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DETERMINATION OF DOSULEPIN AND ITS METABOLITE: APPLICATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

Dosulepin, 11-(3-dimethylaminopropylidene)-6,11-dihydrodibenzo[*b,e*]thiepin hydrochloride, is a thio analogue of amitriptyline and is used for the treatment of anxiety and affective disorders. The present study developed a simple and sensitive procedure for the determination of this compound and its metabolite, northiaden, by a combination of high-performance liquid chromatography with electrochemical detection. Hydrodynamic voltammograms demonstrated an optimal applied potential at 1300 mV for both dosulepin and northiaden. A mobile phase consisting of 0.1 *M* acetate buffer–acetonitrile–perchloric acid–trichloroacetic acid (50:50:2:1.5) provided the best separation of the drugs. The extraction procedure, which used a heptane–isoamyl alcohol (99:1) mixture, was successfully applied with a recovery of over 90%. A preliminary pharmacokinetic study was performed by the proposed method.

INTRODUCTION

Dosulepin, 11-(3-dimethyaminopropylidene)-6,11-dihydrodibenzo[*b,e*]-thiepin hydrochloride (Fig. 1), is a dibenzothiepine derivative which has been reported to be clinically effective for depression with anxiety [1–3]. Many clinical surveys have indicated a potency comparable with the typical tricyclic antidepressants, imipramine and amitriptyline, with a lower toxicity [4,5]. In a recent study, we demonstrated that the compound blocks the uptake of catecholamine, but not serotonin, in rat brain [6]. Other pharmacological studies have shown that the drug represents a new type of tricyclic compound sharing both potent antidepressant and tranquillizing properties [7]. These results agreed with the findings indicating that dosulepin is a useful drug for the treatment of anxiety and affective disorders [8].

In recent therapeutics, it has become important to monitor the fate of admin-

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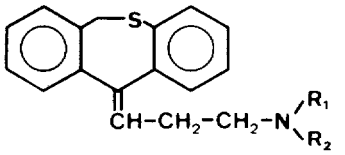


	R_1	R_2
Dosulepin:	CH_3	CH_3
Northiaden:	CH_3	H

Fig. 1. Chemical structure of 11-(3-dimethylaminopropylidene)-6,11-dihydrodibenzo[*b,e*]thiepin (dosulepin) and its metabolite, northiaden.

istered drugs. For this purpose, a simple and sensitive procedure for determination of the drug is required. Dosulepin has been reported to be detectable by gas chromatography combined with mass spectrometry (GC–MS) [9,10]. However, gas chromatography is complicated by the requirement of a volatile derivative. Another procedure for the determination of this compound is high-performance liquid chromatography (HPLC) with a UV detector [11]. HPLC is a convenient tool for separating non-volatile compounds. However, the sensitivity of the UV detector is insufficient to monitor the concentration of dosulepin in blood samples. The present study was undertaken to develop a sensitive procedure for the measurement of dosulepin with its active metabolite, northiaden, in biological samples.

EXPERIMENTAL

Apparatus

A liquid chromatographic system (Model 510, Waters Assoc., Milford, MA, U.S.A.) was used with a six-port injector (Model 7125, Rheodyne, Berkeley, CA, U.S.A.) and a glassy carbon amperometric detector (VMD-501, Yanagimoto, Kyoto, Japan). The analytical column consisted of an Ultrasphere-ODS reversed-phase column (average particle size, 5 μm; 250×4.6 mm I.D.; Altex, Berkeley, CA, U.S.A.). To protect the analytcal column, a short ODS column (10×4.5 mm I.D.) was equipped. The detector potential was finally set at 1.3 V versus the Ag/AgCl reference electrode.

Cyclic voltammography was carried out using a Model CV-1B detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.). The working electrode was of glassy carbon. The scanning rate was set at 100 mV/s.

Reagents

Dosulepin hydrochloride and northiaden hydrochloride were generous gifts from Kaken Pharmaceuticals (Tokyo, Japan). Imipramine hydrochloride, the internal standard, was purchased from Sigma (St. Louis, MO, U.S.A.). Other chemicals for extraction and chromatography were all analytical grade (Wako, Osaka, Japan) and were used without further purification.

The chromatographic mobile phase consisted of 0.1 *M* acetate buffer–acetonitrile–perchloric acid–trichloroacetic acid (50:50:2:1.5, pH adjusted 2.0 by sodium hydroxide. The flow-rate was 1.5 ml/min.

Animals

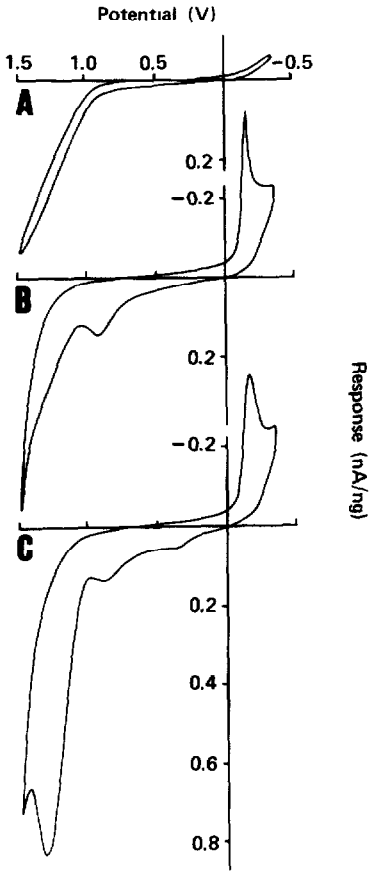
Wistar rats weighing ca. 250 g were used. All animals were kept in a room with a controlled temperature (23 ± 0.5 °C), humidity (55 ± 5%) and light cycle (12 h illumination with the light being turned on at 07:00). They were injected intravenously with 10 mg/kg dosulepin, and sacrificed by decapitation at different times after the injection. The brain was obtained as quickly as possible after decapitation, and the hippocampus was dissected out according to the procedure of Glowinski and Iversen [12]. The samples were stored at −80 °C until assay. Blood samples were collected from the carotid artery when the animals were decapitated. The serum was also stored in a deep freeze after separation.

Extraction procedure

The brain sample (hippocampus, ca. 120 mg) was transferred to a glass-stoppered tube and homogenized in 250 μl of distilled water containing the internal standard (imipramine). The 500 μl of 1 *M* sodium hydroxide and 5 ml of an organic solvent mixture of heptane–isoamyl alcohol (99:1) was added to the tube. The tube was vortexed for 1 min and then centrifuged at 1000 *g* for 3 min to separate the organic layer, of which 4 ml were transferred to another tube. Then 100 μl of 0.1 *M* hydrochloric acid were added to the tube, which was then vortexed for 1 min. After brief centrifugation to separate the layers, a portion of the hydrochloric acid layer was subjected to chromatographic determination. To a tube containing 100 μl of serum sample were added 0.5 ml of 1 *M* sodium hydroxide and the internal standard. The tube was vortexed vigorously, and the same extraction procedure as above was applied.

RESULTS AND DISCUSSION

Pharmacokinetic monitoring is an important requisite for reasonable therapeutics. For this purpose, a simple and sensitive procedure is needed for determining the concentration of drug administered. Therapeutic drugs are generally water-soluble and non-volatile, which means that they are difficult to assay by GC–MS. HPLC is applicable to such substances. Dosulepin, a dibenzothiepine derivative, has been reported to be assayed by HPLC with UV detection [11]. However, the sensitivity was insufficient for precise determination using a small sample. Electrochemical detection (ED) is now employed for heterocyclic substances, such as dibenzazepine [13], benzodiazepine [14] and phenothiazine derivatives [15,16]. The dibenzazepines are classified as tricyclic antidepressants, as are imipramine and desmethylimipramine (desipramine). Dosulepin, a dibenzothiepine derivative, is a structural analogue of the imipramines. However, the dibenzothiepine derivative yielded little electrochemical reaction in a mobile phase consisting of 0.1 *M* acetate buffer and acetonitrile, which had previously been to be effective for imipramine [13].



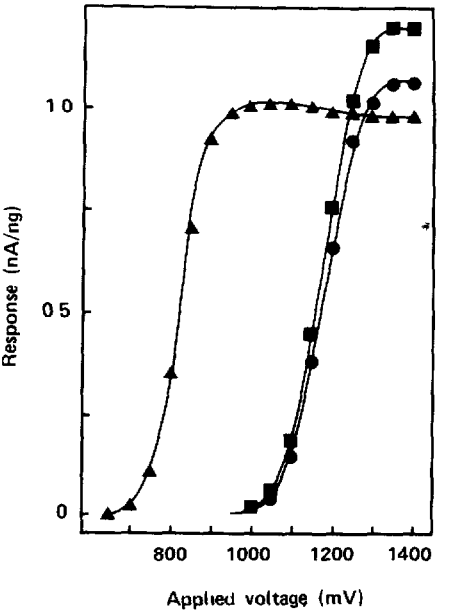


Fig. 2. Cyclic voltammograms of dosulepin. The reaction media were: 0.1 *M* acetate buffer containing 50% acetonitrile (basic mixture, A); basic mixture plus 2% perchloric acid (B); and basic mixture plus 2% perchloric acid and 1.5% trichloroacetic acid (C). The scanning rate was 100 mV/s. Note that both perchloric acid and trichloroacetic acid were required for the electrochemical reaction of dosulepin.

Fig. 3. Hydrodynamic voltammograms of dosulepin (●), northiaden (■) and imipramine (▲).

Cyclic voltammography was carried out to determine an appropriate medium for electrochemical reaction (Fig. 2). A basic mixture of 0.1 *M* acetate buffer containing acetonitrile [13] did not show any oxidative response at applied voltages up to +1.5 V. Addition of perchloric acid (PCA) resulted in a response at 900 mV (Fig. 2B). However, the response was still insufficient to be used for detection in chromatography. Furthermore, a concentration of PCA in excess of 2% yielded high background current, and rendered accurate determination impossible. Another response occurred at 1.3 V when trichloroacetic acid (TCA) was added to the medium (basic mixture plus perchloric acid). The optimal concentration of TCA was estimated as 1.5% according to the maximum signal-to-noise ratio (Fig. 2C).

Hydrodynamic voltammograms of dibenzothiepines (dosulepin, northiaden)

and imipramine are shown in Fig. 3. Both dosulepin and northiaden caused electrochemical responses at 950 mV versus the Ag/AgCl reference electrode. Both responses increased steadily until the applied voltage reached 1.3 V, and then plateaued. These findings were in good agreement with cyclic voltammograms. On the other hand, the electrochemical response of imipramine differed markedly from those of the dibenzothiepines. The internal standard caused a response at 650 mV, which reached a plateau at 950 mV. This result was similar to that obtained in a previous study [13]. A further increase in the applied voltage did not decrease the electrochemical response of the internal standard. At the applied voltage at which the assay was carried out (1.3 V), the electrochemical response of imipramine was broadly similar to those of dibenzothiepine derivatives. This implied that imipramine was electrochemically appropriate as the internal standard for this assay system.

The retention times of substances in a reversed-phase column are influenced by the concentrations of organic solvents in the chromatographic carrier. However, since the electrochemical reaction occurs only in aqueous solution, the choice of organic solvent is restricted. For example, tetrahydrofuran and methanol, which are frequently employed in HPLC–ED, are inadequate for separating dibenzothiepines because the retention times of the substances are not shortened at practical concentrations. Acetonitrile is known to promote reactions involving ionization, and so we used it in the present study with HPLC–ED to shorten the retention times of hydrophobic substances in the reversed-phase column. The effects of acetonitrile on the retention times of dosulepin, northiaden and imipramine were examined to decide the optimal concentration of acetonitrile (Fig. 4). An increase in the concentration of the organic solvent shortened the retention times of all these substances by almost the same amount. A concentration of 50% was optimal for separating the three substances in one chromatographic run. This concentration did not interfere with the present electrochemical reactions.

The detector response (peak height) varied with the amount of dosulepin or northiaden injected. The ratio of dibenzothiepines to the internal standard showed a linear relationship over a wide range of dosages between 1 ng and 1 μg ($r = 0.998$, $P < 0.001$). This means that a simple comparison of the peak height is applicable for calibration of the concentrations of the tricyclic antidepressants and their metabolites.

The recoveries of dosulepin and northiaden were over 90% after adjusting for solvent loss, and the ratios of these substances to imipramine were constant in the dose range from 1 ng to 1 μg.

A typical chromatogram of a blood sample is shown in Fig. 5. In this case, a rat was injected intravenously with 10 mg/kg dosulepin. The animal was killed 1 h after the injection and a blood sample was obtained from the carotid artery. After extraction, 20 μl of the final aqueous layer was injected into the column. While a relatively high voltage was applied to the electrode, no biological substances such as monoamine transmitters and aromatic amino acids interfered with the quantitation of the dibenzothiepine derivatives. A small amount of northiaden, a metabolite of dosulepin, occurred in the blood of the animal injected with the

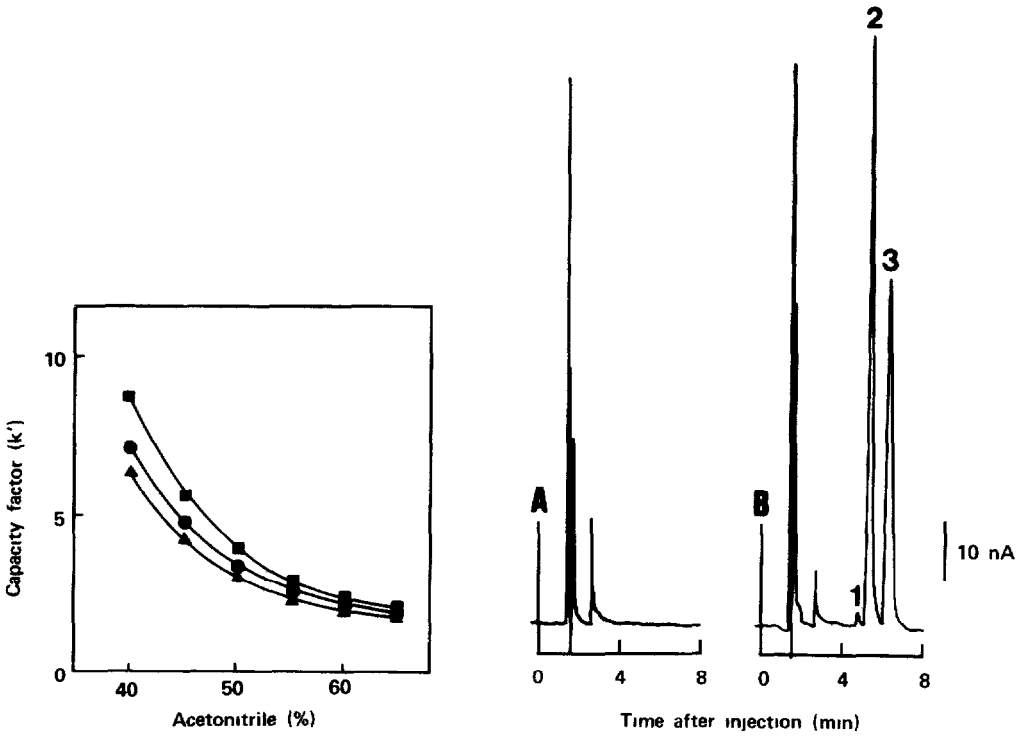


Fig. 4. Effects of acetonitrile on the retention times of dosulepin (●), northiaden (▲) and imipramine (■).

Fig. 5. Typical chromatograms obtained by an injection of a blood sample from rats injected with physiological saline (A) and injected intravenously with 10 mg/kg dosulepin at 1 h before sacrifice (B). For details of the chromatographic conditions, see Experimental. Peaks: 1 = northiaden; 2 = dosulepin; 3 = imipramine (internal standard).

parent drug. One chromatographic run was completed within 8 min, and 60 samples could be assayed in 8 h of routine work.

The pharmacokinetics of dosulepin were examined in rats up to 12 h after giving an intravenous injection of dosulepin (Fig. 6). The concentrations of the drug were always higher in the brain tissue than in the blood. For example, at 30 min after the injection, the intracerebral concentration was estimated to be 40.5 μg/g wet tisue (the highest level), as opposed to only 0.7 μg/ml in the blood. Determination was possible in the blood up to 24 h later (3 ng/ml). The time course of dosulepin disappearance was estimated after giving an intravenous injection of the drug at a dose level of 10 mg/kg. The disappearance curves were linear in both the brain and blood with correlation coefficients of -0.994 and -0.991, respectively ($P < 0.001$). The biological half-lives of dosulepin were estimated to be 1.69 and 2.50 h for the brain and blood, respectively, by the damping Gauss–Newton method.

Desmethylated metabolites of tricyclic antidepressants are pharmacologically active. Desipramine has been reported to exert a stronger blocking property on

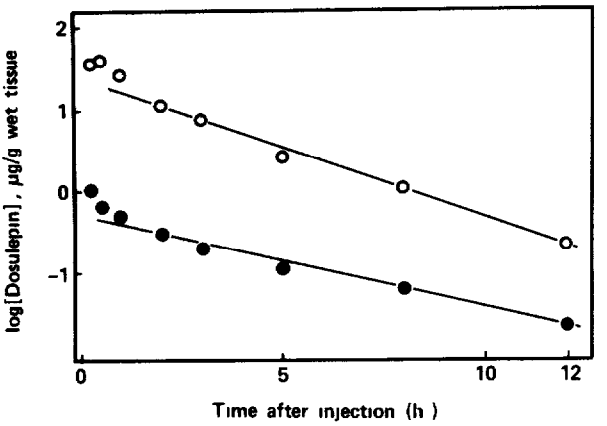


Fig. 6. Disappearance curves of dosulepin from the blood (●) and brain samples (hippocampus, ○) of rats. The biological half-lives were estimated to be 1.69 and 2.5 h for the brain and blood, respectively.

amine uptake mechanisms, especially that of dopamine [15]. On the other hand, our recent study has demonstrated that northiaden also has an the effect on the dopamine and noradrenaline uptake mechanisms [6]. The side-effects of tricyclic antidepressants have frequently been discussed in terms of their desmethyl metabolites. Thus, simultaneous determination of these metabolites is clinically required. Following intravenous injection of dosulepin, northiaden, a metabolite of dosulepin, was detected in both the brain and blood. The concentration was the highest at 30 min after the injection in the blood (44.6 ng/ml). This value seems to be lower compared with that of desipramine after imipramine administration [14]. The precise mode of action will be discussed elsewhere [17].

In conclusion, the procedure described here was found to be useful for monitoring the concentrations of dosulepin, a tricyclic antidepressant, and its desmethylated metabolite.

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