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Note

Gas chromatographic–mass spectrometric analysis and preliminary human pharmacokinetics of sertraline, a new antidepressant drug

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The involvement of monoamine neurotransmitters in depressive disorders has long been recognized [1, 2]. The tricyclic antidepressants, the most widely used therapeutic agents, are thought to act primarily by inhibiting the reuptake of released norepinephrine to facilitate synaptic transmission [3, 4]. They are also reported to inhibit serotonin uptake and to increase the sensitivity of its post-synaptic receptors [5, 6]. The mixed pharmacological action of the tricyclics is implicated in their low therapeutic index and their limiting anticholinergic and cardiovascular side-effects [7, 8]. Recent studies suggest that serotonin is particularly involved in mood regulation and that compounds selectively blocking its synaptosomal uptake mechanism would be more effective antidepressants [8, 9]. Newly discovered agents such as zimeldine, fluvoxamine, fluoxetin and, more recently, sertraline were designed to be selective serotonin uptake inhibitors [10, 11].

Sertraline [1*S*,4*S*-N-methyl-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-1-naphthylamine] exhibited better selectivity for inhibiting serotonin uptake than all the other agents [12]. It potentiates pharmacological effects dependent on serotoninergic activity without enhancing those involving catecholamines [13]. It was shown to be well tolerated in mice, rats and dogs with no locomoter stimulant effects in rats or untoward cardiovascular effects in dogs [13]. Sertraline promises to be an efficacious drug possessing a favorable therapeutic index for the management of depressive illness. An analytical method for sertraline quan-

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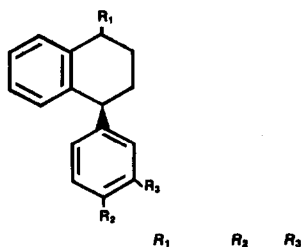

	R_1	R_2	R_3
1. Sertraline	CH_3HN—	Cl	Cl
2. Internal Standard	CH_3HN'''''	Br	H

Fig. 1. Structure of sertraline and the internal standard.

tification in plasma was needed to support clinical studies. This report describes the method and summarizes the drug's preliminary pharmacokinetics in man following single oral doses.

EXPERIMENTAL

Materials

All solvents were glass-distilled (Burdick & Jackson, Labs., Muskegon, MI, U.S.A.) and all reagents were ACS grade and were used as received. Sertraline and the internal standard (CP-53,631) (Fig. 1) were obtained from Pfizer. The glassware was soaked overnight in chromic acid, thoroughly rinsed, dried, and then silylated with hexamethyldisilazane (Pierce, Rockford, IL, U.S.A.) as described previously [14]. Standard solutions of sertraline and of the internal standard were prepared monthly in ethyl acetate (0.1 mg/ml) and were kept at 4°C. From these, dilute solutions were prepared daily in methanol.

Assay

Plasma samples (3 ml) containing no more than 40 ng/ml sertraline were vortexed for 10 s with 15 ng of the internal standard methanolic solution (0.1 ml) and then with 0.25 ml of aqueous 1 *M* sodium hydroxide. The mixture was extracted with diethyl ether (2×5 ml) and the combined extracts were transferred to a 15-ml centrifuge tube and evaporated to dryness by a dry nitrogen stream. Each sample was dissolved in 0.2 ml of acetonitrile and mixed with 1 ml of 0.1 *M* hydrochloric acid. The mixture was extracted with hexane (2×2 ml) and the hexane extracts were discarded. The aqueous phase was basified with 0.4 ml of 6 *M* sodium hydroxide and re-extracted with diethyl ether (2×2 ml). The combined ether extracts were transferred to a 15-ml centrifuge tube and evaporated to dryness. The residues were dissolved in 20 μl of ethyl acetate, and aliquots (2–5 μl) were injected into the chromatograph. Plasma samples containing more than 40 ng/ml sertraline were diluted with control human plasma and reassayed.

Instrumentation

Analysis was carried out on an LKB-9000 combined gas chromatographic–mass spectrometric (GC–MS) system equipped with an accelerating

TABLE I

ANALYSIS OF PLASMA SAMPLES CONTAINING KNOWN AMOUNTS OF SERTRALINE: INTRA-ASSAY PARAMETERS

Five plasma samples fortified at each concentration.

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (mean) (%)
1	1.0 ± 0.07	7.0	100
2	2.1 ± 0.13	6.2	105
6	5.9 ± 0.18	3.1	98
10	10.7 ± 0.32	3.0	107
15	15.0 ± 0.45	3.0	100
20	20.0 ± 0.20	1.0	100
40	40.0 ± 0.20	0.5	100

voltage alternator unit set to monitor the ions at m/z 274 and 286. The ionization energy was 20 eV. The samples were introduced by GC on a 0.8 m × 2 mm I.D. glass column packed with 3% Silar 10C on Gas Chrom Q 80–100 mesh. The injector, column and separator temperatures were set at 200, 255 and 290°C, respectively. The flow-rate of the carrier gas, helium, was kept at 40 ml/min. Under these conditions, the retention times of sertraline and its internal standard were 3.9 and 4.3 min, respectively. The signals from the mass spectrometer were recorded on a two-pen recorder (Hitachi Model 196-0010).

Calculations

Plasma sertraline concentrations were calculated from the peak height at m/z 274 relative to the internal standard peak at m/z 286 with reference to a standard curve constructed from plasma samples fortified with known amounts of sertraline. The assay parameters are shown in Tables I and II.

TABLE II

ANALYSIS OF PLASMA SAMPLES CONTAINING KNOWN AMOUNTS OF SERTRALINE: INTER-ASSAY PARAMETERS

Ten sets of fortified plasma samples were assayed. Each set (one sample per concentration) was assayed on a different day over a six-month period.

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	Coefficient of variation (%)	Accuracy (mean) (%)
1	1.1 ± 0.08	7.3	110
2	2.0 ± 0.12	6.0	100
6	5.8 ± 0.16	2.8	97
10	10.1 ± 0.22	2.2	101
15	14.9 ± 0.29	1.9	99
20	20.0 ± 0.22	1.1	100
40	40.1 ± 0.18	0.5	100

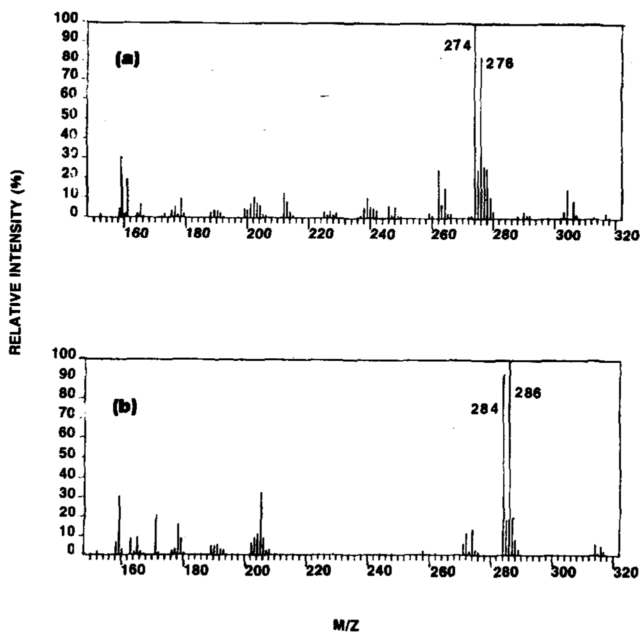

Fig. 2. Mass spectra of sertraline (a) and the internal standard (b). Both spectra were taken at 20 eV.

Human studies

The hydrochloride salt of sertraline was administered in capsules to healthy male volunteers at the dose level of 50, 100, 125, 150, 175, 200, 250, 300, 350 and 400 mg. Each dose level was tested in three different subjects. The subjects were fasted overnight before dosing and food was withheld for an additional 2 h post-dose. Blood samples were drawn into heparinized tubes at 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h. Plasma was obtained by centrifugation and frozen until assay.

RESULTS AND DISCUSSION

Examination of the electron-impact mass spectra of sertraline and the internal standard, recorded at 20 eV (Fig. 2), shows the most abundant ion to be the $[M-CH_3NH_2]^+$ ion. This ion was used to monitor drug levels at 20 eV. Its absolute abundance was reduced at higher ionization energy, leading to a loss of the sensitivity of the assay when ionization energy higher than 20 eV was applied. For sertraline, the $[M-CH_3NH_2]^+$ ion is the dichlorophenyl dihydronaphthalene ion at *m/z* 274. For the internal standard, the $[M-CH_3NH_2]^+$ ion is the bromophenyl dihydronaphthalene ion at *m/z* 284 and its corresponding bromine isotopic abundance ion at *m/z* 286. Comparable results were obtained by monitoring either ion. Monitoring the ion at *m/z* 286, however, afforded a marginal

TABLE III

PHARMACOKINETIC PARAMETERS

Values are average of three subjects. T_{max} = time at which the peak plasma concentration was observed; C_{max} = observed peak plasma concentration divided by the dose; $AUC_{0-48\,h}$ was calculated by trapezoidal approximation and divided by the dose.

Dose (mg)	T_{max} (h)	C_{max} (mg/ml per mg dose)	$AUC_{0-48\,h}$ (ng h/ml per mg dose)
50	6	0.19	5.3
100	8	0.16	4.2
125	8	0.18	5.0
150	8	0.14	3.8
175	8	0.24	5.6
200	6	0.28	7.1
250	8	0.18	4.2
300	8	0.26	6.2
350	8	0.31	7.8
400	10	0.22	6.2
Mean ± S.D.	7.8 ± 1.1	0.22 ± 0.06	5.5 ± 1.3

improvement in background noise. No peaks at the retention times of the drug or the internal standard were present in control plasma samples analyzed according to the method described. The addition of the internal standard did not contribute any additional peaks at the retention time of sertraline. Calibration curves for sertraline in plasma were linear from 1 to 40 ng/ml. The correlation coefficients over a six-month period averaged 1.000 ± 0.05% (n = 10). The precision of the assay was 7% or better and its accuracy ranged from 97 to 110% (Tables I and II). Sertraline is metabolized via N-demethylation and various other routes [15]. The primary amine metabolite does not elute from the standard GC column under the present assay conditions. None of the other metabolites co-elutes with sertraline and none yields an abundant ion at m/z 274 by electron ionization. The present method is, therefore, specific for the quantitative determination of sertraline.

Plasma concentrations in 30 subjects receiving single oral 50- to 400-mg sertraline doses were plotted to calculate the pharmacokinetic parameters. The drug was slowly absorbed from oral doses. The maximum plasma concentration was not reached until 6–10 h post-dose (Table III). The distribution and elimination of sertraline were also slow. The terminal elimination phase did not occur until 12 h after dose. Accurate calculation of the terminal half-life for elimination from plasma was not possible because only three concentration values (12, 24 and 48 h) were available for calculation. Based on these limited data, the average half-life was estimated to be about 24 h. Despite the slow absorption, distribution and elimination, sertraline appeared to be consistently absorbed. As shown from Table III, the peak plasma concentration (C_{max}) and the area under the plasma concentration curve (AUC) were generally proportional to the dose over the range 50–400 mg.

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