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Note

Sensitive and quantitative determination of plasma doxepin and desmethyldoxepin in chronic pain patients by gas chromatography and mass spectrometry

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Doxepin (N,N-dimethyl-3-dibenz[*b*,*e*]oxepin-11-(6H)-ylidene-1-propanamine) is a tricyclic antidepressant with anti-anxiety and antihistamine (sleep, sedation) properties. Although documentation is limited, it has also been found effective in chronic pain states which are often accompanied by depression [1]. The purpose of this study was to develop a precise and selective gas chromatography—mass spectrometry (GC—MS) method to analyze doxepin and its major metabolite desmethyldoxepin and to correlate the peripheral plasma concentrations with therapeutic analgesic, antidepressant, and sleep effects of doxepin. These clinical effects were observed in a double blind comparison between doxepin and placebo in 30 depressed, chronic pain patients [2].

Many analytical methods have been utilized to measure tricyclics in general and doxepin in particular. Gas—liquid chromatography (GLC) with flame ionization detection [3], GLC and capillary GLC with nitrogen—phosphorus detection [4—6], high-performance liquid chromatography (HPLC) (7, 8], radioimmunoassay [9], and GC—MS with mass fragmentography detection [10, 11]. These methods have proven to be adequate for high concentrations of doxepin but GC—MS offers the necessary precision, accuracy, selectivity, sensitivity and low sample volume requirements for clinical analyses. The method presented in this paper provides the necessary precision, selectivity, low sample volume (i.e., 1.0 ml) and a sensitivity of less than 1 ng/ml for the presented clinical studies of pain patients.

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MATERIALS AND METHODS

Standards and reagents

Doxepin hydrochloride (76.0% *trans*, 12.8% *cis* isomer and desmethyldoxepin *trans* isomer) were supplied by Pfizer (Central Research, Groton, CT, U.S.A.). Amitriptyline and nortriptyline were used as the internal standards and supplied by Wyeth Laboratories (New York, NY, U.S.A.). Hexane, methanol and isoamyl alcohol were distilled in glass (Burdick and Jackson Labs., Muskegon, MI, U.S.A.). Trifluoroacetic anhydride (Regis Chemical, Morton Grove, IL, U.S.A.), sodium hydroxide and hydrochloric acid were all ACS reagent grade. All glassware was rinsed previously with 10% isoamyl alcohol in hexane. This rinse prevents adsorption of the secondary and tertiary amines to the glassware.

Sample extraction and derivatization procedure

Blood samples were collected into Vacutainer tubes, kept on ice and centrifuged within 1 h. Plasma was transferred to plastic tubes and stored at −80°C for up to six months, prior to analysis, without loss of drug. After thawing, 1.0 ml of plasma was transferred to a 12-ml screw top test tube with a PTFE-lined cap and 50 μl of the internal standard (I.S.) (amitriptyline—nortriptyline, 60 ng:50 μl) in water was added. The mixture was made alkaline by the addition of 50 μl of 6 *N* sodium hydroxide followed by the addition of 5 ml of hexane—isoamyl alcohol (95:5, v/v). The tubes were shaken on an automatic shaker for 20 min and the phases were separated by centrifugation at 1700 *g*. The organic phase was then transferred to a graduated conical centrifuge tube. To selectively extract the tricyclics, 500 μl of 0.1 *N* hydrochloric acid was added to the organic phase and the samples were placed on the shaker for 10 min. The hydrochloric acid was then transferred to a 1.0-ml reacti-vial (Regis Chemical) and reduced to dryness at 45°C under a stream of ultra pure nitrogen. Derivatization of the desmethyldoxepin was achieved by the addition of 300 μl of a 5% trifluoroacetic anhydride (TFAA) solution and reacted at room temperature for 30 min. Higher concentrations of TFAA or higher reaction temperatures tend to degrade tricyclics or give multiple reaction products [11]. The reaction converted virtually 100% of desmethyldoxepin to its N-trifluoroacetyl derivative without degradation losses of doxepin. The reaction mixture was then taken to dryness under ultra-pure nitrogen at room temperature, redissolved in 25 μl ethyl acetate and 10 μl were injected directly into the GC—MS system for analysis.

Gas chromatography—mass spectrometry analyses

A Finnigan 3300 quadrupole mass spectrometer coupled to a Finnigan 9500 gas chromatograph and a 6000 data system were used to perform the analyses (Finnigan Instruments, Sunnyvale, CA, U.S.A.). The following chromatographic conditions were used: glass column, 1.1 m × 2 mm I.D., packed with 3% OV-17 on 100—120 Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). The column temperature was maintained at 220°C; injector, 260°C; separator, 300°C. The carrier gas (helium) was maintained at a flow-rate of 20 ml/min. The column was prepared for analyses by priming

with three injections of 10 μg each of doxepin and desmethyldoxepin trifluoroacetyl derivative. As previously reported [12], this is an essential step to avoid variable adsorption of the secondary and tertiary amines and ensure optimal chromatographic reproducibility. The mass spectrometer was operated at an electron energy of 70 eV, filament emission of 800 μA, and an ion source temperature of 75°C. The data system was programmed to scan the ions *m/e* 58.1 (doxepin), 165.1 (doxepin and desmethyldoxepin), 232.2 (nortriptyline) and 234.2 (desmethyldoxepin) at a rate of 1 sec per scan.

In parallel with each set of patient samples, a set of calibration samples were processed. Plasma (1 ml) was spiked with 10, 20, 50 and 100 ng of doxepin and desmethyldoxepin. These samples were analyzed first in duplicate and from the resulting area integration values the ratios vs. the internal standard were generated, plotted and used to quantitate subsequent clinical samples. It is essential to include adequate calibration points in the normal therapeutic range of 20—100 ng/ml doxepin, and 10—60 ng/ml desmethyldoxepin, to ensure validity of reported values. We used an internal standard ratio calibration curve generated daily to quantitate our daily set of samples. This curve varied by only ± 7% over a week's period of analyses.

Clinical study

A double blind comparison between doxepin and placebo in 30 depressed patients with chronic lumbar or cervical pain was undertaken. Dependent variables included Hamilton depression scale, Profile of mood states, Global assessment, Visual analog scale ratings of average pain severity, Percent time pain felt, Muscle tension, Effects of pain on sleep, mood, and activity, as well as plasma β-endorphin and enkephalin-like activity. Plasma levels of doxepin and desmethyldoxepin as described in this study were also correlated with these variables [2].

RESULTS AND DISCUSSION

A typical mass fragment chromatogram of a patient's sample is shown in Fig. 1. The ions chosen were based upon our analysis of the mass spectra of the reference material shown in Fig. 2. We used *m/e* 58.1 for doxepin and amitriptyline and *m/e* 232.2 and 234.2 for desmethyldoxepin and nortriptyline, respectively. The utility and validity of the developed methodology for providing clinically significant data was verified in a double blind study of patients receiving varying amounts of doxepin for pain. Significant improvements in doxepin-treated patients compared to placebo patients were observed in several variables including Hamilton depression scale, global assessment, percent time pain felt, muscle tension, sleep and mood. Maximal therapeutic effects occurred when combined plasma doxepin and desmethyldoxepin levels were near 70 ng/ml as determined by the assay technique described. Presented in Figs. 3 and 4 are linear correlations of the daily oral doxepin dosage taken by patients versus the concentration of doxepin (Fig. 3) and desmethyldoxepin (Fig. 4) found in their plasma utilizing our newly developed GC—MS procedure. After analyses were complete, it was determined that Hamilton depression scores which are used to determine the depth of depres-

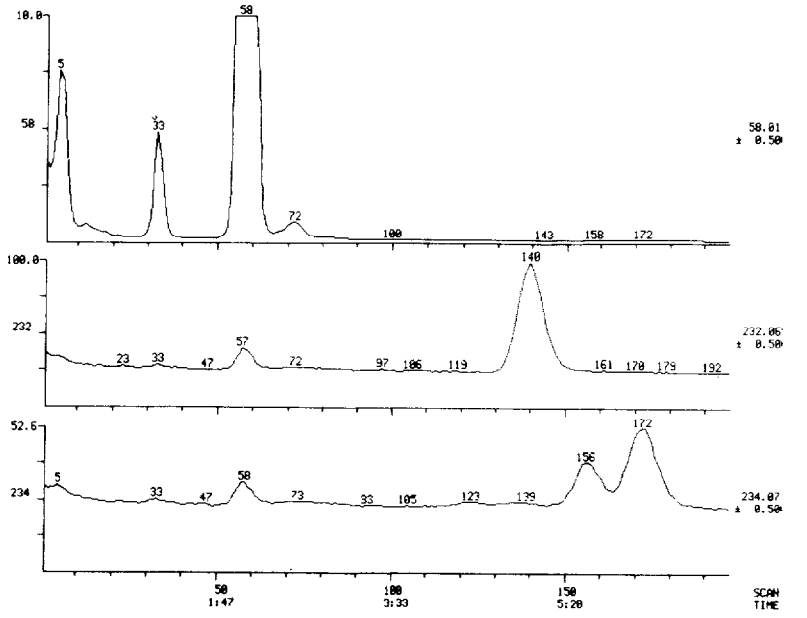

Fig. 1. Selected ion monitoring chromatogram of amitriptyline (I.S.), scan 58; doxepin, scan 72; nortriptyline (I.S.), scan 140; *cis*-desmethyldoxepin, scan 156; and *trans*-desmethyldoxepin, scan 172. This injection represents 0.4 ml (equivalent) of patient's plasma onto the column.

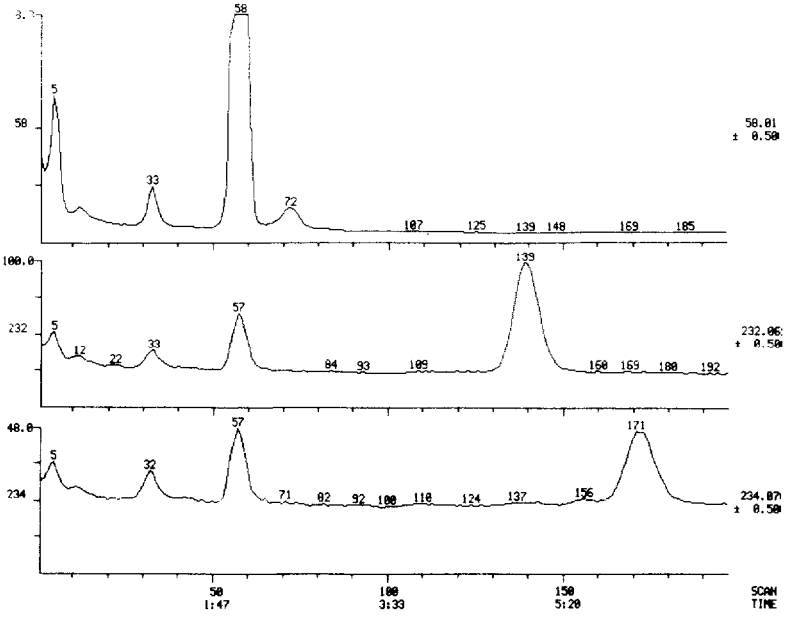

Fig. 2. Selected ion monitoring chromatogram of amitriptyline (I.S.), scan 58; doxepin, scan 72; nortriptyline (I.S.), scan 139; and *trans*-desmethyldoxepin, scan 171. This is a blank plasma spiked with drug.

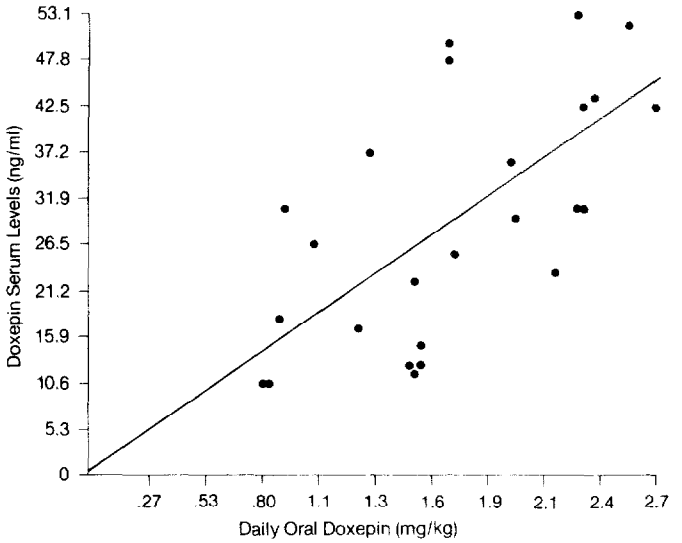

Fig. 3. Doxepin plasma levels (ng/ml) in depressed, chronic pain patients at varying daily oral doxepin doses. $y = 0.5 + 17.1\ x$ (x = oral dose), $r = 0.88$, $p < 0.001$, $n = 39$. Linearity suggests consistent absorption and metabolism.

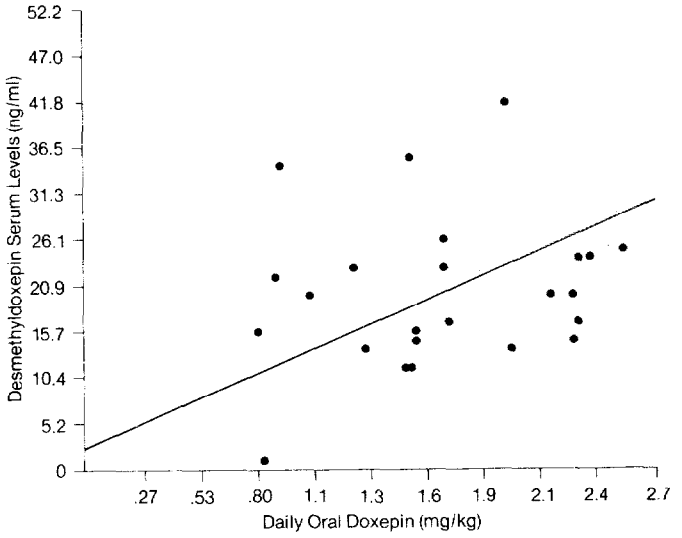

Fig. 4. Desmethyldoxepin plasma levels (ng/ml) in depressed, chronic pain patients at varying daily oral doxepin levels. $y = 2.25 + 10.3\ x$ (x = oral dose), $r = 0.72$, $p < 0.001$, $n = 39$. Values include both *cis* and *trans* isomers.

sion were significantly ($p < 0.01$) decreased by doxepin. Doxepin and the desmethyl metabolite were also significantly ($p < 0.01$) associated with a decrease in the percent of time pain was felt.

Therefore, utilizing an improved GC—MS procedure which uses only 1 ml plasma and has a sensitivity of less than 1 ng/ml, we found an 88% correlation of patient plasma doxepin concentration to daily oral doxepin and a 72% correlation with the plasma desmethyl metabolite formation. Therefore, this

newly developed GC—MS procedure can be used to study various clinical effects of doxepin as well as patient compliance to the drug due to its inherent sensitivity and low sample volume necessary for analysis.

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