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RO 15-1788 ANTAGONISES THE CENTRAL EFFECTS OF DIAZEPAM IN MAN WITHOUT ALTERING DIAZEPAM BIOAVAILABILITY

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1 In a double-blind, placebo controlled study, the efficacy of Ro 15-1788, a new benzodiazepine antagonist, in blocking the cognitive, psychomotor and subjective effects of diazepam, was investigated in a group of six healthy male volunteers.

2 The central effects of orally administered diazepam (40 mg) were most pronounced 1 h after dosing and persisted for 9 h with decreasing severity.

3 Concurrent oral administration of Ro 15-1788 (200 mg) completely prevented the impairment in cognitive and psychomotor function observed after diazepam alone.

4 The duration of action of Ro 15-1788 was shorter than that of diazepam.

5 Plasma diazepam levels after administration of the diazepam/antagonist combination were very similar to those observed following diazepam alone.

Introduction

The benzodiazepines are considered to produce their numerous effects on the central nervous system primarily by facilitating the synaptic transmission of the inhibitory neurotransmitter γ-amino butyric acid (GABA) (Haefely *et al.*, 1975; Costa *et al.*, 1976, 1978). Using radiolabelled diazepam it has been possible to demonstrate the existence of high affinity binding sites in the mammalian brain that fulfil many of the criteria of pharmacological receptors (Mohler & Okada, 1977 a, b; Squires & Braestrup, 1977). The presence of similar receptors in the human brain has also been demonstrated (Mohler & Okada, 1978). There is convincing evidence that these benzodiazepine receptors are associated with the post-synaptic GABA receptor complex and that the enhancing effect of the benzodiazepines on GABAergic transmission, and consequently their pharmacological activity, is initiated by interaction between the benzodiazepines and their receptor sites (Guidotti *et al.*, 1978).

Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a] [1,4]-benzodiazepine-3-carboxylate; Figure 1) is a recently synthesised imidazodiazepine which potently inhibits the specific high-affinity binding of [^{3}H]-diazepam to brain synaptosomal fractions but which produces, *in vivo*, none of the behavioural or neurological effects typical of the benzodiazepines (Hunkeler *et al.*, 1981).

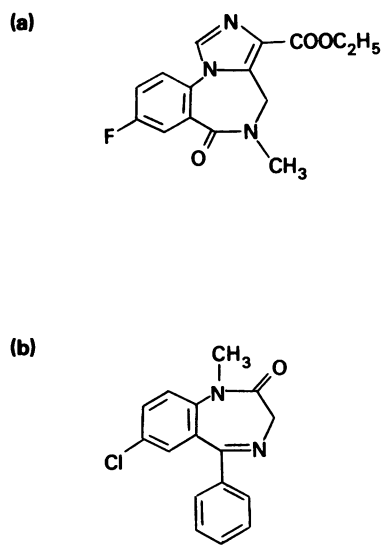

Figure 1 Structural formulae of (a) Ro 15-1788 and (b) diazepam.

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Furthermore Ro 15-1788 blocks the behavioural, neurological and electrophysiological effects of several marketed benzodiazepines in animals (Hunkeler *et al.*, 1981; Polc *et al.*, 1981). Animal studies have shown this compound to be even less toxic than most of the classical benzodiazepines (Hunkeler *et al.*, 1981). Single oral doses up to 600 mg are well tolerated in man and are devoid of any demonstrable pharmacological activity (unpublished data). The marked central effects of 3-methylclonazepam in man have also been effectively antagonised by Ro 15-1788 (Darragh *et al.*, 1981, a, b).

Methods

Subjects

Six healthy male volunteers aged between 19 and 34 years (mean 24 years) and within 10% of their ideal body weight (mean 69.5 kg, range 62.7–80.0 kg), participated in this study. A complete physical examination, including 12-lead electrocardiogram and laboratory screen, was conducted before and after the study. Each subject gave written informed consent to participate in this study, the protocol for which was approved by the Institutional Review Board. Subjects were excluded if there was any abnormality on physical examination or laboratory findings, a history of allergy (including drug hypersensitivity), a history of drug abuse, or intake of any medication within 2 weeks of enrolment in the study, or a score of more than 13 or less than 3 of the Eysenck Personality Inventory. Smoking, alcohol and caffeine-containing drinks were not allowed for the duration of each study period.

Treatments

The following dosing combinations were investigated:

(a) Diazepam (40 mg) + placebo (Ro 15-1788)
(b) Diazepam (40 mg) + Ro 15-1788 (200 mg)
(c) Placebo (diazepam) + placebo (Ro 15-1788)

Experiment design

This was a double-blind, balanced, triple crossover investigation. Each subject underwent three experimental conditions corresponding to the administration of treatments (a), (b) and (c) above. The order of administration was randomised using two 3 × 3 latin square designs, and a 14-day washout period was allowed between successive doses. Subjects were required to report to the Institute at 19.00 h on the day preceding each study day and remained under supervision until all assessments had been completed. 'Lights-out' was from 23.00 to 08.00 h, to ensure compliance with dietary restrictions and to stabilize circadian variation. Subjects fasted from 23.00 h until 1.5 h after dose administration. Each treatment combination was administered orally with a glass of water.

Psychometric tests

The following psychometric procedures were used in the assessment of drug effects:

(1) *Digit Symbol Substitution Test (DSST)* This subtest of the Weschler Adult Intelligence Scale (WAIS) was administered according to the WAIS manual (Weschler, 1955). In order to minimize learning, four equivalent versions were used. This test requires intense cognitive effort over a short period and is reliably affected by the benzodiazepines (Wittenborn, 1978).
(2) *Thurstone's perceptual speed test (PST)* In this test subjects were required to compare visual configurations, embedded in distracting material and to identify two figures as identical under conditions of time pressure. More information regarding this test may be found in Thurstone (1966).
(3) *Signalled reaction time (SRT)* Subjects were required to respond to the onset of a light stimulus, preceeded at a random interval (1–2.5 s) by an auditory 'ready' signal, by pressing a button. Ten practice trials were given followed by 20 test trials.
(4) *Digit copying test (DCT)* This test is similar to the DSST except that instead of substituting symbols, subjects were required to copy the digits themselves. The score is the number of digits correctly copied in 60 s. This test requires little mental effort and is mainly an index of psychomotor speed and integration. In previous studies, we have found DCT to be reliably affected by benzodiazepines (unpublished data).
(5) *Subjective assessments* Subjects were required to rate subjective mood on a series of 16 bipolar visual analogue scales (100 mm), as described by Bond & Lader (1974). These scales load onto three factors: alertness, calmness and contentedness and have been found to be particularly useful in assessing drug-induced shifts in mood (Bond & Lader, 1973; Tyrer, 1976).

To minimize learning effects in the psychometric tests, the subjects were intensively trained on 2 days before the first dose administration. The test battery was administered by the same operator at 1, 2.5, 6, 9 and 25 h after each dose.

Plasma diazepam levels

Blood for diazepam assay was taken pre-dose and 1, 2, 3, 4, 6, 8, 12 and 24 h after administration of both

diazepam and the diazepam/Ro 15-1788 combination. Plasma diazepam was measured by radioimmunoassay as described by Dixon & Crews (1978). Comparative bioavailability of diazepam following the two treatments was assessed by comparing the areas under the plasma levels/time curves (AUCs) using a t-test for paired data. The AUCs, from $t = 0$ to $t = 24$ h, were determined by the trapezoidal rule. Pharmacokinetic parameters were calculated on an IBM 360 computer using the CSTRIP programme of Sedman & Wagner (1976).

Analysis of psychometric data

The condition of homogeneity of variance having been satisfied, data were submitted to a series of two-way analyses of variance. Significant times by treatments interactions were further investigated by means of a series of one-way analyses of variance, comparing either diazepam or combination scores with corresponding placebo scores. Only results from the one-way comparisons are reported.

Results

Performance tests

The results of the performance tests were illustrated in Figures 2, 3, 4 and 5. Diazepam significantly impaired performance on all four tests at 1 and 2.5 h. At 6 h DSST and DCT scores were still impaired while at 9 h drug effects were observed on the DCT and SRT measures. No significant effects were seen on any measure 25 h after dosing.

When Ro 15-1788 was administered concurrently with diazepam, impairment was prevented on all four measures up to 6 h after dosing. Scores following the combination at 9 h, however, were lower than placebo scores for DSST and DCT, with DCT still showing significant impairment at 25 h.

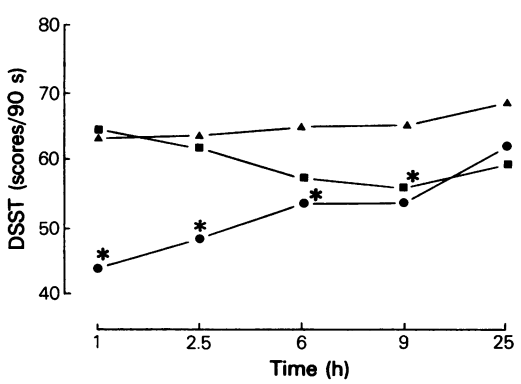

Figure 2 Effect of diazepam (●), diazepam + Ro 15-1788 (■) and placebo (▲) on digit symbol substitution (mean scores $n = 6$). $^*P < 0.05$ compared with placebo.

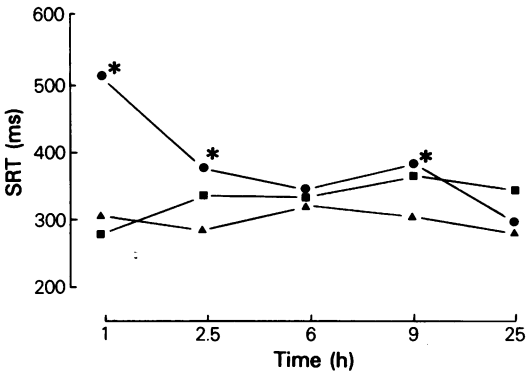

Figure 3 Effect of diazepam (●), diazepam + Ro 15-1788 (■) and placebo (▲) on simple reaction time (mean scores $n = 6$). $^*P < 0.05$ compared with placebo.

Subjective ratings

Significant mood changes observed following the two treatments, as compared with placebo scores, are illustrated in Figure 6. The most marked changes in mood occurred 1 h after diazepam, with 10 of the 16 scales reflecting significant changes. These shifts indicate drug-induced sedation, incoordination, lethargy and mental slowness, which persisted, with decreasing severity for 9 h after dosing. The marked changes in mood seen 1 h after diazepam alone were not observed following concurrent administration of Ro 15-1788, with only the incompetence scale showing change. This represents almost complete antagonism of the subjective effects of diazepam at this time, and is in good agreement with the performance data. With time, however, more

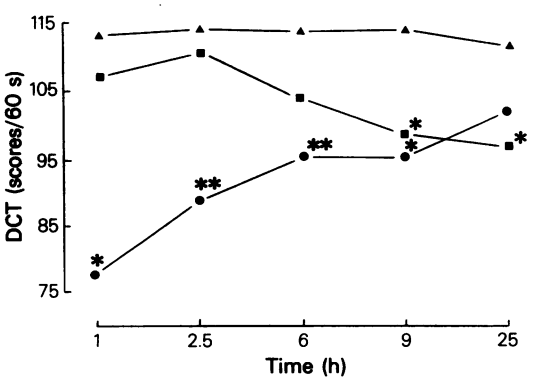

Figure 4 Effect of diazepam (●), diazepam + Ro 15-1788 (■) and placebo (▲) on the digit copying test (mean score $n = 6$). $^*P < 0.05$, $^{**}P < 0.01$ compared with placebo.

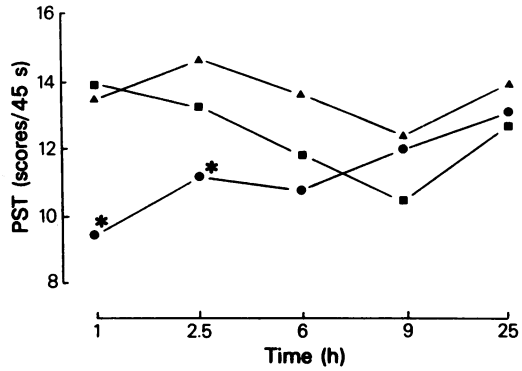

Figure 5 Effect of diazepam (●), diazepam + Ro 15-1788 (■) and placebo (▲) on the perceptual speed test (mean scores $n = 6$). * $P < 0.05$ compared with placebo.

marked shifts in mood occurred following the combined dose, so that by 6 h some changes were observed which reflected sedation, ataxia, mental slowness and lethargy. At 9 h subjects still rated themselves as feeling more clumsy and lethargic following the combination. By 25 h no significant drug effects were observed.

Plasma diazepam

Mean plasma diazepam levels for the six subjects, following either diazepam alone or the diazepam/Ro 15-1788 combination, are illustrated in Figure 7. The two curves are virtually superimposable and statistical analysis confirmed that the mean AUCs did not differ significantly for the two treatments. The mean terminal elimination half-lives of diazepam following the two treatments were not significantly different (19.4 ± 6.2 h following diazepam alone, 20.1 ± 7.9 h following the combined dose).

Discussion

The results of this study establish Ro 15-1788 as an effective antagonist of the cognitive, psychomotor and subjective effects of diazepam in man. The impairment in performance observed following diazepam alone was similar to that reported elsewhere (McNair, 1973; Kleinknecht & Donaldson, 1975; Wittenborn, 1979). The data indicate that the most severe performance decrements and mood changes occurred when plasma levels of diazepam were highest, with recovery from the sedative effects being observed during the elimination phase. It is of interest that performance, as assessed by DSST and PST, showed more rapid recovery than on the SRT and DCT, both of which have predominantly motor components. Concomitant administration of Ro 15-1788 effectively prevented significant impairment in performance due to diazepam for up to 6 h after-dosing, although marked changes in mood were apparent earlier. The findings are in good agreement with our previous observations (Darragh *et al.*, 1981a) where this antagonist effectively blocked the potent central effects of 3-methyl-clonazepam in healthy volunteers for up to 2.5 h. The duration of action of Ro 15-1788 in these studies was relatively short, possibly reflecting its short elimination half-life of less than 2 h (unpublished data).

Figure 6 Summary of directional shifts in mood compared with placebo, following diazepam alone or diazepam in combination with Ro 15-1788. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Comparison	*Time after dose administration (h)* 1	2.5	6	9
Diazepam (*v* Placebo)	Drowsy*** Feeble** Muzzy*** Clumsy*** Lethargic*** Mentally slow** Dreamy** Incompetent*** Bored* Withdrawn**	Drowsy* Feeble* Muzzy* Clumsy** Dreamy* Bored***	Muzzy* Clumsy**	Clumsy* Mentally slow*
Diazepam + Ro 15-1788 (*v* Placebo)	Incompetent*	Clumsy* Dreamy** Incompetent*	Drowsy* Feeble* Muzzy* Clumsy* Lethargic* Mentally slow** Incompetent*	Clumsy* Lethargic*

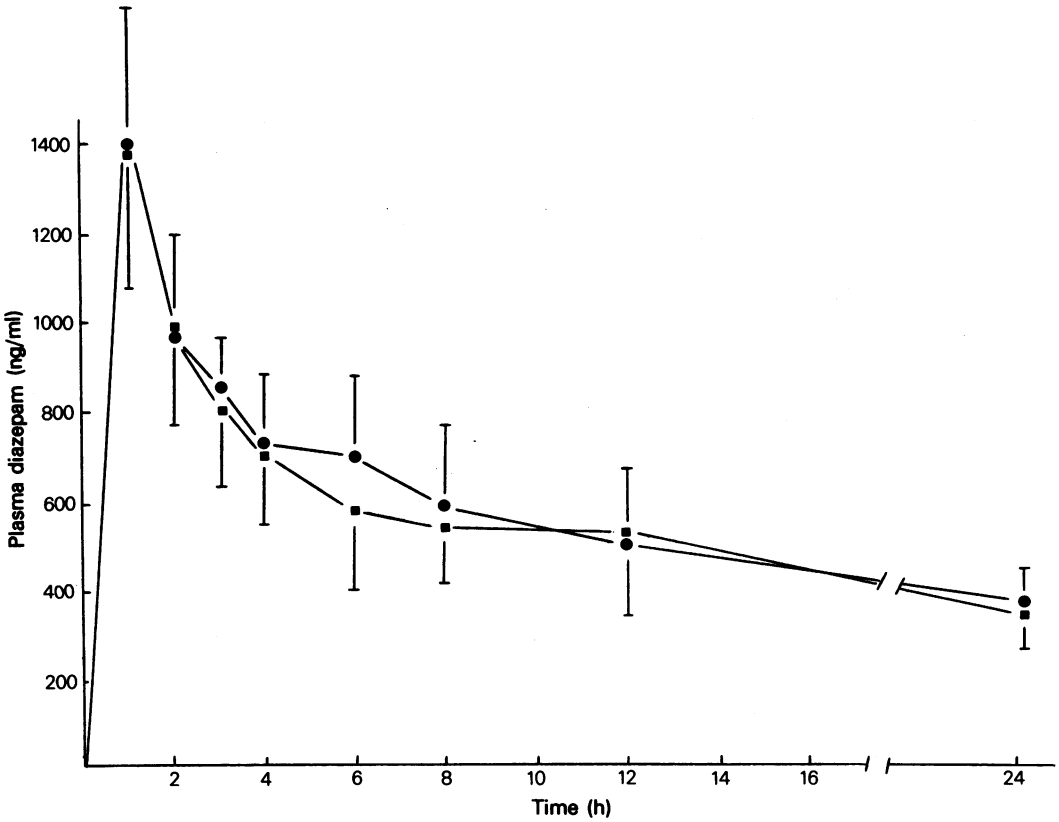

Figure 7 Plasma levels of diazepam following administration of diazepam alone (40 mg) (●) and diazepam (40 mg) + Ro 15-1788 (200 mg) (■) (mean ± s.d., $n = 6$).

The ability of Ro 15-1788 to antagonise the central effects of diazepam does not appear to be mediated by interference with the bioavailability of the parent compound. However the possibility that the antagonist may modify the bioavailability of *N*-desmethyldiazepam, the major active metabolite of diazepam, cannot be ruled out.

The results of the present study correlate well with the animal data reported by Hunkeler *et al.* (1981), despite evidence for species differences in benzodiazepine receptor distribution (Tallman *et al.*, 1980). The efficacy of Ro 15-1788 in blocking the binding of [^{3}H]-diazepam to its receptor sites, in conjunction with the present findings, suggest that the cognitive, psychomotor and subjective effects of diazepam in man are mediated by the benzodiazepine receptor. Since only normal subjects were investigated in the present study, it is not possible to say whether the anxiolytic effects of the benzodiazepines are also mediated in this way. However, the ability of Ro 15-1788 to antagonise the anti-conflict effects of the benzodiazepines in animals (Hunkeler *et al.*, 1981) suggest that this may be the case.

Previous attempts to antagonise the central effects of the benzodiazepines by cholinesterase inhibitors (Di Liberti *et al.*, 1975; Rupreht, 1980) or naloxone (Bell, 1975: Christensen & Huttel, 1979) have been inconclusive, but have demonstrated the potential value of an effective benzodiazepine antagonist. Clinically, a drug such as Ro 15-1788 would be of benefit in the management of benzodiazepine overdose, and in the rapid reversal of benzodiazepine anaesthesia. As a research tool, such an antagonist should prove useful in further characterisation of the benzodiazepine receptor, which may in turn lead to the synthesis of new and more specific benzodiazepine antagonists. Such antagonists should also prove valuable in the search for endogenous benzodiazepine receptor ligands and in the determination of their relationship, if any, to anxiety states.

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