Mass Fragmentographic Analysis of Clomipramine and Its Mono-Demethylated Metabolite in Human Plasma

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Abstract. A mass fragmentographic method for the quantification of clomipramine (CIM) and mono-demethyl-clomipramine (DCIM) in human plasma was developed. The deuterium labelled analogues of the compounds were used as internal standards. The sensitivity of the method allows the determination of CIM and DCIM in plasma after oral doses with a standard deviation less than 7% at concentrations of 25 ng/ml. The method was applied to the analysis of drug concentrations in plasma of clomipramine treated healthy volunteers and depressed patients. After acute treatment the level of DCIM in plasma was low as compared to chronic treatment.

Key words: Clomipramine — Mono-demethyl-clomipramine — Mass fragmentography — Plasma.

Clomipramine (CIM) is a recently introduced tricyclic antidepressant drug which inhibits 5-hydroxytryptamine (5-HT) uptake in brain neurons (Carlsson et al., 1969). Controlled clinical trials have demonstrated its antidepressant effect (Jimenez, 1968; Rickels et al., 1974) and several reports also suggest a therapeutic effect on patients with obsessive-compulsive neurosis (Marshall, 1971). Most tricyclic antidepressant drugs are metabolized with marked individual differences resulting in a wide range of plasma levels in patients taking a fixed dose (Hammer and Sjöqvist, 1967). For one of the antidepressants, nortriptyline, a therapeutic range for the drug level in plasma has been demonstrated (Åsberg et al., 1971). For such reasons the development of sensitive and specific analytical methods for the antidepressant drugs are required and in the case of the tertiary amines also for the demethylated metabolites that are also biologically active. This paper presents a mass fragmentographic method for the analysis of CIM and its demethylated metabolite demethyl-clomipramine (DCIM) in plasma. The mass fragmentographic (MF) technique has a higher sensitivity than the liquid chromatographic method recently described (Mellström and Eksborg, 1976). The MF method also has a high reproducibility since deuterated analogues can be used as internal standards.

METHODS

The study was performed on 5 patients and 5 healthy volunteers. The patients were acutely admitted to the clinic because of depressive symptoms. Three were men (age 40–63) and 2 were women (age 31 and 44). The volunteers were all women (age 21–46). No subject received any drug for at least 1 week before the study.

Following a placebo period lasting from 1–4 days, treatment of the patients was initiated with clomipramine (Anafranil, Geigy) 50 mg/day p.o. with daily increment of 25 mg up to a final dose of 100 mg/day divided into 4 doses. The volunteers received a single oral dose of clomipramine, 25 mg.

Blood samples were taken from all the subjects in the morning before breakfast and before the first dose of the day. All the subjects were fasting for at least 10 h before the drug administration. In the patients, samples were taken during the first 2–4 weeks of treatment. From the volunteers, blood was collected before and during the 24 h period following clomipramine administration.

The blood samples were taken by venipuncture into heparinized tubes. Plasma and red cells were separated by centrifugation at 1000 g for 10 min. Within 1 h plasma was frozen at −20 °C pending the analysis.

Extraction and Derivatization Procedure. The internal standards CIM-D₃ (750 ng) and DCIM-D₃ (750 ng) were added to a 2 ml plasma sample. After the addition of 1 ml 5% NaOH the sample was shaken with 15 ml heptane for about 20 min. The heptane layer was transferred to another tube and the amines extracted into 3 ml 0.05 M HCl. The heptane phase was then discarded and the water phase made alkaline by adding 0.2 ml 5% NaOH. Isopropyl alcohol in toluene (15:85, v:v), 0.5 ml, was added to the water phase and after thorough mixing and centrifugation for 10 min the tube was placed in a deep freeze. After the water layer was
frozen the organic layer was transferred to a conical test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 30 μl ethyl acetate and stored at -20°C pending the analysis. After the MF analysis of CIM (see below) the sample was again evaporated to dryness. DCIM was derivatized by adding 50 μl of 1% PFPA in toluene and reacting for 1 h at room temperature. The toluene was then evaporated and the residue dissolved in 30 μl toluene and the sample was stored at -20°C pending the analysis of DCIM.

Synthesis of DCIM-D3. This compound was prepared from didemethylclomipramine in a two step synthesis. Didemethyl-clomipramine was condensed with ethylchloroformiat according to Weiss (1965). The N-carboetoxy compound formed was without further purification reduced with lithium-aluminium deuteride (LAD) in tetrahydrofuran (2.5 h reflux). The mixture from the reduction was separated with preparative thin layer chromatography (SiO₂, acetic acid/ethanol/ethylacetate/water, 17:17:56:10). DCIM-D₃ was recovered in a yield of about 10%. The mass spectrum of the PFPA derivative of DCIM-D₃ is shown in Figure 2.

Chemicals Used. All solvents were of analytical grade. CIM-HCl (Anafranil), DCIM-HCl and didemethyl-CIM-HCl were gifts from Ciba-Geigy AB, Sweden. CIM labelled with 3 deuterium atoms in the methyl group and pentafluoropropionic anhydride (PFPA) were obtained from Produktkontroll AB, Stockholm, Sweden.

GC-MS Analysis. A Pye 104 gas chromatograph coupled to an LKB 2091 mass spectrometer with an accelerating voltage alternator (AVA) unit was used. The column (1.5 m x 2 mm) was packed with 3% SE-30 on chromosorb W 80/100 mesh. The column temperature was 230—240°C and the helium flow rate 20—30 ml/min. The volume of sample injected was 1—3 μl. The mass spectrometer settings were: Separator temperature 270°C, ion source temperature 280°C. Ionization potential 22.5 eV. Trap current 100 μA.

For the analysis of CIM the magnetic field was adjusted to m/e 317—the molecular ion of CIM-D₃—with the accelerating voltage set at 3.0 KeV which was intermittently increased to allow alteration between m/e 317 and 314, the molecular ion of CIM. The setting for DCIM analysis were m/e 449 and 446.