite was then eluted, the eluate reduced in volume and subjected to further chromatography in solvent system D. The metabolite was finally eluted from these plates with toluene/methanol 1 : 1. After

Figure 2 Plasma kinetics of total radioactivity (a) and the unchanged drug (b) in human volunteers after oral (▲ 50 mg; ● 100 mg; ○ 200 mg) or intravenous (■ 20 mg) administration of $-N-^{14}CH_3$ labelled and $[^3H]$ -meptazinol. The plasma concentrations of the unchanged drug after oral administration were all below the limit of sensitivity for detection.

Figure 3 Urinary excretion of radioactivity (a, ^{14}C -; b, 3H -) by human volunteers after oral (▲ 50 mg; ● 100 mg; ○ 200 mg) or intravenous (■ 20 mg) administration of $-N-^{14}CH_3$ labelled or $[^3H]$ -meptazinol.

Figure 4 Thin layer chromatograms of urinary metabolites of meptazinol after β -glucuronidase treatment. A, solvent system A, ethanol/chloroform/heptane/water 20 : 10 : 10 : 5; B, solvent system B, chloroform/isopropanol/ammonia 60 : 50 : 1; C, solvent system C, chloroform/acetone/acetic acid/water 60 : 20 : 20 : 5. a, Expected location of meptazinol; b, expected location of 7-oxo-meptazinol (Wy 24694).

reduction to dryness and dissolution in bromoform the infra-red spectrum was determined.

Estimation of the extent of N-demethylation

The administration of both the [$1\text{-}^{14}\text{C}$] labelled drug and the tritiated compound provided a convenient and sensitive way of investigating the extent of its N-demethylation. A lower recovery of radiocarbon compared to tritium in the urine and faeces of subjects dosed with this dual labelled material would represent the extent of N-demethylation of the compound.

Results

After oral administration of the drug, absorption appeared to be fairly rapid as shown by the maximum plasma concentration of radioactivity

occurring within 1.5 h of dosing (Figure 2). The drug was also efficiently absorbed since only a small amount of the radioactivity appeared in the faeces [$<10.0\%$] and recovery in the urine [$>65\%$] was good. However, plasma levels of unchanged meptazinol remained below the limit of detection (20 ng/ml) even after doses of 200 mg.

After intravenous administration, the highest levels of the unchanged drug seen in two subjects who had received 20 mg of the compound were 53 and 60 ng/ml. Subsequent elimination of the drug took place in an apparently mono-exponential manner with a half-life of approximately 2 hours.

Excretion of radioactivity took place predominantly via the urine irrespective of dosage route. The process was rapid, over 50% of the dose being recovered in the first 9 h after dosing (Figure 3). Less than 10% of the dose was voided in the faeces.

Metabolism of the compound after both intravenous and oral dosage was extensive as indicated by less than 5% of the dose being recovered from the urine as toluene soluble radioactivity (which represented essentially unchanged drug). However, after treatment with β -glucuronidase more than 40% of radioactivity was rendered toluene soluble.

Further studies on the biotransformation of the compound were confined to subjects who had taken the drug orally. Differentiation between glucuronide and sulphate conjugates of meptazinol was accomplished by the attempted hydrolysis in the presence of glucaro-1, 4-lactone (150 μM), a potent inhibitor of β -glucuronidase. This resulted in very little material being rendered solvent soluble indicating that the major metabolite was the glucuronide and not the sulphate conjugate. Further examination of the metabolism of the compound showed that it did not undergo N-demethylation since no significant loss of radiocarbon relative to tritium was observed. A minor metabolite constituting about 5% of the total dose was found in the toluene extract of β -glucuronidase hydrolysate. Comparative thin layer chromatography of this metabolite with 6-ethyl - 6 - (3 - hydroxyphenyl) - 1 - methyl-hexahydroazepin - [2H] - 2 - one (Wy 24694) showed the two compounds to behave very similarly (Figure 4). In addition the infra-red spectrum of the metabolite closely resembled that of Wy 24694 (Figure 5). Further confirmation of this tentative identification was obtained from the extensive loss of tritium relative to radiocarbon in this metabolite. Tritium in the original meptazinol molecule had been located on the 7-position and tritium deficiency in the metabolite therefore suggested that reaction had taken place at this position.

Figure 5 Infra-red spectra of (a) a minor metabolite of meptazinol and (b) authentic 7-oxo-meptazinol (Wy 24694).

Discussion

The results of these studies have shown a striking difference in the plasma levels of meptazinol achieved after intravenous compared with those attained after oral administration of the compound. Peak plasma concentrations observed after intravenous dosage of 20 mg of the drug were approximately 60 ng/ml but after ten times that dose given orally plasma concentrations remained below the limit of detection of 20 ng/ml.

Other major analgesics such as morphine have, in the past, been reported to be rather poorly absorbed after oral administration. However, many such reports were based on observations of very low blood levels of the unchanged drug (Way & Adler, 1962). In recent years it has become apparent that there may be several other explanations for low blood levels. For example, extensive localization of the drug in tissues, or near complete metabolism during the drug's initial passage through the liver. Oral administration of meptazinol has been shown to result in only low plasma levels of the free drug despite its rapid and efficient absorption. Metabolic studies have established that the drug is extensively conjugated with glucuronic acid, an observation which may provide an explanation for these low plasma levels. Recently Brunk & Delle (1974) have shown that morphine is also efficiently absorbed after oral administration but achieves only low plasma levels due to extensive first-pass metabolism.

Most major analgesics possessing an N-methyl function undergo demethylation. It is possible to measure the rate and extent of this process by determining the urinary concentration of the demethylated product or by measuring exhaled $^{14}\text{CO}_2$ following the administration of the -N- $^{14}\text{CH}_3$ labelled drug. However, both these methods have serious disadvantages. For example, attempting to assay the demethylated product could give a misleading impression of the extent of demethylation if this metabolite underwent further biotransformation to an unidentified product. Complete collection of expired CO_2 is very difficult with large animals and impractical in the case of man. However, the alternative of taking gas samples at various times after dosing is at least laborious and furthermore may not fully define the excretion profile. Thus neither of these methods would appear to be as sensitive, accurate or as convenient as the mixed isotope technique that has been employed in the present study. From the very similar recovery of both isotopes it would appear that meptazinol does not in fact undergo significant demethylation. In this context it is interesting to note that Axelrod (1956) first observed that substrates possessing phenolic functions were demethylated more slowly than when this function was masked or missing. Thus he noted that morphine was demethylated only about half as fast as codeine and similarly 3-hydroxy-N-methyl-morphinan underwent N-demethylation at only about 25% of the rate of

the corresponding 3-methoxy compound. These *in vitro* results were found to correlate well with the extent of demethylation occurring *in vivo*. Thus N-demethylation has been shown to play a less important role in the metabolism of phenolic analgesics as morphine, levorphanol and profadol than for those compounds that either lack a phenolic group altogether such as pethidine and ethoheptazine or that have etherified phenolic groups such as codeine (Beckett & Casy, 1965). The results of the present investigation show that the metabolism of meptazinol is in accord with these general observations.

Since biotransformation generally produces compounds with greater hydrophilicity it was interesting to note during preliminary t.l.c. studies that the minor metabolite of meptazinol was apparently more lipophilic than the parent drug. In view of this observation it was considered possible that this metabolite might possess some

biological activity, an idea reinforced by chemical considerations. Oxidation at either of the carbon atoms adjacent to the nitrogen atom in the azepine ring might be expected to abolish the basic character of the molecule and increase its lipophilicity. The resulting compound would have a structural resemblance to the CNS depressants N-methyl glutethimide and methsuximide. However, after confirmation of the identity of this minor metabolite as 7-oxo-meptazinol, its subsequent biological evaluation showed it to have no apparent CNS activity even after oral doses of 400 mg/kg. Not surprisingly further tests for anticonvulsive and analgesic activity also proved negative.

The excellent technical assistance of Mr Brian Morrison is gratefully acknowledged. We are indebted to Drs Swaisland and Sanford for helpful discussion.

References

- AXELROD, J. (1956). The enzymatic N-demethylation of narcotic drugs. *J. Pharmac. exp. Ther.*, **117**, 322-328.
- BECKETT, A.H. & CASY, A.F. (1965). *Progress in Medicinal Chemistry*. Eds Ellis, G.P. & West, G.B., **4**, p. 177. London: Butterworths.
- BRUNK, S.F. & DELLE, M. (1974). Morphine metabolism in man. *Clin. Pharmac. Ther.*, **16**, 51-56.
- CHANG, S.F., HOWELL, J.P. & GLAZKO, A.J. (1968). Metabolic disposition of profadol (*m*-(1-methyl - 3 - propyl - 3 - pyrrolidinyl) phenol; C1572) in laboratory animals. *Fed. Proc. Fed. Am. Soc. exp. Biol.*, **27**, 654.
- FRANKLIN, R.A., ALDRIDGE, A. & de B. WHITE, C. (1975). Preliminary metabolic studies on the new analgesic meptazinol. *Br. J. clin. Pharmac.*, **2**, 472-473.
- GOODE, P.G. & WHITE, A.C. (1971). Some properties of Wy 22811 a new analgesic compound. *Br. J. Pharmac.*, **43**, 462-463P.
- MAHIN, D.T. & LOFBERG, R. (1966). A simplified method of sample preparation for determination of tritium carbon-14 or sulphur-35 in blood and tissue by liquid scintillation counting. *Analyt. Biochem.*, **16**, 500-504.
- OOSTERLINCK, W. & DE SY, W. (1975). Preliminary clinical experience with meptazinol, a new analgesic. *Curr. Med. Res. Opin.*, **3**, 187-191.
- WAY, E.L. & ADLER, T.K. (1962). The biological disposition of morphine and its surrogates. *Bull. Wld Hlth Org.*, **27**, 359-394.

(Received August 21, 1975)