THE RELATIONSHIP OF PLASMA CHLORPROMAZINE TO ITS 7-HYDROXY AND SULPHOXIDE METABOLITES IN A LARGE POPULATION OF CHRONIC SCHIZOPHRENICS

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1 Blood samples were obtained from eighty-six chronic schizophrenics receiving a wide range of oral doses of chlorpromazine. Plasma concentrations of chlorpromazine, 7-hydroxychlorpromazine and chlorpromazine sulphoxide were estimated using a sensitive gas-liquid chromatographic method and their relationships to oral dose and to global clinical control were investigated.

2 Wide variability was observed in the plasma concentrations of unchanged drug and metabolites between patients receiving similar daily doses.

3 In general the plasma concentrations of the 7-hydroxy and sulphoxide metabolites were of similar magnitude to the concentrations of chlorpromazine.

4 Global symptom control was unrelated to the plasma concentration of unchanged chlorpromazine. However, patients judged to be under good control had relatively higher concentrations of the biologically active 7-hydroxy metabolite in their plasma than patients who were poorly controlled and in whom the biologically inactive sulphoxide metabolite predominated. When the ratio of 7-hydroxychlorpromazine to chlorpromazine sulphoxide was derived for each patient, a highly significant difference was found to exist between the metabolite ratios of patients grouped according to clinical control.

5 It is suggested that a prediction of therapeutic response to chlorpromazine may be provided in the form of the ratio of the plasma concentration of biologically active metabolite to the concentration of either the unchanged drug or its inactive metabolite.

Introduction

The problem of inter-patient variation in response to chlorpromazine has endured for twenty years. Despite numerous clinical pharmacological studies, it has not yet proved possible to predict with any degree of certainty how an individual patient will react to the drug when given orally over a wide dose range. It would appear that oral dose bears only a crude relationship to clinical efficacy. Increasing the daily dose to more than 0.5 g enhances the effectiveness of chlorpromazine in general (Cole & Davis, 1969), but still only about 75% of schizophrenic patients will recover from one particular episode (N.I.H. Study Group, 1967). Brodie (1964) has suggested that assessing the plasma level of a drug as a guide to dose adjustment might diminish inter-patient

differences in the response to treatment. Investigations on relatively small numbers of patients have revealed wide differences in the plasma concentration of unchanged chlorpromazine within groups receiving the same oral daily dose (Curry, 1968; Curry, Marshall, Davis & Janowsky, 1970; Curry, 1971). In view of these differences, and because biologically active metabolites of the drug are known to exist, it was decided to investigate the relationship of plasma chlorpromazine and its sulphoxide and 7-hydroxy metabolites to oral dose and global control of symptoms in a large population of in-patient chronic schizophrenics.

Methods

Clinical material

Eighty-six chronic schizophrenics, all patients in Fulbourn Hospital, Cambridge, were chosen as

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subjects for the study. Their average duration of stay in hospital after the original diagnosis of schizophrenia was 17 years and the average period of chlorpromazine treatment was 9.6 years. The average daily oral dose of chlorpromazine was 280 mg.

Senior nurses to whom the patients were well known were asked to rate the patients in terms of global control of schizophrenic symptomatology within one of three grades—good, moderate or poor.

Blood for biochemical analysis was taken 3 h after the last oral dose of chlorpromazine, at a time when the plasma concentration has just passed its peak (Curry *et al.*, 1970; Sakalis, Curry, Mould & Lader, 1972). Blood (5–10 ml) was taken by cubital venepuncture into lithium-heparin tubes and the plasma separated in the dark within 1 h of collection. Any sample showing significant haemolysis was discarded. Plasma was stored in the dark at -20°C until assay.

Plasma assay procedure

Extraction and measurement of chlorpromazine, its sulphoxide and 7-hydroxychlorpromazine was carried out by a modification of previously published work by Curry (1968), who had suggested that the extraction of the more polar metabolites could be achieved by the use of diethylether. Accordingly the following procedure was utilized for the analysis of plasma.

Plasma (4 ml) was made alkaline by the addition of 1.25 M sodium hydroxide (1 ml) and extracted for 15 min with peroxide-free ether (10 ml). After centrifuging, the ether layer was carefully removed and the plasma extracted with a further 5 ml ether. The combined ether extracts were shaken with 0.05 N hydrochloric acid (2 ml) for 15 minutes. An aliquot (1.8 ml) of the acid was taken and made alkaline by addition of 1 M NH_4OH (0.2 ml) and shaken with toluene-isoamyl alcohol (95–15, v/v, 50 μl). The aqueous layer was carefully removed and the organic layer centrifuged in a finely tapered tube. Analysis of 5 μl injections of the extract was performed on a Pye Model 104 gas chromatogram incorporating a Ni electron capture detector. The conditions of analysis were as follows: column, 7 feet, 3% OV17 on Gas Chrom W (H.P.) 100–120 mesh; carrier gas filtered 'white' spot nitrogen, 60 ml/min; column temperature 295°C ; detector operated at 150 μs pulse space at a temperature of 320°C . Plasma metabolites were measured by the ratio of their peak heights or areas to that of an internal standard. The internal standard employed was 2:6 dichlorpromazine hydrochloride (Flint, Ferullo, Levandoski & Hwang, 1971) which was

added to plasma before extraction at a concentration of 6 ng/ml.

Under these conditions of analysis, chlorpromazine, the internal standard, 7-hydroxychlorpromazine and chlorpromazine sulphoxide gave well defined peaks with retention times of 1.75 min, 2.6 min, 4.4 min and 5.0 min respectively (Fig. 1). Recoveries (mean $\pm$ s.e.) of these compounds added to plasma in the concentration range 10–100 ng/ml and taken through the entire procedure were $95 \pm 1\%$ for chlorpromazine, $103 \pm 3\%$ for 7-hydroxychlorpromazine and $92 \pm 4\%$ for the chlorpromazine sulphoxide. The lower limit of sensitivity of the detector for these compounds injected in 5 μl was 0.5 ng for chlorpromazine and its sulphoxide and 1 ng for 7-hydroxychlorpromazine.

Similar concentrations of other potential metabolites [desmethyl chlorpromazines (mono- and di-) and their sulphoxides, 7-hydroxy-desmethylchlorpromazines (mono- and di-), 6-hydroxychlorpromazine, 8-hydroxychlorpromazine] when added to plasma and taken through the entire procedure if extracted in sufficient amounts to give measurable peaks, did so with retention times which differed from the three compounds mentioned above. Analysis of plasma from patients generally showed only three significant peaks corresponding to chlorpromazine, 7-hydroxychlorpromazine and chlorpromazine sulphoxide, although occasionally a specimen was found to contain measurable amounts of monodesmethylchlorpromazine (Figure 1). The retention time of this compound was 2.1 min and the mean recovery from plasma was $86\% \pm 4\%$ s.e.

Results

Enormous variability in plasma concentrations of chlorpromazine was observed (Fig. 2) and confirms earlier reports. There was a highly significant overall correlation ($P < 0.005$) between plasma chlorpromazine values and daily dose of drug, however, this relationship was apparently lost at doses greater than 400 mg/day (Figure 2). A similar picture of wide variability was found with chlorpromazine sulphoxide (Fig. 3) but once again a highly significant correlation ($P < 0.005$) existed between plasma concentration and oral dose. In twenty-three patients plasma 7-hydroxychlorpromazine was also assayed and in most of these patients the concentration of this metabolite was of similar magnitude to that of unchanged chlorpromazine and chlorpromazine sulphoxide.

When related to global symptom control interesting differences emerged between the average plasma concentrations of chlorpromazine

Fig. 1 Gas-liquid chromatograph traces of control plasma (obtained from a drug-free subject) to which known amounts of chlorpromazine (CPZ), monodesmethylchlorpromazine (NOR), 7-hydroxychlorpromazine (7-OH-CPZ), sulfoxchlorpromazine (CPZ-SO) and dichlorpromazine (diCPZ) were added *in vitro* prior to assay (a) and of plasma taken from a patient receiving oral chlorpromazine to which only dichlorpromazine was added (b). The standard compounds were added to the control plasma to give a final concentration of 50 ng/ml and the concentration of the internal standard (diCPZ) was 6 ng/ml in both cases.

Fig. 2 Relationship of plasma chlorpromazine concentration (ng/ml) to total daily oral dose of chlorpromazine (mg) in 86 chronic schizophrenics. Average values at each dose level are shown connected by a continuous line.

Fig. 3 Relationship of plasma chlorpromazine sulphoxide concentration (ng/ml) to total daily oral dose of chlorpromazine (mg) in 86 chronic schizophrenics. Average values at each dose level are shown connected by a continuous line.

and of its metabolites (Table 1). The concentration of unchanged drug did not differ significantly between the three categories of patient, but the concentration of chlorpromazine sulphoxide was significantly ($P < 0.05$) lower in patients considered to show good control compared to patients showing poor control (Table 1). The reverse situation existed for 7-hydroxychlorpromazine, with higher average concentrations being found in the well controlled group. In view of the wide inter-patient variability in the concentrations of these plasma parameters, it was considered that a useful manner of expressing results was as the

ratio between metabolites or of metabolite to unchanged drug within each patient. Thus the ratio of 7-hydroxychlorpromazine to chlorpromazine averaged 1.2 in patients whose symptoms were well controlled but only 0.75 in patients whose symptoms were poorly controlled (Table 1). This difference did not quite reach statistical significance ($0.1 > P > 0.05$), but the average ratio of 7-hydroxychlorpromazine to chlorpromazine sulphoxide was 2.2 in well controlled patients, highly significantly greater ($P < 0.01$) than in poorly controlled patients in whom the ratio was 0.4 (Table 1).

Table 1 Average plasma concentrations (ng/ml) of chlorpromazine (CPZ) and its metabolites [7-hydroxychlorpromazine (7-OHCPZ) and chlorpromazine sulphoxide (CPZ SO)] and their relationship to global clinical control

Global control	CPZ	CPZ SO	7-OHCPZ	$\frac{7-OHCPZ}{CPZ}$	$\frac{7-OHCPZ}{CPZ SO}$
Poor	59 ± 20 (10)	61 ± 21 (10)	26 ± 11 (4)	0.75 ± 0.02 (4)	0.4 ± 0.1 (4)
Moderate	46 ± 9 (26)	41 ± 9 (26)	34 ± 4 (11)	1.0 ± 0.2 (11)	0.8 ± 0.1 (11)
Good	30 ± 6 (36)	19 ± 4 (36)	42 ± 7 (8)	1.2 ± 0.3 (8)	2.2 ± 0.3 (8)

Values shown are means ± s.e. for the number of patients given in parentheses.

Discussion

A relationship between the plasma concentration of chlorpromazine and clinical response has been found during the first two weeks of drug treatment (Curry, Lader, Mould & Sakalis, 1972). This relationship was, however, lost in patients treated for longer periods and it was suggested that some at least of the therapeutic efficacy of chlorpromazine might be attributed to the formation of pharmacologically active metabolites. It has been known for some time that certain of the metabolites of chlorpromazine, including 7-hydroxychlorpromazine, possess pharmacological activity comparable to that of the parent compound (Manian, Efron & Goldberg, 1965). Unlike 7-hydroxychlorpromazine, the other major metabolite chlorpromazine sulphoxide is without biological activity in the antagonism of amphetamine-induced stereotypy in rats (Lal & Sourkes, 1972). In a study of five chronic schizophrenics, Kaul, Ticku & Clark (1972), using a dansylation technique, detected 7-hydroxychlorpromazine in the plasma of four patients who were receiving a daily oral dose of chlorpromazine of 600 mg or more. In one patient who was receiving 400 mg of chlorpromazine daily, there was no detectable 7-hydroxy metabolite, all blood samples being taken 2 h after the last oral dose. The majority of our patients in whom 7-hydroxychlorpromazine was assayed were receiving less than 400 mg of chlorpromazine daily, and in no patient with a measurable plasma concentration of unchanged drug was the 7-hydroxy metabolite undetectable. The results presented above indicate that this biologically active metabolite is present in the plasma of patients receiving oral chlorpromazine over a wide dose range and thus account must be taken of its possible contribution to the clinical efficacy of the drug. A recent clinical investigation (Sakalis, Chan, Gershon & Park, 1973) provided some evidence of a relationship between clinical response and the concentration of 7-hydroxychlorpromazine in eight schizophrenic patients. Patients who

responded poorly to the drug had relatively high plasma levels of chlorpromazine sulphoxide.

The present results, obtained from a large population of schizophrenics under chronic treatment with chlorpromazine, emphasize the unpredictability of plasma concentrations of the drug and its metabolites in any particular patient from the oral dose. The wide variation in plasma levels undoubtedly reflect individual differences not only in intestinal absorption and metabolism but also in reliability of drug taking. The metabolic variable, however, would seem to be of definite importance to the clinical response and it could be that the induction of hydroxylating enzymes leading to a relative preponderance of the 7-hydroxy metabolite relative to the biologically inactive chlorpromazine sulphoxide plays an important role in determining the therapeutic response within individual patients. The lack of correlation between clinical state and plasma concentration of unchanged drug suggests that a more accurate prediction of therapeutic response may be provided in the form of the ratio of the plasma concentration of biologically active metabolite to the concentration of either the unchanged drug or its inactive metabolite.

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