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Note

Determination of dextromethorphan in serum by gas chromatography

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Dextromethorphan (I) [(+)-3-methoxy-17-methylmorphinan] is a widely used antitussive agent of synthetic origin. It is sometimes rated the equal of codeine for this utility [1], though this is not universally true [2]. Very little is known about the pharmacokinetic behavior of I. One difficulty has been the lack of a sensitive, specific method for its determination in biological fluids. Most existing methods do not have the sensitivity for analyses in the low nanogram range [3—5]. The reported gas chromatographic (GC) methods [4, 5] are capable of detecting I in biological materials at levels in the low microgram range. However, it was not established that this sensitivity was adequate for the analysis of actual samples. I was reportedly found in human blood using a fluorometric method [3]. The specificity of this method was not established, and the interference, if any, of metabolites was not reported. Due to the lack of a sensitive determination for I, it was suggested that bioavailability studies be conducted using plasma levels of (+)-17-methylmorphinan-3-ol (dextrorphan), an active metabolite [6]. Although it was assumed that I would not interfere because of the extremely small amounts present, the interference of other metabolites was not determined. (+)-Morphinan-3-ol, for example, has a similar fluorescence spectrum [7]. Recently a sensitive, specific radioimmunoassay was described [8] which was capable of detecting approximately 1 ng/ml of I in plasma. No I was detected in the plasma of one human subject given a single 0.5-mg/kg dose. In a study involving seven subjects [2], 43% of a 60-mg dose of [N-methyl-^{14}C]I · HBr was excreted in the urine in 24 h and only 0.12% was recovered in feces. The maximum amount of radioactivity in plasma corresponded to about 20 ng/ml of I. These figures would have included any metabolite containing an N-methyl group, such as dextrorphan.

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This report describes a sensitive GC method for the determination of I in serum, and the application of this method to sera from dogs and humans after single doses of I · HBr.

EXPERIMENTAL

Reagents

All chemicals were analytical-reagent grade. Benzene, isopropyl alcohol, methylene chloride, and isooctane were a glass-distilled chromatographic grade obtained from Mallinckrodt (St. Louis, Mo., U.S.A.). Dextromethorphan · HBr was obtained from Hoffmann-LaRoche (Nutley, N.J., U.S.A.) and diphenylpyraline · HCl was purchased from K & K Labs. (Plainview, N.Y., U.S.A.). (+)-3-Methoxymorphinan was prepared from I and cyanogen bromide (Eastman-Kodak, Rochester, N.Y., U.S.A.) using the procedure of Clark [9]. The tartrate salt was recrystallized twice from ethanol—water, m.p. 209—211°. The structure was verified by elemental analysis and mass spectrometry. Dextrorphan tartrate and (+)-morphinan-3-ol were obtained from Hoffmann-LaRoche and Boehringer (Ingelheim am Rhein, G.F.R.), respectively. Pentafluorobenzyl chloroformate and trichloroethyl chloroformate were obtained from Pierce (Rockford, Ill., U.S.A.) and Aldrich (Milwaukee, Wis., U.S.A.), respectively.

Apparatus

The samples were analyzed using a gas chromatograph (Model 7610A, Hewlett-Packard, Avondale, Pa., U.S.A.) equipped with a ^{63}Ni electron-capture detector. A silanized glass column (120 cm × 4 mm I.D.) packed with 3% OV-25 on 80—100 mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.) was used at 250°. The injection port and detector were operated at 265° and 300°, respectively, with a 150-μsec pulse interval for the detector. The flow-rate of the carrier gas (5% methane in argon) was 50—55 ml/min. Under these conditions, the retention times of the pentafluorobenzyl carbamates of I and diphenylpyraline were 7.5 and 8.9 min, respectively, and the respective trichloroethyl carbamates eluted at 8.2 and 9.8 min.

Procedure

Two-milliliter aliquots or less of serum were diluted with 2 ml of distilled water in a 13-ml glass-stoppered centrifuge tube and a quantity of the internal standard, diphenylpyraline · HCl, roughly equivalent to the mid-point of the standard curve was added to all samples and standards. The mixture was made alkaline with 0.5 ml of 2 *M* potassium hydroxide and extracted vigorously with 4.0 ml of benzene—isopropyl alcohol (9:1, v/v) for 5 min. The upper layer was transferred to a 13 × 100-mm test-tube after centrifugation and one drop of acetic anhydride was added and mixed. After 5 min, 2.0 ml of 0.05 *M* sulfuric acid was added and the mixture was agitated for 1 min on a rotary tube mixer. After initially discarding most of the benzene, the sample was centrifuged and all remaining benzene was carefully removed. The remaining aqueous sample was made alkaline with 0.3 ml of 2 *M* potassium hydroxide and extracted with 2.0 ml of benzene—isopropyl alcohol (9:1, v/v) for 1 min on a rotary mixer.

After centrifugation, the benzene layer was transferred to a 2-ml glass ampoule and evaporated under a stream of nitrogen in a warm sand bath. A 0.25-ml aliquot of 0.5% (v/v) pentafluorobenzyl chloroformate (trichloroethyl chloroformate was used for the human samples) in methylene chloride and 0.15 ml of 5% (w/v) aqueous sodium bicarbonate were added to the sample, and the ampoule was sealed and heated at 80° in a sand bath for 2 h.

After cooling, the ampoule was scored, broken about 1 cm above the stricture, and the contents were evaporated to dryness under a stream of nitrogen in a warm sand bath. One milliliter of isooctane and 0.5 ml of 0.5 *M* potassium hydroxide in methanol—water (3:1, v/v) were added and mixed for 1 min on a rotary mixer. After centrifugation, the upper layer was transferred to a vial with PTFE-lined screw cap and concentrated before analysis if necessary. A standard curve was prepared daily (peak height ratio vs. concentration) by analyzing in duplicate at least three standards in serum. Linearity was observed through 150 ng/ml, the maximum level usually required.

In vivo studies

Three fasted male mongrel dogs received 1.0-mg/kg doses of I · HBr in aqueous solution by three routes of administration — intravenous (i.v.), intraperitoneal (i.p.), and per os (p.o.), with at least one week between doses. Blood samples were collected from the jugular vein 0.5, 1, 2, 3, 4, and 6 h after drug administration.

Twelve fasted male human volunteers, 21—30 years and 60—100 kg, were given single 20-mg doses of I · HBr p.o. The drug was administered as part of an experimental liquid formulation also containing 60 mg of pseudoephedrine · HCl and 200 mg of guaifenesin. Venous blood samples were taken 0.5, 1, 1.5, 2.5, and 5 h after drug administration.

Serum was prepared from all blood samples and stored at −15°.

RESULTS AND DISCUSSION

GC analysis of I

The absence of reactive groups on I makes the use of common acylating agents impossible. The tertiary nitrogen is susceptible to derivatization with chloroformates [10], with the loss of a methyl group, and this approach has been used in the development of a technique for the determination of a number of tertiary amines [11—13]. We have found this general method, with some modification, applicable to the determination of I in serum, with detection limits of < 1 ng/ml. Pentafluorobenzyl chloroformate is preferred for greater sensitivity, but trichloroethyl chloroformate has been used with similar results. Solvent extractions combined with a back-extraction with dilute aqueous acid provided adequate purification prior to derivatization. Sodium bicarbonate was superior to sodium carbonate as a catalyst for the derivatization reaction, and an aqueous bicarbonate solution, resulting in a two-phase system, gave higher yields than the solid reagent. The utility of alcoholic alkali for the reduction of interference resulting from the derivatizing reagent [13] was confirmed.

The derivatizing reagent also reacts with (+)-3-methoxymorphinan (II) to

form the same derivative, necessitating the removal of II with acetic anhydride. When 1.0 μg of II was added to control serum, less than 0.5% was recovered using this procedure. The interference of dextrorphan and (+)-morphinan-3-ol was likewise negligible (< 0.5%). The total amount of I + II can be estimated by omitting the acetic anhydride. This has not resulted in substantially larger values than for I alone, and it is known that II is not a major metabolite [14]. The analysis of ten 1-ml control serum samples spiked with 50 ng/ml I resulted in a mean of 50.7 ± 2.4 (S.D.).

Canine serum I

Serum I was determined in dogs after i.v., i.p., or p.o. administration of 1-mg/kg doses of I · HBr (Table I). The mean concentration of I in serum 0.5 h after i.v. administration was 78 ng/ml. The mean terminal $t_{1/2}$ after i.v. administration was 2.3 h. Values of 2.5 and 3.9 h were reported for two dogs treated with 2 mg/kg I i.v. [8]. After i.p. administration, there was a marked reduction in the amount of I present in serum with a further decrease to only about 2 ng/ml after oral administration. This contrasts with the results obtained with hydrocodone, where serum drug levels after i.v. and p.o. administration were similar [15]. Poor oral availability could be associated with low absorption, but the fact that i.p. availability was also low suggests a first-pass metabolic effect [16]. The rapid and extensive metabolism of I has been described

TABLE I

SERUM CONCENTRATIONS OF I AFTER INTRAVENOUS, INTRAPERITONEAL, AND ORAL ADMINISTRATION OF 1 mg/kg OF I · HBr IN DOGS

Hours	Serum I (ng/ml as free base)*
Intravenous administration	
0.5	78 ± 2
1.0	52 ± 5
2.0	40 ± 10
3.0	28 ± 6
4.0	21 ± 6
6.0	12 ± 6
Intraperitoneal administration	
0.5	9.5 ± 5.1
1.0	9.8 ± 4.8
2.0	7.7 ± 3.1
3.0	5.5 ± 2.1
4.0	4.5 ± 1.7
6.0	2.9 ± 1.1
Oral administration	
0.5	2.1 ± 0.6
1.0	2.1 ± 0.9
2.0	1.6 ± 0.8
3.0	1.2 ± 0.8
4.0	0.9 ± 0.9
6.0	0.4 ± 0.6

*Mean of three animals

previously [8, 14]. The mean area under the serum concentration vs. time curve for the oral treatment in the present study was 3.8% of the i.v. value. This is somewhat lower than reported values of 7 and 18% [8].

The difference in the results from p.o. and i.p. administration may reflect less than complete absorption and/or gut-wall metabolism, a process known to be important for some compounds [17].

Human serum I

Serum I levels were very low in humans after oral administration. A mean peak value of < 2 ng/ml was obtained after a 20-mg oral dose of I · HBr (Table II). This contrasts with peak drug levels of about 20 ng/ml after a 10-mg oral dose of hydrocodone bitartrate [15] and about 30 ng/ml after a 15-mg oral dose of codeine [18]. Intersubject variability in the present study was high,

TABLE II

SERUM CONCENTRATIONS OF I IN HUMANS AFTER A SINGLE 20-mg ORAL DOSE OF I · HBr

Hours	Serum I (ng/ml as free base)*
0.5	0.1 ± 0.1
1.0	0.7 ± 0.8
1.5	1.1 ± 1.3
2.5	1.8 ± 2.2
5.0	1.4 ± 1.6

*Mean of twelve subjects ± S.D.

indicating possible differences in absorption or metabolism of the drug. Four of twelve subjects had peak serum drug levels of > 2 ng/ml with one value of 7.9 ng/ml and seven had values < 1 ng/ml. This may account for the fact that Dixon et al. [8] were unable to detect I in the plasma of one subject using a radioimmunoassay with a sensitivity of about 1 ng/ml.

Preliminary work indicates that the GC method described above can also be used to analyze urine samples. Although it appears that serum drug levels may be too low in most cases to permit detailed kinetic analyses, this problem can perhaps be circumvented by using excretion data.

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