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Note

Quantitative determination of perphenazine and its dealkylated metabolite using high-performance liquid chromatography

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The introduction of neuroleptics into the treatment of psychotic symptoms has demonstrated, that it may be difficult to achieve a satisfactory balance between extrapyramidal side-effects and the antipsychotic effect. Furthermore, cases have been reported where the therapeutic effect has not been observed despite long-term neuroleptic treatment [1, 2].

In 1968, when Curry [3] published his first gas chromatographic method for the determination of chlorpromazine, many thought that therapeutic monitoring of this drug was just around the corner. During the intervening fifteen years a large number of analytical methods for the quantification of many different neuroleptics in human plasma have been published [4, 5].

Among the pharmacokinetic parameters of clinical consequence discovered in these investigations have been the big inter-individual variations in the plasma concentrations reached during a fixed-dose therapy [1, 6]. However, it has been difficult to exploit this knowledge effectively in the clinical treatment, as few investigations have hitherto documented an adequate correlation between the clinical effect and the plasma concentration of the administered neuroleptic [7]. Some possible reasons for this lack of correlation are: first, inhomogeneity of the material investigated; second, unknown time of delay

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between the measurement of the plasma concentration and the occurrence of the clinical effect at the receptors; third, lack of appreciation of the need for careful design of the method, a design that takes into account application to the clinical situation.

The aims of this paper are two-fold. First, to evaluate the advantages and drawbacks of different analytical methods commonly applied to plasma-monitored neuroleptic treatment. Second, to describe a selective high-performance liquid chromatographic (HPLC) method for the determination of perphenazine and its dealkylated metabolite.

REVIEW OF DIFFERENT METHODS

In our opinion, an analytical method must indicate unequivocally to the clinician whether the lack of a therapeutic response is due to non-compliance or to rapid metabolism to inactive metabolites. We will emphasize the necessity for a chromatographic system to proceed the quantification to ensure a separate estimate of parent compound and one or more metabolites.

A review of the literature indicates that the most frequently applied methods for determination of neuroleptics are gas-liquid chromatography (GLC), HPLC, radioimmunoassay (RIA) and radioreceptor assay (RRA). The two chromatographic methods (GLC and HPLC) give a specific quantification of both the parent compound and the metabolites, and may be classifiable as adequate for the clinical situation.

RIA will give a specific estimate of the active compound in only a few situations, because of a substantial similarity between the chemical structures of the parent compound and the metabolite(s). This will result in cross-reaction between added antibody and parent compound/metabolite(s) and consequently cause false increased values. Neither does the RIA method permit an evaluation of changed metabolizing capacity or non-compliance. We therefore conclude that this method is of little help to the psychiatrist in judging whether the actual medical treatment will result in an acceptable therapeutic effect.

RRA determines specifically the amount of dopamine-receptor-active substances in a specimen. The principle is based on an in vitro competition between active substance in the sample and an isotope-labelled neuroleptic for the binding sites in a suspension of dopamine receptors extracted from brain tissue of an animal. The clinical relevance of such biologically specific estimates depends on two things. First, the binding affinity must be similar to that in human receptors, and second, the blocking in vitro must be equivalent to that in vivo. Besides, a total estimate of active compounds in the sample does not give any information about the ratio between the parent compound and the active metabolites. This might explain the lack of correlation between the antipsychotic effect and the measured plasma concentrations of neuroleptics that form active metabolites, as has been found with the RRA method. We therefore doubt that the RRA method will bring any substantial contribution to the improved monitoring of neuroleptics. We disagree with the many authors [8—10] who argue for the use of the RRA method as an adequate tool to make medical treatment with neuroleptics effective. Arguments in favour of RIA and

RRA methods that make reference to high capacity and low cost price are of minor importance in this connection.

EXPERIMENTAL

Reagents

Ethyl acetate, methanol and methylene dichloride (E. Merck, Darmstadt, F.R.G.) were of analytical grade. Hexane (Mallinckrodt, KY, U.S.A.) was of nanograde purity. Sodium hydroxide and hydrochloric acid were prepared in our laboratory.

Reference substances

Stock solutions (1 g/l) in ethanol of perphenazine (PPZ), of dealkylated perphenazine {1-[γ-(2-chloro-10-phenothiazinyl)propyl]piperazine dihydrochloride, DAPPZ}, and of the internal standard {4-[3-(2,8-dichlorophenothiazin-10-yl)propyl]-1-piperazinethanol, CPPZ} were all from Schering (Bloomfield, NJ, U.S.A.). The solutions were stable under refrigeration for one year.

Extraction procedure

To a centrifuge tube containing 2500 μl of plasma, 7.5 ng of the internal standard were added. To the sample were added 100 μl of 1 *M* sodium hydroxide, and this solution was extracted with 6 ml of organic solvents (ethyl acetate—hexane, 4:2) by vigorously shaking the tube for 30 sec. After centrifugation for 3 min, the organic phase was transferred to a centrifuge tube containing 2 ml of 0.1 *M* hydrochloric acid, using a methanol moistened Pasteur pipette.

The compounds were extracted into the aqueous phase by vigorously shaking the tube for 30 sec. After centrifugation (3 min) the organic phase was discarded. To the aqueous phase were added 100 μl of 6 *M* sodium hydroxide and 3 ml of hexane. By shaking vigorously (30 sec) the compounds were re-extracted into the organic phase. After centrifugation a moistened pipette was used to transfer the organic phase to a tapered tube. The hexane was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 75 μl of solvent, and 20 μl of this solution were injected into the chromatograph.

Liquid column chromatography

A liquid chromatograph (Pye Unicam, Cambridge, U.K.) Type LC 3, equipped with a UV detector Type LC 3, was used. The column (2 cm × 4.6 mm I.D.) was filled with C_{18} material (Perkin-Elmer, U.S.A.) with particle size 3 μm. The mobile phase was methanol—water—methylene dichloride—ammonia (200:40:10:3) at a flow-rate of 1.0 ml/min. The detection was carried out at 257 nm.

Calculations

The plasma concentrations were read from standard curves constructed from chromatograms of plasma samples containing different known amounts of PPZ

and DAPPZ. The peak height ratios PPZ/CPPZ and DAPPZ/CPPZ were plotted against the concentrations.

RESULTS

Chromatograms of plasma extracts are illustrated in Fig. 1. Retention times for PPZ, CPPZ and DAPPZ were 2.1, 2.5 and 5.9 min, respectively. The peaks of PPZ and DAPPZ correspond to plasma concentrations of 1.0 and 2.5 nmol/l, respectively (A). The chromatogram of a plasma blank showed no interfering peaks (B). The left-hand chromatogram (C) illustrates the determination of a plasma sample from a patient receiving oral treatment with 8 mg twice daily. The sample was drawn 12 h after the last dose, and the plasma concentrations of PPZ and DAPPZ were estimated to 3.8 and 1.6 nmol/l, respectively.

The lower limit for safe quantification (sensitivity) was defined as a peak height ten times greater than the fluctuation of the baseline. Defined in this way, we found the sensitivity to be below 0.5 nmol/l for PPZ, extracting 2.5 ml of plasma.

The inter- and intra-assay variations for PPZ and DAPPZ present in various

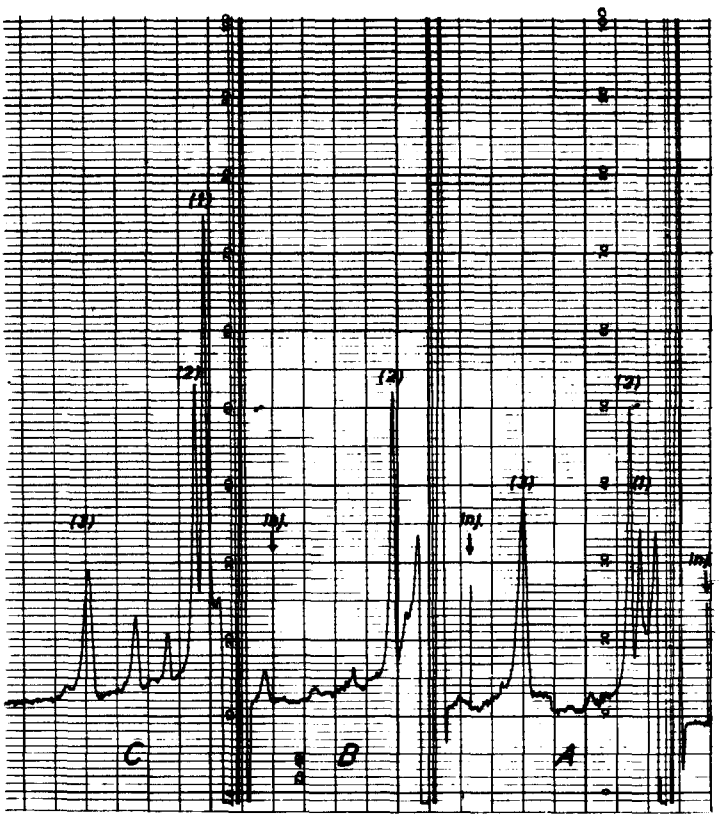

Fig. 1. Chromatograms of three plasma samples. (A) Human plasma with the following additions: 6.25 ng of PPZ (1); 7.50 ng of C-PPZ (2); 15.75 ng of DAPPZ (3). (B) The same human plasma with only 7.50 ng of C-PPZ (2) added. (C) PPZ (1) and DAPPZ (3) in a patient receiving continuous oral PPZ medication.

TABLE I

INTER- AND INTRA-ASSAY TESTS FOR PERPHENAZINE (PPZ) AND DEALKYLATED PERPHENAZINE (DAPPZ) FROM PLASMA

The plasma volume extracted was 2.5 ml in each instance. n = 10.

Concentration added (nmol/l)		Calculated concentrations (mean ± S.D., nmol/l)			
		Inter-assay		Intra-assay	
PPZ	DAPPZ	PPZ	DAPPZ	PPZ	DAPPZ
0.50	1.00	0.46 ± 0.03	1.02 ± 0.08	0.49 ± 0.01	1.00 ± 0.04
1.00	2.00	0.98 ± 0.07	2.00 ± 0.11	1.01 ± 0.04	2.01 ± 0.08
2.00	4.00	2.01 ± 0.10	4.07 ± 0.15	2.00 ± 0.06	4.04 ± 0.04
3.00	6.00	2.98 ± 0.12	6.07 ± 0.10	3.02 ± 0.10	6.03 ± 0.10
4.00	8.00	4.01 ± 0.09	8.05 ± 0.16	3.99 ± 0.06	7.95 ± 0.11
6.00	12.00	6.06 ± 0.19	11.90 ± 0.24	6.02 ± 0.05	11.97 ± 0.20
8.00	16.00	8.01 ± 0.17	16.11 ± 0.30	8.07 ± 0.09	15.95 ± 0.24

concentrations were estimated on ten plasma samples within the therapeutic range (Table I).

Recovery tests were performed by comparing the peak heights of PPZ, CPPZ and DAPPZ from plasma extracts with the peak heights that resulted from injections of ethanolic solutions of equivalent amounts. About 70% recovery from plasma samples was attained.

The analytical selectivity was examined by comparing retention times of other psychotropic drugs with those of PPZ and DAPPZ. Other neuroleptics (except levomepromazine) and diazepam should be avoided, whereas anti-parkinsonian drugs (biperidine, orphenadine), antidepressants (amitriptyline, nortriptyline, imipramine and clomipramine), nitrazepam and tetraethyldisulfiram (Antabus®) did not interfere in the chromatogram.

To ensure that identical results were obtained with the GLC method [11] used in our earlier investigations and the new HPLC method, thirty plasma

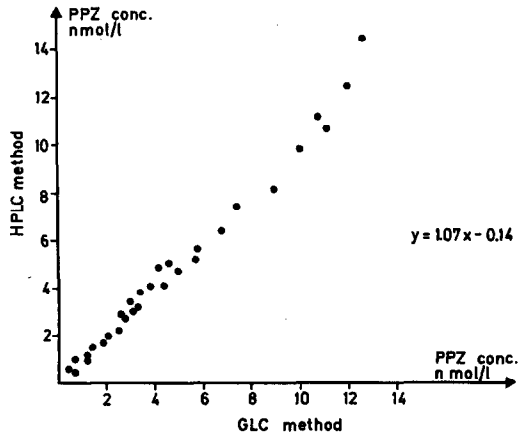

Fig. 2. Correlation of the GC and HPLC methods applied to thirty plasma samples from PPZ-medicated patients.

samples drawn from PPZ-medicated patients were analysed by the two methods. Fig. 2 illustrates the excellent uniformity of the results obtained over a wide concentration range ($r = 0.99$).

DISCUSSION

Previous investigations by our group [12, 13] have revealed that the PPZ plasma concentration should lie in the range 2—6 nmol/l during the entire dose interval to achieve the antipsychotic effect and to reduce the risk of provoking extrapyramidal side-effects. This observation has been confirmed by routine application over a three-year period, so it seems likely that the parent compound (PPZ) is the only active compound at the receptors. However, RRA examinations of the in vitro dopamine receptor activity of different PPZ metabolites indicated that PPZ and the 7-hydroxy metabolite had similar effects. The lipophility of the metabolite is less than that of the parent compound, and so it seems likely that the metabolite does not cross the blood—brain barrier. We therefore consider that quantification of this metabolite is of minor importance.

The metabolite estimated in this method, dealkylated perphenazine (DAPPZ), is different from that selected in our earlier published GLC method. This is for analytical reasons only, because the sulphoxide metabolite estimated in the GLC method has a restricted UV spectrum at 257 nm, which is the optimal UV-absorbing wavelength for PPZ and DAPPZ.

In spite of the biological inactivity of DAPPZ, the simultaneous estimation of this metabolite and of PPZ is essential if the right conclusion is to be reached concerning the reason for poor effect of the treatment. If the PPZ plasma concentration is too low to be detectable, then a metabolite concentration about zero indicates non-compliance, whereas a high metabolite concentration indicates a marked metabolizing capacity. The results in Table I show that the method is sensitive enough to ensure accurate quantification even in the subtherapeutic range. Furthermore, the correlation between detector deflection and concentration values is linear over a wide range for PPZ and DAPPZ, respectively.

Table I illustrates a coefficient of variance not exceeding 7% for both components, a methodological precision which is moderate in relation to biological variations, giving no noise at the steady-state level.

As mentioned earlier, we have monitored PPZ treatment for three years using plasma concentrations as a guide. The results obtained have been so convincing that the capacity of the GLC method was inadequate to comply with the continuously increasing demand. This is the main reason for the development of the HPLC method, which is technologically more robust than the GLC method.

CONCLUSION

A critical review of different analytical methods for quantification of neuroleptics in human plasma is made. The chromatographic methods (GC and HPLC) are classifiable as adequate for the clinical situation, whereas a RIA

and RRA have certain clinical shortcomings because of lack of specificity. A new developed HPLC method for the determination of perphenazine and its dealkylated metabolite in human plasma is described.

The sensitivity, precision and selectivity make the method adequate to estimate, by routine, the concentrations of the parent compound and its dealkylated metabolite in schizophrenic patients. The method has been compared to an earlier published GC method and was found to give almost identical results.

REFERENCES

1 P.L. Morselli, G. Bianchetti and M. Dugas, Ther. Drug Monitor., 4 (1982) 51.
2 R.C. Smith, J. Crayton, H. Dekirmerjian, D. Klass and J.M. Davis, Arch. Gen. Psychiatry, 36 (1979) 579.
3 S.H. Curry, Anal. Chem., 40 (1968) 1251.
4 T.B. Cooper, Clin. Pharmacokinet., 3 (1978) 14.
5 N.-E. Larsen, in E. Usdin (Editor), Clinical Pharmacology in Psychiatry, Macmillan, London, 1981, p. 3.
6 S.G. Dahl, in E. Usdin (Editor), Clinical Pharmacology in Psychiatry, Macmillan, London, 1981, p. 125.
7 P.L. Morselli and E. Zarifian, in Drug Concentrations in Neuropsychiatry, Ciba Foundation Symposia 74, Elsevier, Amsterdam, 1980, p. 115.
8 I. Creese and S.H. Snyder, Nature, 270 (1977) 179.
9 S.R. Lader, J. Immunoassay, 1 (1980) 57.
10 H.Y. Meltzer, J.M. Kane and T. Kolakowska, in J.T. Coyle (Editor), Neuroleptics: Neurochemical, Behavioral and Clinical Perspectives, Raven Press, New York, 1983, p. 255.
11 N.-E. Larsen and J. Næstoft, J. Chromatogr., 109 (1975) 259.
12 L. Bolvig Hansen, N.-E. Larsen and N. Gulmann, Psychopharmacology, 78 (1982) 112.
13 P. Knudsen, L. Bolvig Hansen, K. Højholdt and N.-E. Larsen, Acta Psychiatr. Scand., (1985) in press.