Rapid Ultraviolet Procedure for Measuring Doxepin and Some Doxepin Metabolites in Human Urine

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A method is described for measuring doxepin-related material excreted in urine. Unchanged doxepin and certain doxepin metabolites are oxidized to doxepin ketone with KMnO₄. The doxepin ketone is extracted with n-hexane and quantitated by ultraviolet spectrophotometry at the absorption maximum, 266 nm. This method is fast, reproducible, and measures a greater percentage of doxepin-related material than do existing gas–liquid chromatographic procedures. Doxepin ketone was measured to provide bio-availability data, for comparison of different formulations in humans.

Doxepin hydrochloride, an isomeric mixture of N,N-dimethyl dibenzo[β,e]oxepin Δ₃₁⁻^{14}H₃ γ-propylamine hydrochloride, is a relatively new drug, used in treatment of anxiety and depression (1).

Methods have been published for the detection of doxepin and its demethylated metabolite in plasma and urine by gas–liquid chromatography. Hobbs (2) described a procedure by which he measured doxepin and demethyl doxepin in dog plasma. However, these two components represented less than 2% of the radioactivity in plasma from a dog chronically dosed with ¹⁴C-labeled doxepin. Dusci and Hackett (3) also used a gas–liquid chromatographic procedure to measure only unchanged doxepin in urine from human subjects who received a single 50-mg dose of doxepin. Only 0.4% of the dose was excreted in urine as the parent compound within 24 h and no significant amounts were detected thereafter.

More recently, Devriendt et al. (4) have proposed a series of methods for determining doxepin and its metabolites in biological media. However, all work done by this group was apparently performed on samples of plasma and urine to which doxepin had been added, as no results are given for experimental samples. The three methods of measuring doxepin in urine would not appear to be sensitive enough to detect the amount of unchanged doxepin reported by Dusci and Hackett (3) to be excreted in urine.

We wished to compare the biological availability of different formulations of doxepin. Because available methods measured only a minute percentage of a given doxepin dose, we felt that to provide a valid comparison between two dosage forms, a method was needed that would measure a greater percentage of the doxepin-related material present. We have developed such a method in which the doxepin molecule is oxidized to a neutral ketone. This ketone is then extracted and quantitated by ultraviolet spectrophotometry. This method is not specific—it measures some doxepin metabolites as well as unchanged doxepin. However, it is simpler and measures a greater percentage of doxepin-related material in human urine than do existing gas–liquid chromatography procedures.

Materials and Methods

Ultraviolet analyses were performed with a Cary Model 11-A recording spectrophotometer (Applied Physics Corp., Monrovia, Calif. 91016).

Reagents

Spectroquality n-hexane.

Potassium permanganate, saturated aqueous solution.

Standards. Stock solution was prepared by dissolving 100 mg of doxepin hydrochloride per 100 milliliters in water. We diluted the stock with human urine to provide working standards.

Method

Five milliliters of urine are pipetted into a 40-ml centrifuge tube followed by 1.5 ml of the saturated KMnO₄ solution. The tube is shaken to mix thoroughly and allowed to stand for 5 min. The KMnO₄ oxidizes doxepin to its corresponding ketone, the N,N-dimethyl-γ-propylamine side-chain being replaced by —O.

Doxepin ketone is extracted from the oxidized urine sample by shaking for 10 min with 10 ml of n-hexane. The tubes are centrifuged to sharply separate the phases, and the hexane extract is removed for ultraviolet analysis.
Hexane extracts are scanned in the ultraviolet region from 260 to 300 nm in spectrophotometer cells with a 20-mm light path. Spectroquality n-hexane is used as the solvent blank. Absorbance at 295 nm (trough) is subtracted from the absorbance at 266 nm (peak) to obtain net absorbance.

Human Subjects

Twelve male volunteers were used in a crossover study to compare biological availability of two formulations of doxepin. Initially six subjects received five 10-mg capsules of SK&F 40080-A (the Smith Kline & French formulation of doxepin hydrochloride) and six received five 10-mg capsules of “Sinequan” (the Pfizer formulation of doxepin hydrochloride). Seven days later the subjects were “crossed over,” i.e., the order of administration was reversed. The total urine output was collected at designated intervals during the 48 h after drug administration. Urine volumes were recorded and the samples frozen until analyzed.

Results

Verification of Oxidation Product

A solution of 14C-labeled doxepin hydrochloride was prepared in blank human urine and an aliquot oxidized and extracted as described in methods. Thin-layer chromatograms of the extracted material were scraped in segments and the radioactivity was eluted and measured by liquid-scintillation counting. All radioactivity was found to be in a single spot at the same Rf as authentic doxepin ketone. Ultraviolet spectra of the radioactive and numerous nonradioactive doxepin standards were identical to that of the ketone. Although the above work is strongly indicative of the identity of the oxidation product, further studies would be necessary for absolute proof of structure.

Analytical Variables

Standard curve. A standard curve was obtained by analysis of human urine containing different concentrations of doxepin hydrochloride. Standards were prepared containing 3 to 40 mg of doxepin hydrochloride per liter; a linear relationship exists between concentration and net absorbance over this range. Spectra obtained from analysis of a blank urine sample and the same sample with 10 mg of added doxepin hydrochloride per liter are shown in Figure 1.

Precision. Fresh standards containing 20 and 30 mg of added doxepin hydrochloride per liter were analyzed on five different days during the course of our studies. The coefficients of variation were 1.5% and 1.2%, respectively.

Analysis of control samples. Control urine specimens were collected from subjects before each dose administration. The average net absorbance of all control samples collected immediately prior to the first dose corresponded to a doxepin concentration of 0.3 mg/liter. Control samples collected prior to the second dose had an average net absorbance equivalent to 0.2 mg of doxepin per liter. The range of net absorbance for all control urine samples was equivalent to a doxepin concentration range of 0.1 to 0.7 mg per liter.

Recovery. Recovery of doxepin by our method was compared with recovery of radioactivity in the urine of two men, each of whom received 50 mg (50 μCi) of 14C-labeled doxepin hydrochloride. The percent of dose as determined by radioactivity was assumed to represent the total doxepin-related material in the urine specimens. With our method we could detect an average of 43.5% of the total doxepin-related material present in urine collected 2- to 4-h after drug administration. Thereafter, progressively smaller percentages of total drug-related material were recovered as doxepin ketone. No ketone was detected in samples collected more than 48 h after the drug, although radioactivity measurement indicated that doxepin-related material was still being excreted.

Interference. Cigarette smoking and consumption of coffee had no effect on control urine values. Urines collected from four subjects who smoked 20 to 40 cigarettes and drank three to eight cups of coffee daily had control values equivalent to 0.1 to 0.4 mg of doxepin per liter of urine. A number of commonly occurring drugs were added to urine and checked for possible interference with the analysis of doxepin. These supplemented urines were treated as described in Methods. The net absorbances obtained with urines containing 10 mg of chlorpromazine, chlorpromazine sulfoxide, prochlorperazine, codeine, benzedrine, or phenobarbital per liter were unchanged from control values. Diazepam and chlordiazepoxide produced interfering peaks in the ultraviolet, but these were distinctly different from the ultraviolet spectrum of doxepin ketone. The oxidation product of amitriptyline, however, has ultraviolet characteristics identical to those of doxepin ketone. Wherever a question exists as to whether amitriptyline or doxepin is involved, a more specific analytical technique would be necessary.

Fig. 1. Ultraviolet spectra obtained after oxidation and extraction of human urine samples

a, control urine; b, same urine with addition of 10 mg of doxepin hydrochloride per liter. The spectrum for urine from a person who has taken doxepin is in no way qualitatively different from b.
Bioavailability

Table 1 shows the 48-h recovery in human subjects after administration of SK&F 40080-A or Sinequan, as determined by our method. Recovery varied from less than 4 mg (Subject 12) to 18 mg (Subject 4). Though recovery differed widely among subjects, excretion data for a given individual were similar for either formulation. Average 48-h recoveries differed by just 0.08 mg (0.7%). The peak urinary excretion of doxepin and ketone-convertible metabolites occurred in all but one subject in the 3- to 6-h post-drug collection period. In this collection, the recovery of doxepin-related material ranged from 8 to 33 mg/liter of urine. The average recovery in urine was 15.0% of the dose in the first 12 h after drug administration and 3.1% in the 12- to 24-h collection period. An additional 3.5% of the dose was recovered between 24 and 48 h.

Discussion

Hobbs (2) has reported the presence of the following metabolites of doxepin in rat and dog urine:

a. Demethyl doxepin
b. Doxepin-N-oxide
c. Didemethyl doxepin
d. Hydroxy doxepin
e. Hydroxy doxepin glucuronide
f. Demethyl hydroxy doxepin

Because only the side chain of doxepin is metabolized to form a, b, and c, these metabolites would all be converted to doxepin ketone by the KMnO₄ oxidation as would any parent compound excreted in the urine. Oxidation of d, e, and f would also occur, but the resulting hydroxy doxepin ketone would be too polar to extract into n-hexane.

Assuming that metabolites of doxepin in man are similar to those of rat and dog, several points in our studies may be explained. An increase in the percentage of ring-hydroxylated metabolites of doxepin with the passage of time would explain the decrease in recovery found with later collection periods in the radioactive study. Also, the metabolic pattern may differ among individuals, with some forming greater percentages of hydroxylated metabolites than others. Thus, the more than fourfold difference between individuals in the crossover studies may be more a reflection of metabolite differences than differences in biological availability. In either event, it is apparent from consistent recoveries after successive doses of doxepin that each individual absorbs and metabolizes each dose in a consistent manner.

With previously published methods for measurement of doxepin and (or) its metabolites in biological fluids, only a minute portion of the total doxepin-related material present could be detected. The present method provides a rapid, sensitive means for measuring a significant percentage of doxepin-related material excreted in human urine. When a crossover technique was used, a valid comparison of different doxepin formulations was obtained.

References