Detection of methaqualone and its metabolites in urine

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SYNOPSIS A method is described for the detection of methaqualone and its metabolites in the presence of large doses of other drugs.

Methaqualone hydrochloride, or 2-methyl-3-0-tolyl-4(3H)-quiazolinone, is an orally active, non-barbiturate, mild hypnotic. It is available in Britain as Melsedim (Boots), 150 mg and as Mandrax (Roussel), 250 mg with 25 mg of diphenhydramine. Four cases of addiction to methaqualone were reported by Madden (1966). The physical dependence on methaqualone in these patients has been disputed (Martin, 1966). Ewart and Priest (1967) report a case of methaqualone addiction in which withdrawal was associated with the clinical syndrome of delirium tremens characteristic of the barbiturate-alcohol type of dependence. Further discussion of this problem appears in 'Any questions?' (1967).

McClure (1968) is concerned at the abuse of Mandrax by the drug-dependent population seen at the drug addiction clinic at Welwyn Garden City. Mandrax is used during the daytime when the addicts cannot obtain other drugs, as it gives them a 'buzz' or thrill, apparently not obtained from other hypnotics.

The metabolism of methaqualone has been studied by a number of workers, and methods have been developed for the detection of methaqualone and its metabolites in the plasma, urine, and tissues of a number of animal species, and Eberhardt, Freundt, and Langbein (1962) found four metabolites of methaqualone in man. Cohen, Font du Picard, and Boissier (1962) and Cohen, Wepierre, Font du Picard, and Boissier (1965) used C14-labelled methaqualone to study its distribution and metabolism in the mouse. Akagi, Oketani, and Takada (1963a), Akagi, Oketani, Takada, and Suga (1963b), and Akagi, Oketani, and Yamane (1963c) studied the metabolic fate of methaqualone in rabbit, rat, and man. Geldmacher-Mallingekrodt and Lautenbach (1963) reported the examination of tablets, tissue, and urine in three cases of poisoning with Revonal (Merck) methaqualone. Prabhu, Browne, and Zarosinski (1964) studied the rate of turnover of methaqualone in rat and mouse and demonstrated the major role of the liver in its detoxification.

Nowak, Schorre, and Struller (1966) studied the metabolism of methaqualone in rats, rabbit, dogs, and Rhesus monkeys and showed that hydroxylation is of major importance and that the bulk of the metabolites was excreted as glucuronides. Preuss, Hassler, and Kopf (1966), in a detailed and comprehensive study, isolated some 13 renal excretion products. These studies have shown that biotransformation of methaqualone is largely oxidative in nature, not affecting the basic structure. The benzene ring, and possibly the methyl groups in positions 2 and 2', were hydroxylated.

Our purpose was to devise a rapid method for screening large numbers of urine samples for the presence of methaqualone metabolites. The method was required to be specific even in the presence of high doses of other drugs.

METHODS

ACID HYDROLYSIS OF URINE Urine, 10 ml, and 1.5 ml of concentrated hydrochloric acid are heated on a boiling water bath for two hours. The mixture is then adjusted to pH 10 to 11 with ammonia solution 0.88.

ENZYME HYDROLYSIS OF URINE Urine, 10 ml, 1 ml acetate buffer, pH 5.0, and 1 ml Ketodase (Warner-Chilcott) are incubated overnight at 37°C. The mixture is adjusted to pH 10 to 11 with ammonia solution 0.88.

EXTRACTION The hydrolysed urine is extracted with 25 ml ether for 10 minutes, centrifuged, and the ether phase separated and evaporated to dryness in vacuo.

CHROMATOGRAPHY Chromatoplates are prepared from silica gel H (Merck) and aluminium oxide G (Merck) and stored at 100°C before use. The residues are transferred

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...to the plates in 0-1 ml methanol. The chromatography system used routinely is silica gel H with ether as the solvent (system A). Other systems used were silica gel H with benzene/isopropyl alcohol (100/15) as solvent (system B), and aluminium oxide G with ether/petroleum ether (3/2) as solvent (system C).

LOCATION REAGENTS These are (1) 5% aqueous sulphuric acid, (2) iodoplutinate reagent, (3) Dragendorff reagent, all of which are applied sequentially and are prepared as described by Davidow, Li Petri, Quame, Searle, Fastlitch, and Savitzky (1966), and (4) diazotized sulphanilic acid (Smith, 1960).

RESULTS

When 10 ml of a specimen of urine collected for 24 hours after the ingestion of a single tablet of Mandrax was adjusted to pH 10 to 11 with ammonia solution 0·88 and extracted with ether no unchanged methaqualone or its metabolites could be detected by chromatography in system A, when location reagents 1 to 3 were applied sequentially. When 10 ml of the same urine was enzyme hydrolysed before extraction, four spots could be detected (system A), and are designated EH1, EH2, EH3, and EH4 (Fig. 1). Comparison of extracts from acid- and enzyme-hydrolysed urine show that spots with Rs identical to EH1 to EH4 are produced by acid hydrolysis. Qualitative assessment of the thin-layer plates shows that EH2 appears in equal amounts and that more EH4 is detected after enzyme hydrolysis and more EH3 after acid hydrolysis. EH1 is found only in trace amounts from 10 ml of urine (Fig. 1). Metabolites of methaqualone were detected in the third consecutive 24-hour collection of urine after the ingestion of a single tablet of Mandrax which contains 250 mg of methaqualone and can still be detected after storage for one month at 4°C.

Spots EH1 to EH4 are not detected in ether extracts of 10 ml amounts of a control urine which has been acid or enzyme hydrolysed; nor are they present in urine collected for 24 hours after the ingestion of 25 mg diphenhydramine.

Table I gives a summary of the data obtained following the elution of spots EH1 to EH4. Spots EH1 to EH4 were eluted from a chromatoplate with 0-1N HCl and the ultraviolet absorption was recorded. EH2 and EH3 were eluted and run again in system B. The eluate from spot EH2 was separated

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chromatography System</th>
<th>Location Reagents</th>
<th>Ultraviolet Absorbance in 0-1N HCl</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>EH1</td>
<td>0·35</td>
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<td>-</td>
</tr>
<tr>
<td>EH2</td>
<td>0·56</td>
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<tr>
<td>EH2a</td>
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<td>0·73</td>
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into two parts, EH2a (Rf 0.51), which gave a lemon yellow colour with diazotized sulphonic acid, and EH2b (Rf 0.55) which was red with sulphanilic acid. The eluate from spot EH3 remained as one spot (Rf 0.51), yellow with sulphonic acid. EH2a and EH3 gave brown colours with location reagents 1 to 3 applied sequentially and EH2b a purple brown colour.

The spot EH4, which has an Rf value fractionally higher than methaqualone in system A, was eluted and run again in system C. The eluate, which represented an extraction of 50 ml of enzyme-hydrolysed urine, separated into two spots, EH4a (Rf 0.27) and EH4b (Rf 0.73). Methaqualone in this system has an Rf of 0.73, and, like EH4a and EH4b, is negative with diazotized sulphonic acid. EH4b and methaqualone are brown with the sequential spray reagent and EH4a is purple. A semiquantitative estimate of the relative amounts of EH4a and EH4b in an extract of 50 ml of enzyme-hydrolysed urine suggests that there is 10 times more EH4a than EH4b. This indicates that in an extract from 10 ml urine the spot EH4 is predominantly EH4a and accounts for its having a slightly higher Rf value than methaqualone.

No interference has been encountered in the detection of methaqualone metabolites in urines from patients receiving the following drugs either singly or in combination: heroin, amphetamine, methylamphetamine, methadone, dihydrocodeine, chlorpromazine, dichloralphenazone, orphenadrine, nitrazepam, amitriptyline, trifluoperazine, barbiturate, aspirin, glutethimide, isocarboxazide, chloral hydrate, and tetracycline.

**DISCUSSION**

Mandrax (Roussel) contains in addition to methaqualone a small proportion, one tenth by weight, of diphenhydramine. Our results show that spots EH1 to 4 detected after ingestion of one tablet of Mandrax (Fig. 1) are specifically associated with the methaqualone component. The finding of four spots, EH1 to 4, and further characterization of these spots (Table I), confirms the reports of earlier workers, and in particular the work of Preuss *et al* (1966).

We have presently adopted the enzyme hydrolysis of urine, extraction with ether, chromatography in system A, and the sequential application of location reagents 1 to 3 as our routine procedure. The sequential application of location reagents 1 to 3 has proved to be much more sensitive than Dragendorff reagent alone in the detection of methaqualone metabolites. Although acid hydrolysis is less time consuming, the production of ether-soluble pigments can lead to difficulties in the interpretation of the developed chromatoplates. Enzyme hydrolysis produces an almost pigment-free residue.

Using the above method, which we have found to be specific for methaqualone metabolites even in the presence of large doses of other drugs, we are currently surveying a population of drug addicts to determine the extent of the abuse of methaqualone-containing drugs.

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