Journal of Chromatography, 491 (1989) 432–438
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4752

Note

Mass spectral characterization of urinary pipamperone metabolites and high-performance liquid chromatography assay for pipamperone plasma levels

CLAUS KÖPPEL*

Reanimationszentrum, Klinikum Rudolf Virchow, Standort Charlottenburg, Freie Universität Berlin, Spandauer Damm 130, D-1000 Berlin 19 (F.R.G.)

and

JOACHIM TENCZER and ANGELIKA WAGEMANN

Abteilung Toxikologie, Landesuntersuchungsinstitut für Lebensmittel, Arzneimittel und Tierseuchen Berlin, Invalidenstrasse 60, D-1000 Berlin 21 (F.R.G.)

(First received September 14th, 1988; revised manuscript received March 11th, 1989)

Pipamperone, 1′-[3-(4-fluorobenzoyl)propyl]-1,4′-bipiperidine-4′-carboxamide, is a butyrophenone with moderate neuroleptic activity. Clinically it is used mainly in geriatric patients with psychomotoric agitation, aggressive behaviour or senile dementia [1–5]. The recommended oral dose is three times 20–120 mg per day (package circular of Dipiperon®, Janssen, Neuss, F.R.G.).

Little is known about the pharmacokinetics and metabolic disposition of pipamperone (I, Fig. 1). Its metabolism has been studied in rats [6,7], and the main metabolites were found to be 4-fluorophenylacetic acid (XII), its glycine conjugate (XIII), and 4-fluorophenyl-4-ketobutyric acid (XI) (Fig. 1). A further metabolite (XIV) was generated by elimination of the carboxamide group. Using a gas chromatographic–mass spectrometric (GC–MS) screening procedure, Pfleger et al. [8] identified I, IV, IVa, V and VI in acetylated urine extracts of patients with pipamperone overdose.

In a case of pipamperone intoxication, we were able to identify several previously unknown metabolites, artifacts and derivatives with GC–MS [9]. This finding prompted us to study urinary pipamperone metabolism in patients given

0378-4347/89/$03.50

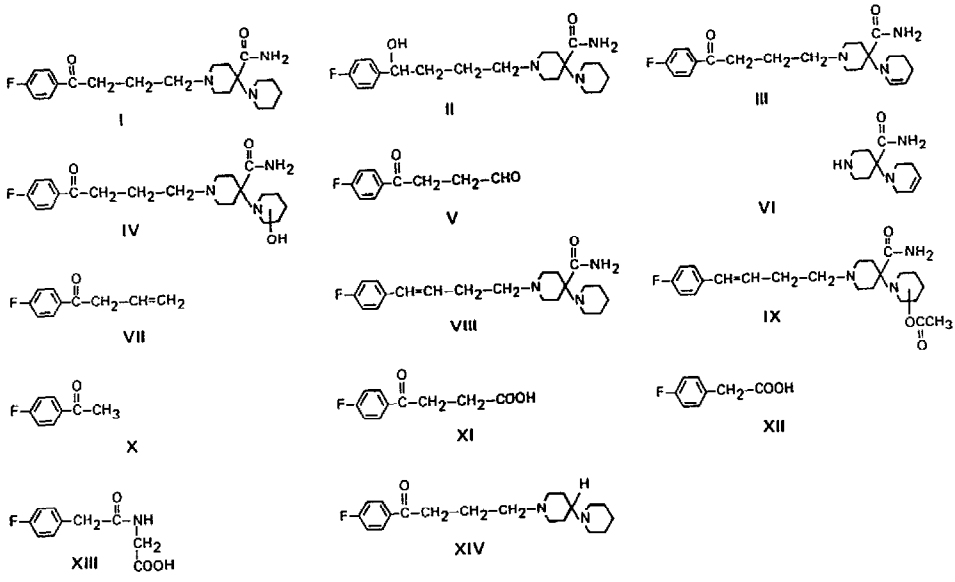

Fig. 1 Pipamperone, its metabolites, artifacts and derivatives.

repeated oral doses of pipamperone. Additionally, pre-dose steady-state plasma levels of pipamperone were determined with high-performance liquid chromatography (HPLC) after oral administration of pipamperone.

EXPERIMENTAL

Clinical studies

Urine was collected for 24 h from five geriatric patients (three females, two males) on an oral dose of three times 20 mg of pipamperone (three 5-ml doses of Dipiperon®-Sirup, Janssen) per day for psychomotoric agitation. All patients were hospitalized because of acute myocardial infarction. Further medication included intravenous nitroglycerine (3 mg/h). Laboratory tests were normal with respect to liver and kidney function. Urine samples were pooled and stored at $-20\,^{\circ}C$ prior to analysis.

Blood was withdrawn from three patients with senile dementia who were receiving three 120-mg doses of pipamperone (three sets of three tablets of Dipiperon) for ten days before intake of the next dose. The samples were immediately centrifuged and stored at $-20\,^{\circ}C$ prior to HPLC analysis.

Reagents and chemicals

Pure samples of pipamperone (I) were generously provided by Janssen. Compound II was synthesized by reduction of I with sodium tetrahydroborate. Reaction of II with acetic anhydride yielded VIII. The purity of the reference compounds was checked by GC–MS. All reagents, analytical-reagent grade or

better, were purchased from commercial sources and used without further purification.

GC–MS analysis of urine samples

A 20-ml sample of urine was extracted twice at pH 3 and subsequently at pH 10 with diethyl ether (Nanograde®, Mallinckrodt, St. Louis, MO, U.S.A.). The two extracts were combined, and the organic solvent was removed with a dry stream of nitrogen. The residue was dissolved in 100 μl of methanol, and a 1–3 μl aliquot was used for GC–MS. Urine was extracted similarly after incubation (37°C) with 0.5 ml of glucuronidase (12 U/ml)/sulphatase (60 U/ml) (Merck, Darmstadt, F.R.G.) at pH 5.5 for 30 min and after hydrochloric acid hydrolysis for 30 min at 100°C. Extracts were analysed directly, after methylation with diazomethane in diethyl ether [10].

Mass spectra were run on a 4021 gas chromatograph–mass spectrometer with an Incos data system (Finnigan, San José, CA, U.S.A.). For GC a fused-silica capillary column (SE 54, 25 m × 0.32 mm I.D.; Macherey & Nagel, Düren, F.R.G.) was used with an injection port temperature of 280°C splitless injection and a column temperature programme of 75–300°C at 15°C/min. The carrier gas was helium at a flow-rate of 1.7 ml/min. The column was directly coupled to the mass spectrometer. Kováts indices were determined by calibration of the column with a mixture of C_{12}–C_{22} *n*-alkanes. The ion source pressure was $4 \cdot 10^{-5}$ Pa in the electron-impact (EI) mode and $3 \cdot 10^{-3}$ Pa in the chemical ionization (CI) mode, using methane. The ion source temperature was 295°C. The multiplier voltage was 1200 V.

All samples were run in the EI (70 eV) and in the CI (30 eV) mode. Structural elucidation was based on reference mass spectra, determination of the molecular ion by CI, fragmentation pattern and formation of the corresponding derivatives after acetylation of the extracts.

HPLC analysis of plasma

Plasma (1.5 ml) was vortex-mixed with 2.0 ml of acetonitrile, allowed to stand for 10 min, vortex-mixed again and then centrifuged for 5 min. The clear supernatant was mixed with 2 ml of Tris buffer (pH 9) and extracted twice with 10 ml of dichloromethane (Nanograde, Mallinckrodt). The solvent was removed with a dry stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase. A 20-μl aliquot was used for HPLC.

The high-performance liquid chromatograph consisted of a Rheodyne 7125 (20-μl loop), a pump 410, a diode array UV detector 235 and an LCI-100 integrating recorder from Perkin Elmer (Überlingen, F.R.G.). The column was 12 cm × 0.4 cm I.D., and used in combination with a precolumn (2 cm × 0.4 cm I.D.), both packed with Nucleosil C_{18}, 5 μm (Macherey & Nagel). The mobile phase was prepared from 5 m*M* heptanesulphonic acid in water–orthophosphoric acid 85% (1000:0.86, v/v) adjusted to pH 3 with 0.1 *M* sodium hydrox-

ide; the eluent was a mixture of this solution and acetonitrile (76:24, v/v). The flow-rate was 1.5 ml/min. A wavelength of 245 nm was used for quantitation and peak purity control by UV spectra (Fig. 2).

Standard stock solutions were prepared in methanol and diluted with the mobile phase.

RESULTS

Gas chromatography–mass spectrometry

The main fragmentation pathways of pipamperone are depicted in Fig. 3. In the mass spectra of pipamperone and its derivatives the molecular ion was small or absent, and a prominent peak due to loss of the carboxamide function (*m/z* 331 in pipamperone) was found (Table I). This ion is the precursor of several other fragment ions. Analogous fragmentation steps were observed in the mass spectra of compounds II–IV, VIII and IX. CI with methane yielded the typical $[M+41]^+$, $[M+29]^+$ and $[M+1]^+$ ions. The exact positions of the hydroxyl function of IV, the O-acetyl function of IVa and IXa and the double bond of III and VI in the piperidine ring system could not be established from the mass spectra.

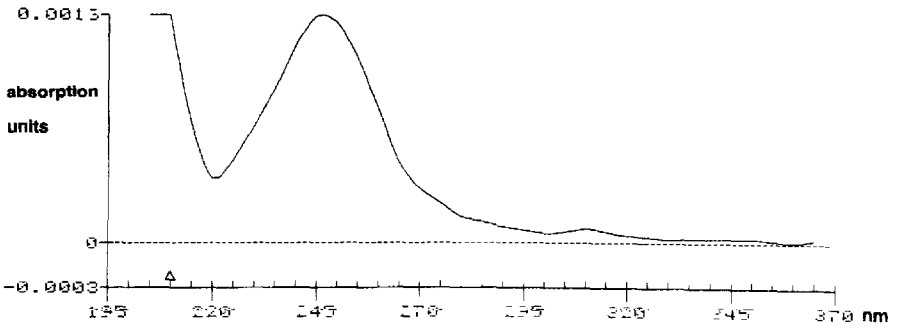

Fig. 2. Diode array UV spectrum of 22 ng of pipamperone.

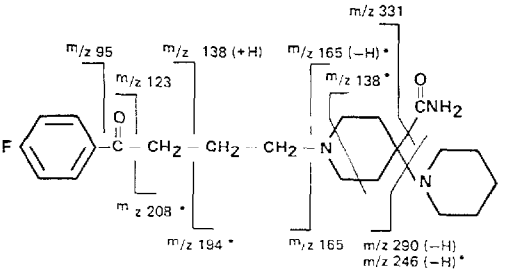

Fig. 3. Fragmentation pattern of pipamperone (molecular ion *m/z* 375) The ions marked with an asterisk have *m/z* 331 as their precursor.

TABLE I

KOVATS INDICES AND MASS SPECTRA OF PIPAMPERONE, ITS METABOLITES, ARTIFACTS AND DERIVATIVES

M^+ = molecular ion; the base peak (100%) is underlined.

Compound	Kavots index	*m/z* (intensity, %)
I	2390	M^+ 375 (<0.1), 331 (79), 292 (22), 290 (16), 246 (17), 220 (13), 208 (18), 194 (43), 165 (100), 154 (8), 138 (90), 123 (62), 110 (40), 98 (22), 84 (21), 55 (11)
II	2240	M^+ 377 (2), 333 (100), 331 (5), 315 (1), 294 (22), 292 (18), 248 (28), 222 (16), 209 (21), 178 (22), 165 (26), 138 (91), 125 (11), 124 (9), 123 (4), 110 (31), 109 (28), 84 (22), 55 (18)
III	2470	M^+ 373 (4), 355 (12), 329 (2), 292 (1), 290 (3), 235 (36), 222 (30), 221 (32), 165 (53), 138 (18), 123 (38), 110 (8), 98 (51), 84 (23), 82 (30), 70 (100)
IV	2810	M^+ 391 (<0.1), 347 (31), 292 (11), 290 (8), 282 (9), 246 (8), 209 (17), 194 (22), 165 (100), 123 (32), 108 (16), 100 (4), 56 (29)
IVa acetylated	2980	M^+ 433 (<0.1), 389 (52), 372 (2), 292 (32), 290 (17), 246 (20), 222 (2), 220 (14), 196 (32), 194 (48), 165 (100), 136 (11), 123 (48), 108 (11), 98 (20)
V	1580	M^+ 180 (22), 152 (2), 136 (12), 135 (21), 133 (5), 125 (40), 123 (21), 109 (12), 195 (22), 56 (100)
VI	1810	M^+ 209 (100), 165 (3), 150 (49), 138 (9), 124 (38), 110 (6), 95 (6), 84 (20), 82 (26)
VII	1395	M^+ 164 (81), 145 (6), 135 (10), 133 (28), 123 (100), 109 (7), 107 (11), 95 (38), 85 (13)
VIII	2340	M^+ 359 (6), 315 (21), 230 (4), 224 (43), 141 (31), 139 (100), 110 (8), 109 (14), 98 (43), 84 (4)
IXa acetylated	2840	M^+ 417 (0.5), 389 (3), 373 (21), 292 (2), 290 (2), 282 (34), 222 (47), 141 (42), 139 (100), 124 (3), 122 (2), 120 (4), 98 (65), 83 (18), 70 (54)
X	1280	M^+ 138 (38), 124 (6), 123 (100), 109 (7), 95 (39), 75 (11)

In underivatized extracts, I, II, III, IV and VII were identified. After acetylation of the extracts, IVa, VIII and IXa were also detected. Compounds V, VI and X could be detected after hydrochloric acid hydrolysis. Pipamperone was stable to hydrochloric acid hydrolysis. Methylation of the extracts gave no evidence for the formation of the methyl derivatives of XI, XII and XIII. Compound XIV could also not be identified.

High-performance liquid chromatography

Fig. 4 shows the chromatograms of a blank plasma sample and of a plasma sample of a patient. The retention time of pipamperone was 7.82 min. The recovery of pipamperone added to blank plasma (30 ng/ml) was $60 \pm 3\%$ (mean $\pm$ S.D.) (external standard method). The detection limit under the described conditions was 10 ng/ml of plasma. Pipamperone standard curves were

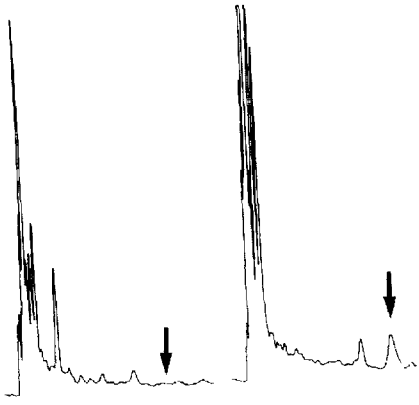

Fig. 4. Blank plasma sample (left) and plasma sample of a patient with a pipamperone level of 25 ng/ml (right). The pipamperone retention time of 7.82 min is arrowed

linear in the range 20–500 ng/ml of plasma ($r^2 = 0.96$). The day-to-day precision was $\pm 7\%$ at a plasma level of 90 ng/ml. No interference with the assay was observed from physiological plasma constituents or co-administered drugs. No metabolites of pipamperone could be detected in plasma. A 10% reduction of the pipamperone plasma level was found when plasma samples were reanalysed after storage for four weeks at $-20\,^{\circ}C$.

Pipamperone predose steady-state plasma levels were 291, 298 and 395 ng/ml in the three patients after a daily dose of 360 mg.

DISCUSSION

The HPLC assay described here allowed detection of pipamperone in plasma after a therapeutic dose with sufficient precision and sensitivity. In urine, the metabolites II, IV and V were identified, as well as unchanged pipamperone. Compounds III, VI, VIII and IX were probably formed by thermal dehydration of the corresponding alcohols. Compound VII was probably generated from pipamperone N-oxide via a Cope elimination. An alcohol analogue of the aldehyde V might be another possible precursor of VII and compound X was probably formed by decarboxylation of 4-fluorophenyl-3-ketopropionic acid.

The main metabolic pathways of pipamperone were reduction of the keto function, hydroxylation of the piperidine ring, oxidative deamination and probably N-oxidation.

In contrast to pipamperone metabolism in the rat, no hydrolysis and elimination of the carboxamide group was observed. It cannot be excluded that the highly polar metabolites XI, XII and XIII, which were detected in rat urine, escaped the extraction procedure. The formation of XII probably requires a multi-step rearrangement [1]. No precursor metabolites of this process could

be identified in urine. Human metabolism of pipamperone is rather similar to that of other butyrophenones, such as haloperidol and melperone [11–13]. However, formation of an N-oxide has previously been observed only in penfluridol metabolism [14]. It remains to be established whether pipamperone metabolites are pharmacologically active. In other butyrophenones such as haloperidol, metabolites seem to play an important role. In patients not responding to neuroleptic therapy with haloperidol, the plasma concentration of reduced haloperidol (a structural analogue of II) seemed to be higher than in responders to therapy, although the haloperidol plasma levels in the two groups did not differ significantly [15].

ACKNOWLEDGEMENTS

We are indebted to Mrs. E. Vejmelka and Mrs. G. Rausch for skilled technical assistance.

REFERENCES

1 W. Soudijn, I. van Wijngaarden and F. Allewijn, Eur. J. Pharmacol., 1 (1967) 47.
2 P.A.J. Janssen and F.T.N. Allewijn, Arzneim.-Forsch., 19 (1969) 199.
3 D.A.P. Janssen, Arzneim.-Forsch., 11 (1961) 819.
4 C.-M. Ideström and B. Cadenius, Psychopharmacology, 4 (1963) 235.
5 K. Heinrich and K. Rumpf, Arzneim.-Forsch., 14 (1964) 587.
6 J. Heykants, Internal Biological Research Report, Janssen Pharmaceutica, Beerse, December 1971.
7 J. Heykants, Internal Biological Research Report, Janssen Pharmaceutica, Beerse, April 1973.
8 K. Pfleger, K. Maurer and A. Weber, Mass Spectra and GC Data of Drugs, Poisons, and Their Metabolites, Vol. II, Verlag Chemie, Weinheim, 1985, pp. 256, 422, 582, 604 and 641.
9 C. Köppel and J. Tenczer, Int. J. Mass Spectrom. Ion Phys., 48 (1983) 213.
10 K. Pfleger, K. Maurer and A. Weber, Mass Spectra and GC Data of Drugs, Poisons, and Their Metabolites, Vol. I, Verlag Chemie, Weinheim, 1985, p. 14.
11 A. Forsman, G. Folsch, M. Larsson and R. Ohman, Curr. Ther. Res., 21 (1977) 606.
12 A. Forsman and M. Larsson, Curr. Ther. Res., 24 (1978) 567.
13 C. Köppel, J. Tenczer and K. Ibe, J. Chromatogr., 427 (1988) 144.
14 S. Pfeifer, Biotransformation von Arzneimitteln, Verlag Chemie, Weinheim, 1979, Vol. 3, p 377.
15 L. Ereshefsky, M.W. Jaun, S.R. Saklad and C.M. Davis, Drug Intell. Clin. Pharmacol., 16 (1982) 472.