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PLASMA CONCENTRATIONS OF BENZODIAZEPINES

ALYSON J. BOND, D.M. HAILEY* & M.H. LADER
Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF

1 Twenty anxious patients were treated with medazepam, diazepam, chlordiazepoxide, amylobarbitone and placebo, each given in flexible dosage for 2-4 weeks.

2 At the end of each treatment, a series of clinical, physiological and behavioural variables were measured and plasma samples were taken for estimation of the appropriate drug and metabolite concentrations.

3 Nordiazepam was shown to be an important metabolite of both medazepam and diazepam: the ratio of medazepam to nordiazepam was 0.14 and the ratio of diazepam to nordiazepam following diazepam administration was 0.72.

4 No significant correlations were found between the plasma concentrations of any of the treatments and the clinical ratings or behavioural measures.

5 Some relationship was shown between levels of medazepam and its physiological effects.

Introduction

Of all the current psychotropic drugs, only lithium is administered with its dosage controlled by estimations of the level of the drug in the body. There have been several studies on plasma concentrations of tricyclic antidepressives (Lader, 1974), and similarly with chlorpromazine (Curry, 1974a). The benzodiazepines have been the most neglected in this respect, partly because of the difficulties of estimating the compounds, partly because of the number of metabolites which many benzodiazepines give rise to, and partly because of the clinical difficulties of standardizing and controlling the treatments. In general, no convincing relationship has been demonstrated between steady state plasma concentrations of these anxiolytics and clinical response (Greenblatt & Shader, 1974). However, the metabolic pathways of the various benzodiazepines have been studied in several species including man. Thus medazepam is metabolized to diazepam and nordiazepam, nordiazepam being primarily formed via the desmethylmedazepam metabolite.

We were engaged in carrying out a detailed comparison of three benzodiazepines–medazepam, diazepam and chlordiazepoxide–with amylobarbitone sodium and placebo with respect to a wide range of clinical, physiological and behavioural variables (Bond, James & Lader, 1974b; Lader, Bond & James, 1974). It seemed opportune to take plasma samples in as standard a way as possible in order to correlate drug concentrations with these other variables.

* Present address: National Standards Laboratory, Queanbeyan, N.S.W. 2620.

Methods

Patients

Twenty patients with anxiety states of at least 6 months duration were studied. Full details are available elsewhere (Lader *et al.*, 1974).

Treatments

Each patient received the following five treatments in balanced order: medazepam, diazepam, chlordiazepoxide, amylobarbitone sodium, placebo. Each treatment was given in flexible dosage schedule for 2-4 weeks under full double-blind procedures. The mean dosage for each drug was: medazepam (27 mg/day, range 10-50), diazepam (11 mg/day, range 2-20), chlordiazepoxide (28 mg/day, range 10-50) and amylobarbitone sodium (140 mg/day, range 60-300).

Measures

The many measures taken on each occasion at the end of each treatment are detailed elsewhere (Bond, James & Lader, 1974a). They included

broad waveband analysis of the EEG under both response and no-response conditions, the EEG evoked response to clicks, heart-rate, pupil size, skin conductance (sweat-gland activity), and behavioural estimates such as tapping speed, reaction time, the digit symbol substitution test, cancellation tasks, card-sorting and simple arithmetic. Clinical assessment included the Hamilton rating scale for anxiety and self-assessment of symptoms using linear scales.

Plasma samples

The patients were instructed to take their usual dose of drug 1 h prior to reporting to the laboratory. Clinical ratings at interview and physiological testing took 1 h whereupon the venepuncture was carried out. Accordingly, the sample was taken 2 h after the last dose of drug. The blood was placed in a sodium heparin bottle, centrifuged, and the plasma separated and stored at -4° C until analysis.

Drug estimations

Medazepam, diazepam and nordiazepam were estimated using electron capture gas chromatography (Baird, Hailey & Malcolm, 1973). Chlordiazepoxide, desmethylchlordiazepoxide and its lactam derivative were estimated by spectrofluorimetry (Schwartz and Postma, 1966). Amylobarbitone sodium was measured by gas liquid chromatography with a tested extraction procedure sensitive enough to analyse therapeutic dose levels (Berry, 1973).

Data analysis

Between-patient correlations were computed using product-moment correlations. Where appropriate within-patient correlations were calculated.

Results

Medazepam treatment

The mean, standard error and range of medazepam concentrations in the twenty patients are set out in Table 1. Individual values are plotted in Figure 1 (note logarithmic scale of concentrations). The concentration of diazepam occurring as the metabolite of medazepam was low but that of the other metabolite, desmethyldiazepam (nordiazepam) was high. This is consistent with earlier work (De Silva & Puglisi, 1970; Mallach, Moosmayer & Rupp, 1973).

Between-patient correlations ($n = 20$) were computed for the three compounds. The only significant correlation ($r = 0.58$; $P < 0.01$) was between diazepam and nordiazepam, implying that the two metabolic pathways (oxidation to diazepam, demethylation and oxidation to nordiazepam) have some sort of relationship in a particular individual, e.g. similar enzyme activity.

Diazepam treatment

The values for diazepam and its major metabolite are given in Table 1. The mean nordiazepam concentration is almost twice that of diazepam. Again, there was a significant between-patient correlation ($r = 0.72$; $P < 0.001$) between diazepam and nordiazepam concentrations.

Chlordiazepoxide treatment

Similar concentrations were obtained for chlordiazepoxide and its metabolites, desmethylchlordiazepoxide and the lactam derivative (Table 1). The correlation between the latter two compounds was particularly high ($r = 0.80$; $P < 0.001$).

Amylobarbitone sodium treatment

Amylobarbitone sodium concentrations average 1,150 ng/ml with a wide scatter (Table 1).

Correlations between treatments

Correlations were computed between patients ($n = 20$) for the concentrations of the drugs and their metabolites during the various treatments. In general the only significant correlations were those between medazepam and its metabolites on the one hand and chlordiazepoxide and its metabolites on the other (Table 2). Thus, the highest correlations were between the concentrations of chlordiazepoxide and its desmethyl derivative combined and various combinations of medazepam and its metabolites. There were no significant correlations between plasma concentrations of diazepam and nordiazepam when the patients were on diazepam treatment and the concentrations of the benzodiazepines on the other treatments.

Correlations between plasma concentrations and other variables

Correlations were computed between the plasma concentrations and the change scores on the other measures, i.e. the scores calculated by taking the scores on each treatment from those pre-

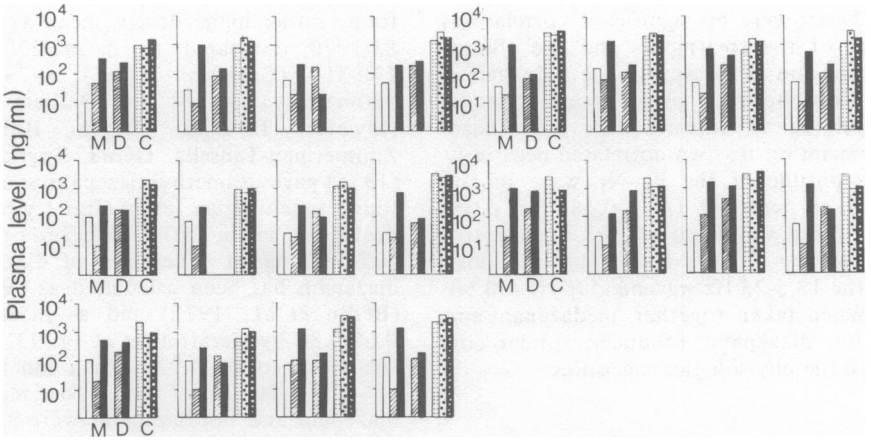

Figure 1 The plasma concentrations of the three benzodiazepines (M, medazepam; D, diazepam; C, chlordiazepoxide) and their metabolites for each of the twenty patients are shown. Note the logarithmic units on the ordinate. □ medazepam; ▨ diazepam; ■ nordiazepam; ⊞ chlordiazepoxide; ⊡ demethylchlordiazepoxide; ■ lactam derivative.

Table 1 Plasma concentrations of administered drugs and their metabolites

		Plasma concentration (ng/ml)		
Drug	*n*	*Mean*	*S.e. mean*	*Range*
Medazepam	20	63	9	10-160
Diazepam		28	7	0-120
Desmethyldiazepam		706	109	210-1680
Diazepam	19	170	20	70-392
Desmethyldiazepam		317	62	83-1070
Chlordiazepoxide	19	1780	200	460-3210
Desmethylchlordiazepoxide		1740	190	850-3540
Lactam derivative		1820	220	670-3800
Amylobarbitone	15	1150	160	100-2200

Table 2 Between-patient correlations between treatments

		Medazepam treatment			
		Nordiazepam	*Medazepam + nordiazepam*	*Diazepam + nordiazepam*	*Medazepam + diazepam + nordiazepam*
Chlordiazepoxide treatment	Chlordiazepoxide	0.54*	0.52*	0.53*	0.52*
	Desmethylchlordiazepoxide	0.55*	0.55*	0.56*	0.56*
	Chlordiazepoxide + desmethylchlordiazepoxide	0.30	0.67**	0.68**	0.67**

* $P < 0.05$; ** $P < 0.01$.

treatment. There were no significant correlations between any of the treatments and the clinical ratings or behavioural measures. The only significant correlations between plasma concentrations and the physiological measures were for medazepam. Medazepam on its own correlated negatively with the amplitude of the P_1–N_1 wave of the auditory evoked response ($r = -0.48$; $P < 0.05$) and the 4-7.5 Hz waveband (θ) of the electroencephalogram ($r = -0.63$; $P < 0.01$) but positively with the 13.5-26 Hz waveband (β) ($r = 0.50$; $P < 0.05$). When taken together, medazepam and its metabolite, diazepam, produced similar correlations with the physiological measures.

Discussion

In this study the patients took each drug for 2-4 weeks. It seems from the majority of studies completed that 2 weeks is the minimum time necessary to be sure of a steady state for diazepam and for its metabolite, nordiazepam. Van der Kleijn, van Rossum, Muskens & Rijntjes (1971) found that both had reached a steady state by 5 days but Berlin, Siwers, Agurell, Hiort, Sjoqvist & Ström (1972) found that although 1 week was sufficient for six of their subjects, one needed longer because his levels were still increasing. Hillestad, Hansen & Melsom (1974b) found that a plateau was reached with diazepam by the end of one week but that 2 weeks was necessary to obtain steady levels of nordiazepam. Most authors agree that there is rapid accumulation of nordiazepam.

Most studies have used fixed doses for investigating plasma concentrations. However, Zingales (1973) studied many patients at different doses over different time periods and found that the dose given correlated with the plasma concentration irrespective of the time interval. We gave the drugs in flexible dosage as we were basically interested in clinical effects but despite this we found some correlations between dose and plasma levels within patients: for both metabolites of medazepam ($r = 0.46$, $P < 0.05$) and for diazepam and its metabolite, nordiazepam ($r = 0.49$, $P < 0.05$). Although diazepam has been given in fixed doses in most studies, plasma concentrations are very variable between subjects. We expected a wider range of values because the drugs were taken in flexible dosage but our concentrations for diazepam, mean 170 ng/ml, range 70-392, fall within a similar range to other studies, e.g. mean 166 ng/ml, range 104-243 (Garattini, Marcucci, Morselli & Mussini, 1973); mean 434 ng/ml, range 264-647 (Dasberg, van der Kleijn, Guelen & van Praag, 1974). For its metabolite, nordiazepam, we found rather higher levels, mean 317 ng/ml, range 83-1070 compared to mean 220 ng/ml, range 120-314 (Garattini *et al.*, 1973) and mean 350 ng/ml, range 14-776 (Dasberg *et al.*, 1974). However, Tansella, Siciliani, Burti, Schiavon, Zimmerman-Tansella, Gerna, Tognoni & Morselli (1975) gave desmethyldiazepam as a hypnotic and found much higher levels after 1 weeks' treatment on 10 mg, range 270-1328 ng/ml or 20 mg, range 327-2850 ng/ml. The ratio of diazepam to nordiazepam has been calculated as between 0.6-1.2 (Berlin *et al.*, 1972) and as mean 0.75, range 0.62-0.86 by Garattini *et al.* (1973) and we found the mean to be 0.72 with a much wider range of 0.21-1.70. The ratios for medazepam, to diazepam and nordiazepam, were also calculated. They were, medazepam to diazepam, mean 4.17, range 0.17-13.0 and medazepam to nordiazepam, mean 0.14, range 0.01-0.41 respectively.

From these ratios for diazepam and medazepam with their common metabolite, nordiazepam, nordiazepam is definitely a very important metabolite of both drugs and this does not seem to be widely recognized in the case of medazepam. There has been some controversy about the clinical effects of nordiazepam based on data from diazepam and clorazepate. Hillestad *et al.* (1974b) state that its clinical effects are negligible but Curry (1974b) states that desmethyldiazepam is 'the most highly anxiolytic benzodiazepine molecule so far examined'. Recent studies have used nordiazepam as a sedative, hypnotic or anxiolytic. Tansella, Zimmerman-Tansella & Lader (1974) compared nordiazepam (10 and 20 mg) with amylobarbitone (200 mg) and placebo given as hypnotics to anxious patients for 7 nights. They found that nordiazepam was superior to both placebo and amylobarbitone on self-ratings of quality of sleep and alertness the following morning. Tognoni, Gomeni, de Maio, Alberti, Franciosi & Scieghi (1975) also found nordiazepam (20-30 mg) was an effective hypnotic for anxious-depressed patients treated with nortriptyline. Neither of these studies compared nordiazepam to another benzodiazepine, so, although it is shown to be an effective hypnotic, it is not known whether it is any more effective than nitrazepam or flurazepam for example. Dasberg (1975) compared nordiazepam (5 mg t.d.s.) to diazepam (5 mg t.d.s.) and placebo but he used normal subjects and not patients. They received the drugs for 7 days and rated nordiazepam as the more effective drug on mood-depressant items (more relaxed, less irritable) and hypno-sedative items (improved sleep) of a self-rating scale.

Dasberg (1975) also confirmed earlier work that nordiazepam persists in the plasma much

longer than diazepam. Diazepam levels fell immediately after withdrawal but nordiazepam levels were much slower to decline, a finding which confirms studies by Garattini *et al.* (1973) who found that after a single oral dose of 20 mg, nordiazepam was still detectable in the blood after 48 h for three out of five subjects, Hillestad *et al.* (1974b) who estimated the respective mean biologic half-lives as diazepam, 54 h and nordiazepam 92 h, and Tognoni *et al.* (1975) who found a range of 26-86 hours.

In the present study, we found no relationship between plasma concentrations and clinical effects in any of the four drugs studied. In fact, such correlations seem to be rare or weak. Tansella *et al.* (1975) found no significant correlations between steady state plasma concentrations of nordiazepam and hypnotic effects as measured by self or nurses' ratings and only one from the fifteen items of the Hamilton rating scale for anxiety. Kanto, Iisalo, Lehtinen & Salminen (1974) found no significant relationships between plasma concentrations of diazepam and its metabolites and clinical effects. In fact, although the concentrations decreased over time, the clinical effects were maintained, a finding similar to those with chlorpromazine (Sakalis, Curry, Mould & Lader, 1972). In a study of flooding during waning diazepam effect, Marks, Visvanathan, Lipsedge & Gardner (1972) found no relationship between serum levels of diazepam and psychotropic effects. Two other studies found some relationship. Curry (1974b) found a significant correlation between nordiazepam concentrations and the symptom rating test score ($r = -0.35$, $P < 0.05$) and Dasberg *et al.* (1974), found significant correlations between plasma concentrations of diazepam and improvement on patient-rated main symptoms ($r = 0.51$, $P < 0.05$) and plasma concentrations of nordiazepam and improvement on gastro-intestinal symptoms on the Hamilton rating scale for anxiety ($r = 0.66$, $P < 0.05$) but autonomic symptoms showed a negative correlation with nordiazepam concentrations ($r = -0.80$, $P < 0.01$), i.e. the patients were made worse. As can be seen, these correlations are not particularly large, numerous or highly significant. Garattini *et al.* (1973) conclude that it is not possible at present to correlate blood levels of diazepam with its therapeutic effects; however, Zingales (1973) suggests monitoring blood levels to maintain steady concentrations and other studies (Dasberg *et al.*, 1974; Hillestad, Hansen, Melstrom & Drivenes, 1974a), propose that there is little functional improvement at levels of diazepam less than 400 ng/ml. Dasberg *et al.* (1974) in fact suggest a 6 mg dose every 8 h is optimum. This sort of proposition seems rather meaningless when one considers firstly that chronic anxiety patients, allowed to take diazepam in flexible dosage, take between 2 and 20 mg a day in varying patterns to obtain maximum symptomatic relief and secondly that all our patients had levels of diazepam less than 400 ng/ml, as did those reported by Garattini *et al.* (1973), Kanto *et al.* (1973) and most of those reported by Berlin *et al.* (1972) and yet the majority showed clinical improvement with few side-effects.

Some relationships were found between plasma concentrations of medazepam and its metabolite, diazepam. It was found that levels of medazepam correlated negatively with the amplitude of the P_1–N_1 wave of the auditory evoked response and negatively with the percentage of slow wave activity on the electroencephalogram but positively with the percentage of fast wave activity. This confirms previous findings with nitrazepam (Bond & Lader, 1972). In fact the benzodiazepines produce definite physiological effects which are useful indicators of drug action. In the present study, many intercorrelations were found between physiological and clinical measures: the drugs which produced significant physiological effects, the benzodiazepines, produced clinical improvement (Bond *et al.*, 1974b) but unfortunately plasma concentrations did not fit into this pattern.

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