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The Effects of a Benzodiazepine Antagonist Ro 15-1788 in the Presence of Stable Concentrations of Midazolam

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The benzodiazepine antagonist Ro 15-1788 was administered in a 10-mg intravenous bolus dose to seven healthy young adult volunteers to evaluate the drug's efficacy and duration of action against midazolam. A steady state serum concentration of midazolam was obtained by an initial fast infusion rate of 6.0 mg/min (duration: 10 min) and a maintenance infusion rate of 0.275 mg/min. After administering the antagonist, all subjects opened their eyes without any command in a median time of 36 s (range: 28–48 s). Their personal, temporal and local orientation was reestablished within 54–120 s (median time: 65 s). The subjects fell deeply asleep, again in a median time of 145 min (range: 115–150 min), which was interpreted as an indication of the returning action of midazolam, which was infused for a total period of 210 min. Ro 15-1788 deserves further study as an antagonist, since it could prove useful in the management of benzodiazepine overdosage and in the reversal of benzodizepine action following surgical anesthesia and in the intensive care. (Key words: Anesthetics, intravenous: midazolam. Antagonists, miscellaneous: benzodizepines, Ro 15-1788. Hypnotics: benzodiazepines, midazolam.)

BENZODIAZEPINE DRUGS are widely used during surgical anesthesia and in the intensive care. The precise mechanisms by which their effects are produced are still incompletely understood. However, they are thought to be mediated through facilitation of the inhibitory synaptic transmission of gamma-aminobutyric acid[1] as a result of the interaction between the benzodiazepines and their receptors.[2]

Several newly synthesized substances inhibit the specific binding of benzodiazepines to brain synaptosomal fractions but produce *in vivo* none of the usual benzodiazepine effects.[3] Of these antagonizing compounds, Ro 15-1788 is the first to undergo clinical investigation. The structural formula (fig. 1) reveals the drug to be an imidazobenzodiazepine. The results of animal studies[3] indicate that Ro 15-1788 acts at the level of the central benzodiazepine receptors to inhibit the usual effects of the benzodiazepine group. Single doses of Ro 15-1788 seem to be well tolerated and nearly devoid of other pharmacologic activity.[4]

This study was undertaken to evaluate the efficacy and minimal duration of action of an intravenous bolus dose of Ro 15-1788 counteracting the action of midazolam at a constant plasma concentration.

Materials and Methods

Seven young healthy subjects (age: 24 ± 3 yr; weight: 69 ± 12 kg; height: 176 ± 9 cm; 3 female and 4 male) participated in the study, which was approved by the hospital research authorities. Informed written consent was obtained from all subjects.

Two indwelling intravenous catheters were placed in the right and left antecubital vein, one for drug administration and the other for blood sampling. The blood sampled was substituted by 100 ml/h Ringer's solution.

Previous studies indicated that a midazolam serum concentration plateau of 0.6 μg/ml is necessary to produce a deep hypnotic effect.[5] This was achieved by a dosage regimen based on pharmacokinetic analysis according to an open two-compartment model[6] and a general method to achieve plasma concentration plateaus[7]: An initial fast infusion rate of 6.0 mg/min lasting 10 min was followed by a maintenance infusion rate of 0.275 mg/min.

The expected concentration (amount)–time–profile was simulated (fig. 2) by computing the convolution integral of the infusion scheme and the pharmacokinetic data after bolus administration.[8] The stimulation depicts constant plasma concentrations to be achieved within 60 min. Steady state conditions are represented by the constant plasma concentration of 0.6 μg/ml (upper curve) and the constant amount of 33 mg midazolam in the peripheral compartment (lower curve). In order to check these simulated data, multiple 5-ml blood samples were taken prior to and during the midazolam infusion. Further blood samples were taken until 24 h after cessation of the infusion. The blood samples were centrifuged and frozen at −20° C until determination of midazolam concentrations by gas chromatography.[9]

Sixty minutes after commencing the midazolam infusion, a 10-mg bolus dose of the benzodiazepine antagonist Ro 15-1788 was injected intravenously over 5 s.

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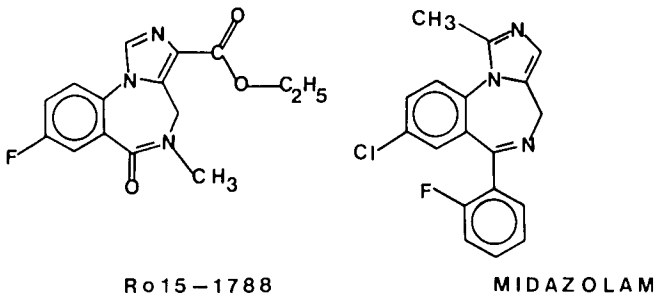

FIG. 1. Structural formula of Ro 15-1788 and midazolam.

The state of awareness of the subjects was measured by a tracing test series (fig. 3) at predetermined times. The test was used to investigate the fine motor response[10] and the subject's ability to concentrate during a short period. The subjects had to trace within the borders of a 4-mm-wide, 80-cm-long winding channel, making as few errors as possible. Every error was counted electronically by a pocket calculator. The product of the performance time and the error counts (time–error–product) was used as test score. Prior to receiving any drugs, the subjets were trained in the techniques of the test until the learning plateau had been reached. After a few rehearsals, within 30 min the subjects performed the test series within a median time of 32 s (range 24–53 s) with a median error count of 7 (range 3–20). The median time–error–product resulted in 209 s (range 101–472 s). The test results of every subject prior to any drug administration are listed in table 1.

The tests were carried out 15, 30, 60, 90, and 120 min after Ro 15-1788 dosage if the subjects remained sufficiently awake to participate.

The subjects were judged to be unconscious or deeply asleep if they didn't respond to verbal commands ("Open your eyes! Put out your tongue!") of normal intensity (52–55 dB). Prior to these commands, the subjects received an acoustic verbal stimulus of maximal loudness (82–87 dB) to provoke an arousal reaction. The subjects were judged drowsy if they slept spontaneously but could be awakened by the stimuli mentioned above.

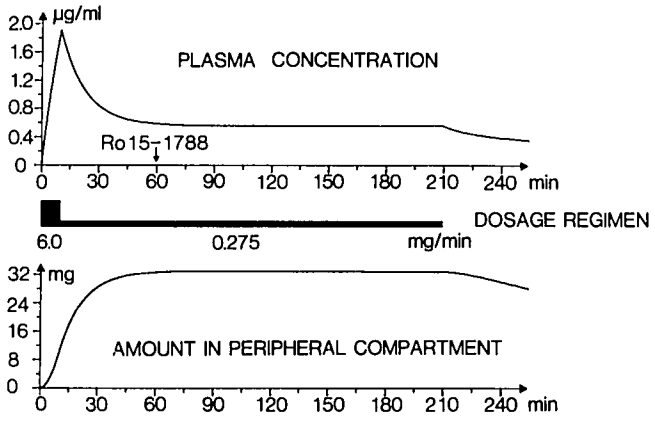

FIG. 2. Computer simulation of the expected midazolam concentration–time–profile (upper curve) and the amount–time–profile in the peripheral compartment (lower curve). The dosage regimen is depicted between both curves.

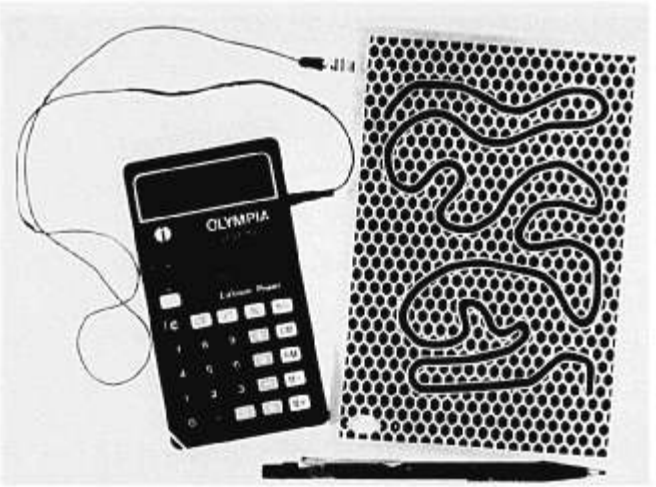

FIG. 3. Tracing test equipment used to evaluate the subject's level of awareness. The subjects had to trace within the borders of the winding channel, making as few errors as possible. The errors were counted electronically by a pocket calculator. The product of the performance time and the error counts (time–error–product) was used as test score.

The scores of the tracing test prior to any drug administration were compared with those after administering Ro 15-17788 by Wilcoxon's signed–ranks test for paired observations. Probability values of less than 0.05 were considered to be of statistical significance.

Results

The midazolam plasma concentrations reached and exceeded the target concentration within 2 min (fig. 4). After the predicted overshoot associated with the equilibration of the peripheral compartment, plasma concentrations decreased to the target level within 50 min of initiation of the midazolam infusion, remaining at approximately 0.6 µg/ml. After cessation of the infusion, plasma concentrations decreased with a mean half-life of 155 min (SD 30 min) and a mean total plasma clearance of 503 ml/min (SD 100 ml/min).

Following midazolam, the subjects fell asleep with loss of eyelid reflex within a median time of 2 min (range: 1–3 min). The corneal reflex couldn't be triggered within a median time of 4 min (range: 2–5 min). The defined unconscious state was reached within a median time of 4 min (range: 2–7 min) and was maintained

TABLE 1. Results of the Tracing Test Series (n = 4) of Every Subject Prior to Any Drug Administration (The last line indicates the over all results (n_{total} = 28). All values are given as median with the range in parentheses)

Subject	Error Counts	Performance Time	Time-Error-Product
1	7.5 (7–9)	28.0 s (25.2–32.8 s)	224 s (176–257 s)
2	3.5 (3–4)	33.8 s (27.6–40.4 s)	106 s (101–162 s)
3	15 (9–20)	26.1 s (23.6–32.2 s)	392 s (290–472 s)
4	7 (6–9)	31.8 s (30.1–34.5 s)	191 s (181–310 s)
5	5 (4–6)	31.9 s (29.9–33.1 s)	150 s (128–199 s)
6	7 (5–10)	25.6 s (24.5–32.7 s)	204 s (123–245 s)
7	5.5 (5–10)	41.4 s (40.0–53.4 s)	265 s (204–400 s)
Median over all tests	7 (3–20)	32.0 s (23.6–53.4 s)	209 s (101–472 s)

until the administration of Ro 15-1788 1 h after commencing the midazolam infusion, which was continued.

Following Ro 15-1788, the subjects opened their eyes without any command in a median time of 36 s (range: 28–48 s). Within a median time of 65 s (range: 54–120 s), they were fully oriented with respect to person, time, and location. They felt as if they had awakened from a normal sleep. The subjects became drowsy again within a median time of 100 min (range: 60–140 min).

They were deeply asleep (unconscious) within a median time of 145 min (range: 115–150 min).

The midazolam infusion was stopped 150 min after the Ro 15-1788 bolus dose. The total infusion period was 210 min, and the total dose of midazolam was 115 mg.

The group results of the tracing test are listed in table 2, whereas the test scores of each individual subjects are plotted in figure 5 as a function of the time elapsed after administering Ro 15-1788. The test series performed 15 and 30 min after the Ro 15-1788 dosage didn't differ significantly from the control values prior to any drug administration, although their median values were slightly higher. However, the individual plot (fig. 5) of the two test series showed no general trend to higher time-error-products. Only one subject (▲) performed the tracing test evidently worse during midazolam infusion and after Ro 15-1788 administration. The scores of the tracing test subsequently performed were significantly ($P < 0.05$) higher than the control values, indicating a returning sedation and incoordination. Subsequent psychomotor tests could not be performed because the subjects were asleep and could not be awakened sufficiently to participate. These findings correlated well with the clinical status, which could be described as drowsy after 60 and 90 min of Ro 15-1788 dosage.

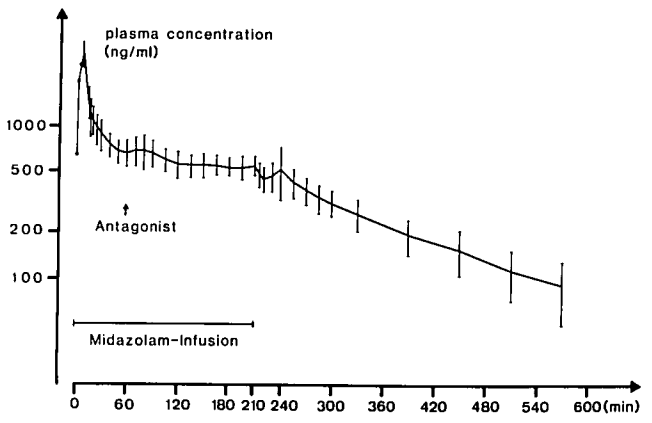

FIG. 4. Midazolam plasma concentration (●: mean values ± SD; n = 7).

Discussion

The onset of Ro 15-1788 action was very prompt, and all subjects were fully oriented within 2 min. The site of action of Ro 15-1788 is presumed to be in the well-perfused cerebral tissues where benzodiazepine receptors have been identified in the rat.[11] Continued alertness of the subjects was demonstrated by the nearly normal tracing test scores obtained at 15 and 30 min after administration of Ro 15-1788.

No subject complained of anxiety when the midazolam action was antagonized. A feeling of anxiety would have given information about a possible additional action of Ro 15-1788 inverse to the anxiolytic action of the

TABLE 2. Results of the Tracing Test Series After Ro 15-1788 Administration in Comparison to the Base Values Prior to Any Drug Application (The test scores are listed as median values with the range in parentheses)

Time after Ro 15-1788	Time-Error-Product	*P* Value	Clinical Status
Base Value	209 s (101–472 s)	—	—
15 min	308 s (289–682 s)	NS	Awake
30 min	352 s (244–1,084 s)	NS	Awake
60 min	1,022 s (395–3,423 s)	<0.05	Drowsy
90 min	1,494 s (592–9,004 s)	<0.05	Drowsy
120 min	No tracing test possible	—	Asleep

NS = not significant (Wilcoxon's signed-ranks test).

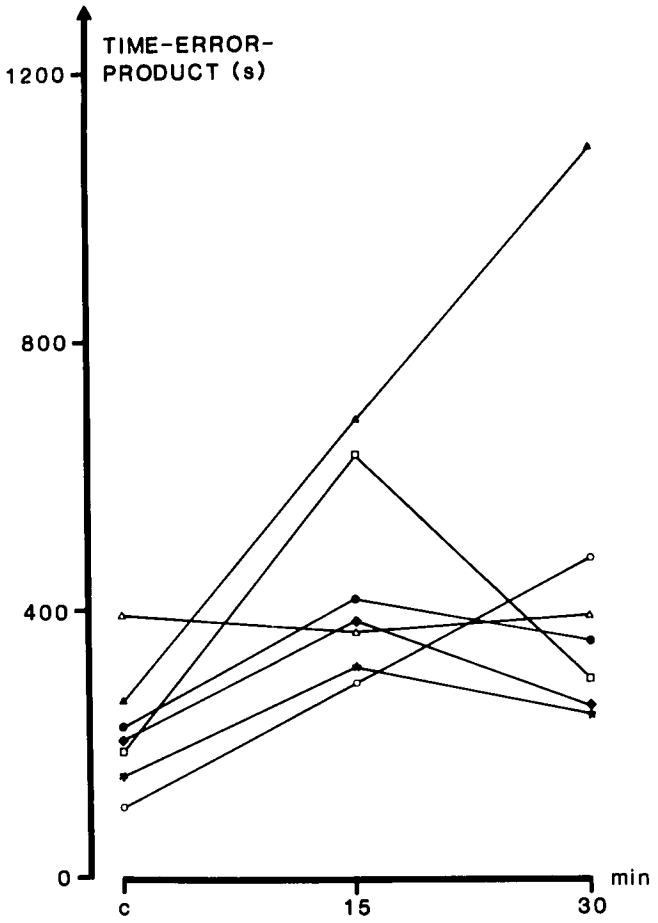

FIG. 5. Time–error–products of each individual subjects as a function of the time elapsed after Ro 15-1788 administration (c = control value prior to any drug administration; each subject is characterized by a special symbol).

benzodiazepines.[12] But, whether the action of Ro15-1788 is purely antagonistic or not could not be studied. It is possible that transient feelings of anxiety and fear were masked by residual anxiolytic effects of high-dose midazolam. Studies using lower benzodiazepine doses may give further information as to whether Ro 15-1788 is a pure benzodiazepine antagonist or an antagonist with inverse agonistic action.

The duration of antagonistic action was relatively long in relation to the short elimination half-time ($t_{1/2}\beta$ = 28 min) and the relatively rapid plasma clearance (2,490 ml/min) of Ro 15-1788 in a single subject.[13]

The drowsiness evident after a median time of 100 min can be interpreted as an indication of the declining efficacy of Ro 15-1788 and the returning action of midazolam as well. The minimal duration of action of this dose of Ro 15-1788 is approximately 90–120 min. A practical impact of this finding is the possibility that patients after receiving a high-dose benzodiazepine may fall asleep again in spite of an initial successful reversal of the benzodiazepine action by Ro 15-1788. Nevertheless, in our opinion, such an efficacious antagonist would be useful in clinical practice—both in the management of overdosage and the reversal of benzodiazepine action in surgical anesthesia and in intensive care.

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