EFFECT OF FLUMAZENIL ON MIDAZOLAM-INDUCED AMNESIA

A. C. McKay, M. S. McKinney AND R. S. J. Clarke

SUMMARY

We have studied the effect of i.v. flumazenil 0.01 mg kg⁻¹ on the amnesia and sedation caused by midazolam 2 mg and 5 mg i.v. in volunteers in order to determine the relationship between the actions of the antagonist on these two effects. Midazolam caused dose-dependent central neural depression as assessed by critical flicker fusion frequency, and dose-dependent amnesia for word cards. In subjects given flumazenil 5 min after administration of midazolam, fusion frequency readings and memory were restored to levels comparable to those before midazolam administration. These two effects of flumazenil were similar in time course and extent, suggesting that they share the same mechanism of action. Flumazenil given alone had no effect on memory. The study has demonstrated anterograde amnesia following benzodiazepine administration and antagonism by flumazenil. There was neither retrograde amnesia nor retrograde antagonism of amnesia.

KEY WORDS


The specific benzodiazepine antagonist flumazenil has little agonist activity, but binds competitively to the benzodiazepine receptor, blocking the actions both of benzodiazepines and of inverse agonists such as beta-carbolines [1]. The major limitation to its routine use would appear to be its short elimination half-life [2] (49–58 min), which is shorter than that of clinically used benzodiazepines, so that resedation is at least a theoretical possibility.

It has long been recognized that benzodiazepine-induced sedation is accompanied by anterograde amnesia, in that patients may appear to be fully aware of events and conversations, but are found subsequently to have no memory. With the exception of lorazepam, the amnesia is reliable only when the drugs are given i.v. [3]. The duration of amnesia is dose-dependent [4].

In recent years, midazolam has become the drug of choice in most situations in acute care in which the use of a parenterally administered benzodiazepine is indicated. This water soluble imidazo-benzodiazepine is associated with a low incidence of pain on i.v. injection and of venous sequelae [5] and has a comparatively short elimination half-life, with no significant pharmacologically active metabolites [6]. In 1980, Dundee and Wilson [7] showed that, when given i.v. in a dose of 5 mg, midazolam caused dense anterograde amnesia of 5–15 min duration, followed by a variable period of less profound amnesia.

Clearly, it is important to be able to predict the nature, extent and duration of the effect of flumazenil on the amnesia produced by i.v. midazolam, particularly in comparison with its effect on the level of sedation. We have therefore adapted the method of Dundee and Wilson to study, first, the effects of two commonly used clinical doses of midazolam, administered i.v., on memory of precisely timed stimuli presented over a period of a few minutes after injection, while at the same time assessing the degree of central neural depression; second, the effects on these observations of subsequent i.v. administration of flumazenil in the recommended dose range; and third, the effects of this dose of flumazenil when not preceded by midazolam.

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SUBJECTS AND METHODS

We studied six healthy volunteers (mean age 34 yr, mean weight 68 kg) on six morning sessions, separated by at least 1 week, so that each received once, in random order, all of the following drug combinations: midazolam 2 mg–saline (M2S); midazolam 5 mg–saline (M5S); midazolam 2 mg–flumazenil (M2F); midazolam 5 mg–flumazenil (M5F); saline–flumazenil (SF); saline–saline (SS). Subjects were unaware of the sequence.

The Leeds Critical Flicker Fusion Frequency Monitor [8] was used to assess the subjects’ degree of central neural depression. This instrument has four light-emitting diodes which are placed 60 cm horizontally in front of the subject and, when activated by the observer, flicker with a frequency which increases or decreases at a rate of 1 Hz s\(^{-1}\) between 10 Hz and 50 Hz. The subject presses a button when the flicker appears subjectively to fuse into a steady light if frequency is increasing, or to become apparent if frequency is decreasing. The machine records this as the critical flicker fusion frequency (CFFF). Because the study was designed to measure rapidly occurring changes in the level of central neural arousal, one ascending and one descending frequency reading only were obtained at each CFFF measurement point and the mean of these calculated.

A sequential plan of the study is shown in figure 1. After four trial runs made over a period of 10 min, subjects recorded an initial CFFF score immediately before time 0, the start of the session. The first of the two drug treatments, midazolam 2 mg, midazolam 5 mg or saline in 2.5 ml was given over 20 s, beginning 1 min after the start of the session. The second treatment, either 0.01 mg kg\(^{-1}\) flumazenil or saline in 5.5–8.0 ml, was given over 40 s after a further 5 min.

At time 0 and after 0.5, 2.0, 2.5, 4.0, 4.5, 7.0, 7.5, 9.0, 9.5, 11.0 and 13.0 min, subjects were shown postcards, one at each time, on each of which one word was written in large letters. Thus two cards were shown before the first drug treatment, four between treatments and the remaining six after the second treatment. The cards were displayed for 3 s and the subjects read the words aloud. No other conversation took place. The words were concrete nouns chosen to occur at a frequency of between 1 in \(10^5\) and 1 in \(10^6\) in general use [9]. Sets of word cards were rotated so that the same combination of sets of words and treatment group was not used more than once, but the 12 words within each set were used always in the same order.

After 3, 5, 8 and 10 min from time 0—that is, twice between drug treatments and twice after the second treatment—further CFFF scores were obtained.

The sequence of injections, word card display and CFFF recordings lasted 13 min, after which subjects rested quietly until 2 h after time 0. A final CFFF score was obtained.

Subjects were subsequently shown, one by one and in random order, a set of 24 word cards containing the 12 previously shown, mixed with 12 others. They were asked to identify those already seen. Memory of cards was scored as one point for complete recognition of a card and half a point for uncertain or hazy recognition, giving a maximum possible score of six points per treatment group per word card. New sets of dummy cards were used for each subject on each occasion.

The CFFF data were analysed as a whole using the Kruskal–Wallis test. Where significant differences were shown, further analysis of individual inter-group differences was carried out using the Mann–Whitney \(U\) test. The memory data were analysed using Fisher’s exact test of
probability. Analysis was two-tailed, and a significance level of \( P < 0.05 \) was used for all tests.

RESULTS

Critical flicker fusion frequencies

There were no significant differences among the six treatment groups in mean CFFF scores taken before commencement of the sessions. At 3 min (2 min after the administration of midazolam 2 mg or 5 mg or saline), CFFF scores in the two groups given midazolam 5 mg (M5 groups) had decreased \( (P < 0.01) \) below those in the two saline (S) groups. However, although there was a reduction in CFFF scores in the two groups given midazolam 2 mg (M2 groups), these scores did not differ significantly from either the S or the M5 groups. The same pattern was seen at the 5-min reading (table I).

At 8 min (2 min after administration of either flumazenil 0.01 mg kg\(^{-1}\) or saline), mean CFFF score had recovered in the M5F group to the extent that it was no longer different from that in the S groups, while in the M5S group, which did not receive flumazenil, the score was significantly less than that in both the S groups and the M5F group. Again, similar but non-significant changes were seen in the M2 groups. A similar pattern of changes was seen in all groups at 10 min.

By 2 h after the beginning of the session, there were no significant differences in CFFF scores among the six treatment groups.

With regard to changes within the treatment groups during the period of the study, there were no significant changes in CFFF scores in either of the two S groups. In the M5S group, the four scores after midazolam administration were significantly less than the initial values, while in the M5F group, scores were reduced significantly at 3 and 5 min, but after flumazenil administration were again comparable to initial values. Again, changes in the M2 groups were similar but less marked. By the final reading at 2 h, mean CFFF scores in all groups were not significantly different from initial values.

Memory

The summed amnesia scores of the six subjects for each of the 12 word cards in all six treatment groups are shown graphically in figure 2. In the two S groups, most subjects recognized most of the words when they were shown again 2 h later, with no significant differences between these two groups. In all four midazolam groups, recognition of the two cards shown before any drug was given was almost complete. However, for word cards shown after midazolam administration but before the second drug treatment, memory was reduced markedly in the two M2 groups and virtually abolished in the two M5 groups. In all four midazolam groups, these scores were significantly different \( (P < 0.01) \) both from the S groups and from the pre-midazolam scores. In the M2S and M5S groups, memory did not improve for the remaining six cards. In the two groups given midazolam followed by flumazenil, memory of word cards shown after administration of flumazenil was markedly improved, so that scores in these groups were now comparable to those in the S groups and to the pre-midazolam scores, but significantly different from both the pre-flumazenil scores and the post-saline scores in the MS groups.

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**Table I. Mean (SEM) critical flicker fusion frequency scores in the six treatment groups at the times shown.** SS = Saline—saline; SF = saline—flumazenil; M5S = midazolam 5 mg—saline; M5F = midazolam 5 mg—flumazenil; M2S = midazolam 2 mg—saline; M2F = midazolam 2 mg—flumazenil. Drug 1 = midazolam 2 mg or 5 mg or saline; Drug 2 = flumazenil 0.01 mg kg\(^{-1}\) or saline.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>SS</th>
<th>SF</th>
<th>M5S</th>
<th>M5F</th>
<th>M2S</th>
<th>M2F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>35.63 (1.086)</td>
<td>33.71 (1.191)</td>
<td>33.78 (0.795)</td>
<td>34.60 (0.673)</td>
<td>34.24 (0.846)</td>
<td>34.92 (0.879)</td>
</tr>
<tr>
<td>Drug 1</td>
<td>34.71 (1.024)</td>
<td>33.94 (0.956)</td>
<td>25.32 (1.736)</td>
<td>25.38 (1.963)</td>
<td>29.63 (0.937)</td>
<td>30.51 (1.505)</td>
</tr>
<tr>
<td>5</td>
<td>34.68 (1.322)</td>
<td>33.69 (1.117)</td>
<td>27.19 (2.412)</td>
<td>27.03 (1.805)</td>
<td>29.98 (1.684)</td>
<td>31.47 (0.943)</td>
</tr>
<tr>
<td>Drug 2</td>
<td>34.15 (1.109)</td>
<td>32.75 (0.950)</td>
<td>26.59 (2.130)</td>
<td>32.04 (0.686)</td>
<td>30.18 (1.969)</td>
<td>32.96 (1.177)</td>
</tr>
<tr>
<td>10</td>
<td>33.80 (0.672)</td>
<td>32.89 (0.858)</td>
<td>27.05 (2.239)</td>
<td>32.34 (0.977)</td>
<td>30.42 (2.257)</td>
<td>32.42 (1.013)</td>
</tr>
<tr>
<td>120</td>
<td>32.98 (1.277)</td>
<td>32.97 (1.193)</td>
<td>30.89 (0.758)</td>
<td>32.53 (1.107)</td>
<td>32.75 (0.892)</td>
<td>34.60 (1.488)</td>
</tr>
</tbody>
</table>
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FIG. 2. Total amnesia scores of the six subjects for each of the 12 word cards in all six treatment groups. The times of administration of saline (S), midazolam (M) 2 mg or 5 mg or flumazenil (F) 0.01 mg kg^{-1} are indicated by the arrows.

DISCUSSION

This study has shown that the amnesic effects of two doses of midazolam administered i.v. were antagonized by subsequent administration of i.v. flumazenil 0.01 mg kg^{-1}. The time course and relative extent of the anti-amnesic action of flumazenil were similar to those of its anti-sedative action. A further finding was that flumazenil alone had no effect either on the level of central neural arousal or on memory.

Benzodiazepine-induced amnesia has been studied extensively, often in situations relevant to the use of the drugs in clinical anaesthesia, and some of its characteristics are well established [10]. In most studies, the model of memory function used has been that of Atkinson and Shiffrin [11], which assumes that memory consists of three components: a very transient sensory memory, a limited capacity short term memory of some seconds duration and a long term memory store with an effectively unlimited capacity. It has been
found consistently that only the last of these is impaired reliably by benzodiazepines [12]. Further, it is established that material already in the memory store is not affected—that is, there is no retrograde amnesia, the presence of which might have suggested that a process such as retrieval of information or the speed of decay of the memory trace was being affected. These observations have led most authors to conclude that the drugs act on the "consolidation" process, whereby recently acquired material is transferred from short term memory to the long term memory store.

In this study, midazolam produced closely related, dose-dependent amnesia and sedation. Similar observations have led some workers to argue that the amnesic effect is not specific but part of the global sedative effect of benzodiazepines [13, 14], perhaps through prevention of rehearsal of new material. However, in several studies, mainly in the clinical anaesthesia field, benzodiazepines have been compared with opioids [12, 15], butyrophenones [16], phenothiazines [17, 18] and barbiturates [19, 20], and have been found in each case to have a greater amnesic effect at approximately equi-sedative doses. Thus the sedation produced by benzodiazepines is different from that of comparable doses of other groups of drugs in that it is accompanied by anterograde amnesia, a specific effect upon the learning process.

In the present study, the close relationship between benzodiazepine-induced sedation and amnesia is confirmed further in that both were antagonized similarly by flumazenil. In the M2F and M5F groups, both CFFF and amnesia scores had returned to pre-midazolam values by 3 min after administration of flumazenil. This suggests that these two actions are mediated by the same receptor in the CNS, and that some of the GABA-ergic inhibitory pathways which are facilitated by benzodiazepines are involved in the control of the laying down of long term memory. These pathways are located in the cerebral cortex and hippocampus in addition to other regions [21], while the currently available evidence on the localization of memory in the mammalian brain suggests that, while the hippocampus is widely involved in learning, the site of long term storage is elsewhere—perhaps in the cortex [22].

This study provides further evidence that the established theory of the mechanism of benzodiazepine-induced amnesia is correct. As expected, there was no evidence of a retrograde amnesic action of midazolam, the two cards shown before its administration being recognized in almost every case. In addition, flumazenil prevented amnesia only of word cards shown after its own administration—there was no "retrograde reversal" of amnesia. It appears, therefore, that material presented before flumazenil was given was already lost by the time this occurred, 1.5 min after the sixth card was shown. At the time that the memory assessments were carried out, 2 h after the commencement of the study, CFFF scores were not significantly different from initial values, and there were no inter-group differences. Thus it is unlikely that the presence of a residual drug effect contributed to the memory scores obtained at this time. It seems, therefore, that the amnesic action of midazolam occurs through an effect on a process that normally begins within 1–2 min following the presentation of material.

Our findings confirm that flumazenil alone in this dose had no effect on central neural arousal as measured by the CFFF score, and also indicate that it had no effects on memory. However, three of the six subjects in the SF group reported slight anxiety or dysphoria lasting less than 30 s following injection of flumazenil, a finding which has been noted by others [23, 24].

Other workers have also reported antagonism of benzodiazepine-induced amnesia by flumazenil. However, in most cases this was noted only as part of a general assessment of the effects of the antagonist and the method of memory testing was either inadequately controlled [25], unspecified [26] or designed in such a way that amnesia and sedative effects could not be separated [27].

Ghoneim, Dembo and Block [28] compared the effects of flumazenil and placebo on immediate and delayed word list recall and recognition and on subjective ratings of mental and physical sedation in patients who had received diazepam sedation for dental surgery. Their results revealed a difference between the effects of flumazenil on subjectively-rated mental sedation, which was restored to pre-surgery scores, and the effects on physical sedation and memory, which were restored only partly, over a period of up to 2 h after administration of flumazenil. In our own study, no difference was found between sedation and amnesia, perhaps largely because of differences in the methods of assessment of memory and sedation. The method of memory assessment, card recognition, used in the present
study is undoubtedly less taxing than the word list recall and recognition tasks used by Ghoneim and colleagues, and it is possible that a more difficult task might have revealed residual amnesia following flumazenil administration. However, this method has been found in the past to be a reliable method of assessing drug-induced amnesia in the clinical setting [3, 4, 15], especially when the aim is to study rapid changes over a short time as in this study. The critical flicker fusion frequency test has been shown to provide a sensitive assessment of central processing of perceptual information [29], and it proved well suited to the rapid, frequent measurements required in the present study. However, it clearly gives a view of cerebral function from a different angle than the subjective ratings used by Ghoneim’s group.

From the clinical point of view, it would appear from these findings that, when flumazenil is used to antagonize midazolam-induced sedation, the ability to remember events occurring during the period of antagonism is also restored. This is an advantage when the antagonist is used following benzodiazepine sedation in dentistry or endoscopy. However, the clinical situation which is most closely mimicked by the design of this study is the use of flumazenil for temporary reversal of benzodiazepine-induced sedation for neurological assessment, and in this case it is unfortunate that, on this evidence and with these doses of midazolam and flumazenil, patients may probably remember these unpleasant experiences.

REFERENCES

for postoperative recovery; A randomised clinical trial in patients undergoing minor surgical procedures under midazolam anaesthesia. *Anaesthesia* 1986; 41: 1001-1006.

