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Plasma concentrations of clobazam and its *N*-desmethyl metabolite; protection against pentetrazol-induced convulsions in mice

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The anticonvulsant effects of acute administration of clobazam and its principal metabolite *N*-desmethylclobazam were studied in mice. Pentetrazol, given by slow intravenous infusion 1 or 2 h after the anticonvulsant dose, was used as the convulsant stimulus. Log dose response relationships for both clobazam and *N*-desmethylclobazam appeared linear, but there was no correlation between plasma concentrations of clobazam and protection. However, correlation between plasma concentrations of *N*-desmethylclobazam and protection was significant in both cases.

Monitoring plasma concentrations of benzodiazepines in order to predict pharmacological response is often considered impracticable (Mandelli et al 1978), as this relationship is complicated by such factors as uneven distribution, tolerance to the benzodiazepine effects and the occurrence of active metabolites, this latter especially with those benzodiazepines that undergo demethylation, e.g. diazepam and clobazam.

The principal metabolite of clobazam in man and certain animal species is *N*-desmethylclobazam (Volz et al 1979). During chronic treatment in man, this accumulates to steady-state levels some eight times higher than those of the parent compound (Rupp et al 1979) as a result of its much longer half-life. As it also has an affinity for the benzodiazepine receptor, which appears to be similar to that of clobazam itself (Hunt 1979), it is likely that desmethylclobazam makes a significant contribution to the overall effect of clobazam treatment.

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In this study, we show that the anticonvulsant effects of clobazam in mice, when measured 2 h after acute administration, show a significant correlation with the plasma levels of desmethylclobazam but not those of the parent compound itself.

Methods

Clobazam (Hoechst UK) was dissolved in a vehicle having the composition: propylene glycol 0·4 ml, ethanol 0·1 ml, benzyl alcohol 0·015 ml, sodium benzoate 50·0 mg, benzoic acid 2·25 mg, distilled water to 1·0 ml. *N*-Desmethylclobazam (Hoechst UK) was dissolved in dimethylsulphoxide (DMSO).

Adult male mice (80) (Tuck No. 1), 25–40 g, were randomly assigned to two groups, one group for the clobazam study and the other for the desmethylclobazam study. The first group, comprising 50 mice, was subdivided into groups of 10. Four of these subgroups received a subcutaneous dose of clobazam, either 1, 2·5, 5 or 10 mg kg^{-1}; the remaining 10 mice were given vehicle alone. All mice received the same injection volume of 2·5 ml kg^{-1}. In the metabolite study, 30 mice were subdivided into groups of 5. Five of these subgroups received a subcutaneous dose of desmethylclobazam, either 2·5, 5, 10, 20 or 40 mg kg^{-1}; the remaining 5 mice were given DMSO alone. In this study, all mice received an injection volume of 1·25 ml kg^{-1}.

One hour after the desmethylclobazam dose and 2 h after the dose of clobazam, the mice were tested with pentetrazol (leptazol, metrazol, pentylenetetrazole). Pentetrazol (Sigma, London; 10 mg kg^{-1} in 165 mM

NaCl solution) was given by slow infusion into a tail vein of the unrestrained mouse at a constant rate of 0·3 ml min^{-1} until a clonic convulsion was elicited. The minimum convulsant dose (MCD pentetrazol) was thus obtained for each mouse and the mean ± s.e. mean was calculated for each group.

With the exception of the mice dosed with clobazam 1 mg kg^{-1}, blood samples were taken from all mice receiving clobazam or desmethylclobazam immediately after they had been given the convulsant. Mice were anaesthetized with 2% halothane (ICI Ltd; 1 litre min^{-1}) and up to 1 ml of blood was taken from the abdominal aorta. These blood samples were collected in heparinized tubes, centrifuged and the plasma stored at −20 °C until required.

Plasma concentrations of clobazam were assayed by gas-liquid chromatography, using a modification of the method of Greenblatt (1980). As the method was not suitable for *N*-desmethylclobazam concentrations above 2 mg ml^{-1} a modification of the high-performance liquid chromatography method of Brachet-Liermain et al (1982) was used.

Results

In Fig. 1A, the log of the clobazam dose is plotted against protection, where protection represents the difference between the mean MCD pentetrazol for the experimental group dosed with clobazam and the control group receiving vehicle only. Protection increased with the log of the clobazam dose, from 7·8 ± 2·3 mg kg^{-1} pentetrazol (mean ± s.e. mean) 2 h after 1 mg kg^{-1} clobazam, to 41·5 ± 3·8 mg kg^{-1} pentetrazol after 10 mg kg^{-1} clobazam. This relationship was linear between clobazam doses of 2·5 and 10 mg kg^{-1}. At doses lower than 1 mg kg^{-1}, the MCD pentetrazol was not significantly different from controls, whereas above 10 mg kg^{-1} clobazam the protection was so great that the end-point of the test (a clonic convulsion) could no longer be reliably detected.

Analysis of the plasma concentrations of both clobazam and desmethylclobazam in these mice showed that the clobazam concentrations 2 h after a 2·5, 5 or 10 mg kg^{-1} dose were very low. In three mice, no clobazam was detectable and in a further six the values were less than 10 ng ml^{-1} and thus below the limits of accurate measurement. The remaining twenty-one values ranged from 13 to 90 ng ml^{-1} and for these the correlation between log clobazam concentration and MCD pentetrazol was extremely poor ($r = 0·026$; $P > 0·1$). In contrast, the levels of desmethylclobazam at this time were much higher, ranging from 1290 to 4160 ng ml^{-1} and the correlation between log desmethylclobazam concentration and MCD pentetrazol was highly significant ($r = 0·79$; $P < 0·01$; $n = 30$). A scatter diagram of log plasma desmethylclobazam values against MCD pentetrazol was plotted and the slope of the regression line, calculated by the method of least squares, was 44·7 ± 6·5 (mean ± s.e. mean).

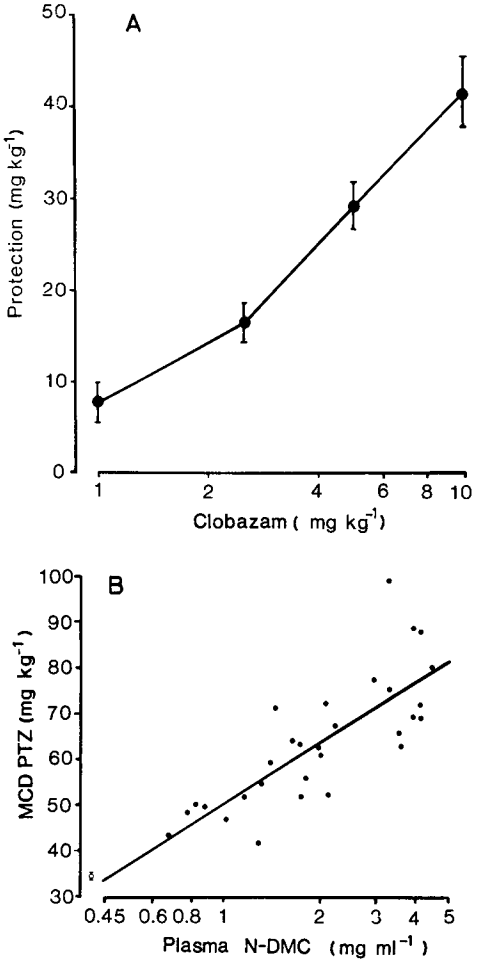

FIG. 1. Effects of subcutaneous administration of clobazam in mice. A: Log clobazam dose plotted against protection after 2 h. Each point shows mean ± s.e. mean (n = 10). B: Plasma concentrations of *N*-desmethylclobazam (N-DMC) measured in the same mice and plotted as a scatter diagram against MCD PTZ (n = 30). The open circle represents the mean (± s.e. mean) for control animals (n = 10).

After administration of desmethylclobazam, mice were clearly protected in a log dose-dependent manner, at doses ranging from 2.5 to 40 mg kg^{-1} (Fig. 2A). The protection, measured after 1 h, ranged from 12·3 ± 2·9 to 41·2 ± 4·2 mg kg^{-1} pentetrazol (mean ± s.e. mean). Analysis of the plasma values of desmethylclobazam once again revealed a highly significant correlation between MCD pentetrazol and log plasma concentration ($r = 0·897$; $P < 0·01$). The slope of this regression line was 42·8 ± 4·5 (mean ± s.e. mean).

There was no significant difference between the slopes of the regression lines for desmethylclobazam

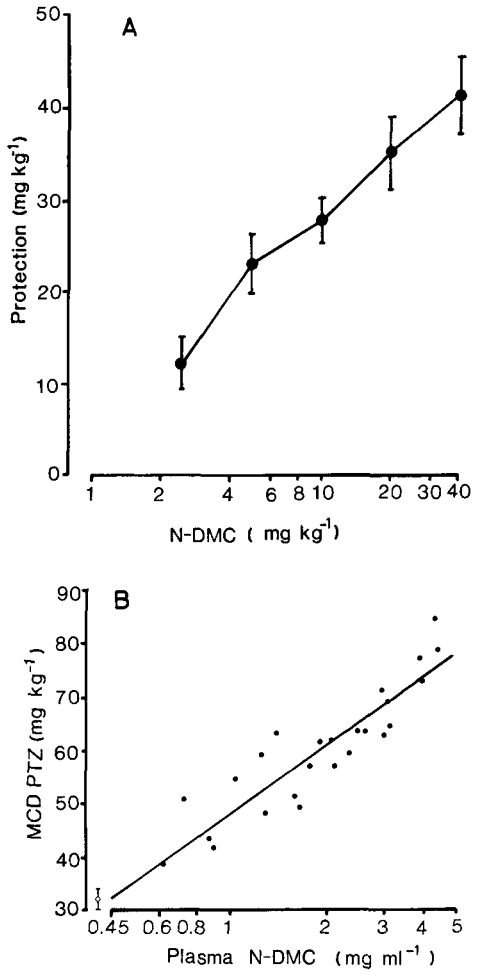

FIG. 2. Effects of subcutaneous administration of *N*-desmethylclobazam in mice. A: Log *N*-desmethylclobazam (N-DMC) dose plotted against protection after 1 h. Each point shows mean ± s.e. mean (n = 5). B: Plasma concentrations of N-DMC measured in the same mice and plotted as a scatter diagram against MCD PTZ (n = 25). The open circle represents the mean (± s.e. mean) for control animals (n = 5).

against MCD pentetrazol after clobazam and desmethylclobazam administration (t = 0·247; $P > 0·1$; Student's t-test).

Discussion

Our results show that although the log dose response relationships for both clobazam and its metabolite, desmethylclobazam, appeared linear (Figs 1 and 2), plasma values of clobazam were low and furthermore there was no correlation between these and the response. However, plasma values of desmethylclobazam were well correlated with the protection resulting from administration of either compound.

The slopes of the regression lines on the scatter diagrams plotted (Figs 1B, 2B) were not significantly different from each other and neither were the intercepts on both axes. This suggests that any residual levels of clobazam, 2 h after administration, played no part in the response.

Caccia et al (1980b) have shown that in mice, but not in rats, significant concentrations of desmethylclobazam are found after clobazam administration. They have invoked this difference to explain the greater duration of protection afforded by clobazam in mice, as the brain concentration and brain half-life of clobazam were similar in both species, whereas desmethylclobazam has a half-life (approx. 200 min) over three times that of the parent compound in mice. Fielding & Hoffmann (1979) had previously shown that desmethylclobazam was itself active against pentetrazol-induced seizures in the mouse, although at higher doses than clobazam. It has since been demonstrated that brain levels of the metabolite are similar after administration of either clobazam or desmethylclobazam in doses which caused protection (Caccia et al 1980a).

Thus our results confirm the findings of Caccia and his colleagues, regarding the significance of desmethylclobazam in the response to clobazam treatment. Furthermore, we have shown, by monitoring plasma concentrations and response in the same animals, that there is a good correlation between plasma desmethylclobazam concentration and anticonvulsant activity.

It seems possible that, on an acute basis, the prediction of anticonvulsant response by monitoring plasma metabolite concentrations of benzodiazepines may be justified, particularly in cases where one metabolite makes a predominant contribution to the overall effect.

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