

ORIGINAL ARTICLE

MIDAZOLAM-INDUCED ANTINOCICEPTION: POSSIBLE INTERACTION WITH MORPHINE RESPONSE IN TAIL-FLICK TEST

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Background – Benzodiazepines have been used with opiates to give better pain relief than opiates alone. However, the interaction between the two groups of drugs is controversial. The aim of this study was to investigate whether midazolam potentiated the antinociceptive effect of morphine.

Methods – Male albino mice were used in this study. Antinociception was measured using the tail-flick test.

Results – Midazolam and morphine caused dose-dependent antinociceptive effects in mice. The combination of midazolam and morphine showed an increase in analgesia. The benzodiazepine receptor antagonist, flumazenil, decreased the response induced by midazolam or midazolam plus morphine but not that of morphine alone. However, the opioid receptor antagonist naloxone, reduced the antinociception induced by morphine, midazolam, or a combination of the two drugs. Methysergide or propranolol increased the analgesic effect of midazolam; ketanserin, phenoxybenzamine and atropine did not.

Conclusion – Midazolam induced antinociception through both benzodiazepine and opioid receptors.

Keywords • flumazenil • mice • midazolam • serotonin receptor antagonists • tail-flick test

Introduction

Receptor sites for benzodiazepines have been shown in the spinal cord, with the highest level within the lamina II of the dorsal horn.¹ Benzodiazepine sites appear to be linked to the gamma-aminobutyric acid (GABA)-A receptor complex,^{2, 3} and benzodiazepines enhance GABA-mediated presynaptic inhibition of primary afferent terminals.⁴ They interact with the antinociceptive effect of morphine and other opioid drugs.^{5 - 9} Systemic administration of benzodiazepines induces sedation and muscle relaxation, which may complicate the interpretation of responses to nociceptive stimulation.¹⁰ Diazepam and other benzo-

diazepines are frequently administered together with opiates to give better pain relief.^{11 - 13} Benzodiazepines have also been reported to be of some value in the treatment of chronic pain.¹⁴ The neurophysiological mechanisms involved in the benzodiazepines effect on nociception have been studied in animal models,^{5, 7, 15} but the interactions between benzodiazepines and morphine in producing analgesia are controversial. For example, diazepam has been found to decrease,⁹ increase,¹⁶ or have no effect on morphine-induced antinociception. The use of benzodiazepine agonists with opioids has gained wide acceptance among anesthesiologists. Midazolam is an ultra-short-acting benzodiazepine and is used clinically in preoperative medication, as an induction agent for general anesthesia, and for intravenous sedation. It has anxiolytic, hypnotic, anticonvulsant, muscle relaxant, and anterograde amnesic properties characteristic of the

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benzodiazepines.^{17, 18} In the present study, the antinociceptive effect of midazolam in the presence or absence of morphine was investigated.

Materials and Methods

Animals

Male NMRI albino mice (25 – 30 g) were used in the study. Animals were housed in plastic cages in an animal room maintained at $23 \pm 1^\circ\text{C}$ on a 12-hour dark cycle (light period, 07:00 – 19:00). Food and water were available at all times except during experiments. Each animal was used once only and was sacrificed immediately after the experiment. Seven animals were used in each experiment. All experiments were performed in accordance with institutional animal use guidelines.

Drugs

The chemicals used were: morphine sulphate (MacFarlan Smith Ltd, England), naloxone hydrochloride (Sigma Chemical Co, England), midazolam (Sigma Poole, UK), flumazenil (Ro 15 – 1788; Roche, Switzerland), phenoxybenzamine (SK&F, USA), methysergide (Sandoz, Switzerland), ketanserin (Sigma Chemical Co, USA), atropine (Merck, Germany) and propranolol (Sigma Poole, UK). Morphine and naloxone were dissolved in saline. The drugs were prepared immediately before use and injected at a volume of 10 mL/kg. All drugs were injected intraperitoneally except morphine, which was administered subcutaneously. Antagonist doses and pretreatment times were usually the same as those previously shown to be pharmacologically active.^{18, 19}

Antinociception recording

Antinociception was assessed using the tail-flick test, with the tail-flick apparatus (type 812, Hugo Sachs Elektronik, Germany). The tail-withdrawal latency (sec) was measured before administration of any drug or vehicle. Normal response latencies were usually between 2.5 and 3.0 sec and a 10 sec cut-off was used to prevent tissue damage. The response was tested 15, 30, 45, and 60 minutes after drug administration. Antinociception was quantified as the percentage of maximum possible effect (%MPE) using the method of Keil and DeLander.²⁰ The following formula was used to calculate %MPE: %MPE =

$$100 \times [(test-control \text{ latency}) / (10 \text{ sec control latency})].$$

Statistical analysis

ANOVA and the Newman-Keuls tests were used to analyze the data. Differences between means with $p < 0.05$ were considered statistically significant. Each point represents the mean \pm SEM of recordings for seven mice.

Results

Effect of morphine or midazolam on tail-flick latency

Subcutaneous (SC) administration of morphine (3, 6, and 9 mg/kg) induced dose-dependent antinociception [$F(3, 24) = 79.2, p < 0.0001$]. The maximum response was obtained with 9 mg/kg of the drug (Figure 1). Intraperitoneal (IP) injection of the benzodiazepine receptor agonist midazolam (0.03, 0.3, 3, and 6 mg/kg) also induced dose-dependent antinociception [$F(4, 30) = 11.9, p < 0.0001$]. The maximum response was obtained with 6 mg/kg of the drug (Figure 2).

Effect of midazolam on morphine-induced antinociception

Figure 3 shows the response to the administration of different doses of morphine (3,

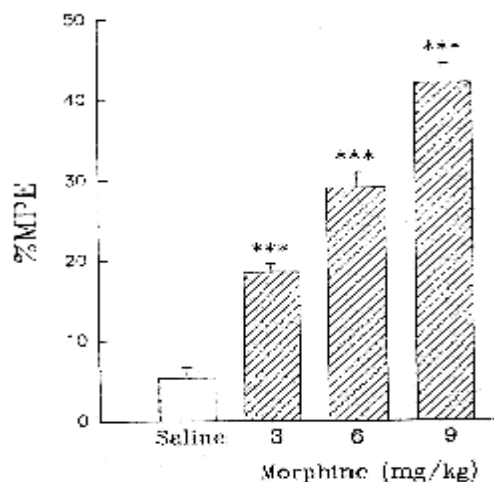


Figure 1. Antinociceptive effect of different doses of morphine in the tail-flick test. Mice were injected subcutaneously (SC) with saline (5 mL/kg) or morphine (3, 6, or 9 mg/kg). Antinociception was recorded for 60 min after morphine injection. Each point is the mean \pm SEM of percentage of maximum possible effect (%MPE) for seven mice. *** $p < 0.001$ vs saline.

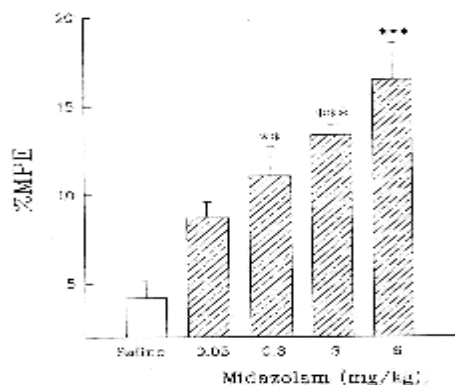


Figure 2. Antinociceptive effect of different doses of midazolam in the tail-flick test. Mice were injected intraperitoneally (IP) with saline (5 mL/kg) or midazolam (0.3, 0.3, 3, or 6 mg/kg). Antinociception was recorded for 60 min after midazolam administration. Each point is the mean \pm SEM of %MPE for seven mice. ** $p < 0.01$, *** $p < 0.001$ vs saline.

6, or 9 mg/kg SC) in the presence or absence of a low dose of midazolam (0.03 mg/kg IP). Two-way ANOVA showed that the combination of morphine with midazolam did not have any effect [F (4, 60) = 0.66, $p > 0.05$].

Figure 4 shows the response to a combination of a higher dose of midazolam (4 mg/kg IP) with different doses of morphine. However, two-way ANOVA showed no interaction between the two drugs [F (4, 60) = 0.998, $p > 0.05$].

Effects of receptor antagonists on antinociception induced by midazolam in the presence or absence of morphine

One-way ANOVA indicates that pretreatment of animals using the benzodiazepine receptor antagonist flumazenil (1 and 2 mg/kg, IP) 15 min before administration of midazolam (6 mg/kg, IP) or 20 min before morphine (4 mg/kg) or prior to the combination of midazolam and morphine reduced the response induced by midazolam [F (2,18) = 10.4, $p < 0.001$] and midazolam plus morphine [F (2, 18) = 7.4, $p < 0.01$] but not morphine alone [F (2, 18) = 0.1, $p > 0.05$]. Flumazenil alone also induced antinociception [F (2, 18) = 5.2, $p < 0.05$] (Figure 5).

Pretreatment of animals with the opioid receptor antagonist naloxone (1 and 2 mg/kg, IP)

15 min before the administration of midazolam (6 mg/kg, IP), 20 min before morphine (4 mg/kg) or prior to midazolam plus morphine reduced the response induced by midazolam [F (2, 18) = 12.25, $p < 0.001$], morphine [F (2, 18) = 16.3, $p < 0.001$] and midazolam plus morphine [F (2, 18) = 18.3, $p < 0.001$]. Naloxone alone did not induce any response [F (2, 18) = 0.26, $p > 0.05$] (Figure 6).

Effect of serotonergic, cholinergic and adrenoceptor antagonists on midazolam-induced response

One-way ANOVA indicates that when the animals were treated with different doses of ketanserin (1 and 2 mg/kg) [F (2, 18) = 2.4, $p > 0.05$], atropine (5 and 10 mg/kg) [F (2, 18) = 0.16, $p > 0.05$] 15 min, or phenoxybenzamine (5 and 10 mg/kg) [F (2, 18) = 0.72, $p > 0.05$] 60 min before midazolam administration (6 mg/kg), the antinociception induced by midazolam was unchanged. While one-way ANOVA indicated that administration of methysergide (1 and 2 mg/kg) [F (2, 18) = 13.6, $p < 0.05$] or propranolol (5 and 10 mg/kg) [F (2, 18) = 3.6, $p < 0.05$] 15 min before midazolam administration increased the midazolam response (Table), two-way ANOVA did not show any interactions between propranolol and midazolam [F (2, 36) = 0.21, $p >$

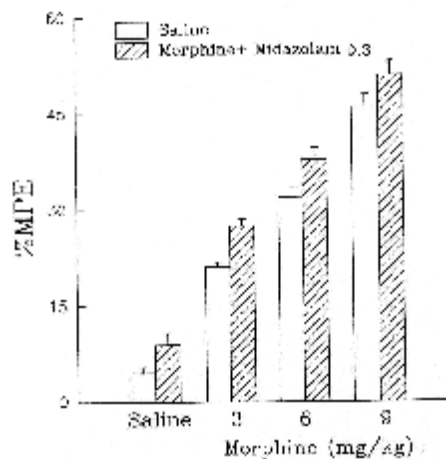


Figure 3. Antinociceptive effect of different doses of morphine in the presence or absence of lower dose of midazolam in the tail-flick test. Mice were injected with saline (5 mL/kg IP) or midazolam (4 mg/kg IP) 5 min before administration of morphine (3, 6, 9, or 6 mg/kg SC). Antinociception was recorded for 60 min after morphine administration. Each point is the mean \pm SEM of %MPE for seven mice.

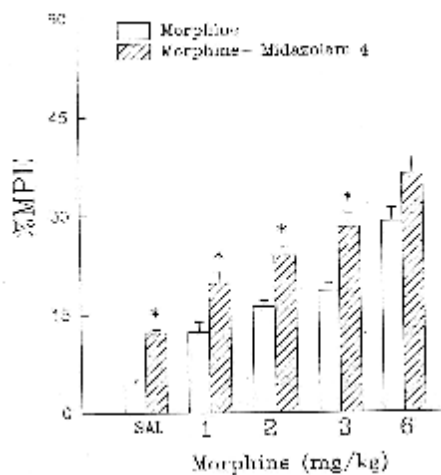


Figure 4. Antinociceptive effect of different doses of morphine in the presence or absence of higher dose of midazolam in the tail-flick test. Mice were injected with saline (5 mL/kg IP) or midazolam (0.03 mg/kg IP) 5 min before administration of morphine (1, 2, 3, or 6 mg/kg SC). Antinociception was recorded for 60 min after morphine administration. Each point is the mean \pm SEM of %MPE for 7 mice. * $p < 0.05$ vs saline.

0.05]. Two-way ANOVA showed an interaction between methysergide and midazolam response [$F(2, 36) = 4.54, p < 0.05$].

Discussion

The present study investigated the antinociceptive response to midazolam in the presence or absence of morphine using the tail-flick test. Different doses of midazolam, a benzodiazepine receptor agonist,¹⁹ induced antinociception dose dependently. Flumazenil, a benzodiazepine receptor antagonist,⁸ reduced the midazolam-induced effect. Therefore, the benzodiazepine receptor mechanism is apparently involved in the response induced by midazolam. These results agree with those obtained by others^{21, 22} indicating that benzodiazepines can elicit antinociception. Contradictory results show that systemic administration of benzodiazepine agonists in animals can decrease^{8, 9} or increase²³ antinociception induced by systemically administered morphine.

It has been proposed that when both drugs are administered at the supraspinal level, midazolam decreases the antinociceptive potency and duration of action of morphine. Thus, different

mechanisms may be involved in antinociception at the levels of spinal cord and in the brain.²⁴ Our results showed that systemic administration of a combination of midazolam and morphine increased the response to low doses of morphine. However, two-way ANOVA did not indicate any potentiation. The antinociception induced by morphine was not reduced by a benzodiazepine receptor antagonist, indicating that the response induced by midazolam, but not morphine, was mediated through a benzodiazepine receptor mechanism. When animals were treated with the opioid receptor antagonist naloxone, the response induced by either midazolam, morphine, or a combination of the two drugs was reduced. This indicates that the effect of midazolam may, at least partly, be mediated through an opioid receptor mechanism. Whether these different effects of midazolam and naloxone are the result of different responses to the drugs at spinal and supraspinal levels needs to be further investigated. The results may be consistent with those of others

Table. Effects of different receptor antagonists on midazolam-induced antinociception in the tail-flick test. Mice were treated intraperitoneally (IP) with saline (5 mL/kg), or atropine (5 and 10 mg/kg) 15 min, methysergide (1 and 2 mg/kg) 15 min, propranolol (5 and 10 mg/kg) 15 min, ketanserin (1 and 2 mg/kg) 15 min or phenoxybenzamine (phenoxy; 5 and 10 mg/kg) 60 min prior to midazolam (6 mg/kg) administration. Antinociception was recorded for 60 min after morphine injection. Each point is the mean \pm SEM of %MEP for 7 mice.

Drug treatment	%MPE of mean \pm SEM	
	Saline	Midazolam
Saline	4.5 \pm 0.7	16.9 \pm 0.9
Atropine 5 mg	5.6 \pm 0.6	16.5 \pm 0.8
Atropine 10 mg	4.7 \pm 0.6	17.2 \pm 0.9
Saline	4.5 \pm 0.7	16.8 \pm 1.5
Methysergide 1 mg	5.3 \pm 0.6	22 \pm 1.2 [†]
Methysergide 2 mg	7.6 \pm 0.5*	26 \pm 1 [‡]
Saline	4.5 \pm 0.7	16.4 \pm 0.8
Propranolol 5 mg	5.7 \pm 0.7	17.8 \pm 0.6
Propranolol 10 mg	6.2 \pm 0.6	19 \pm 0.5
Saline	4.5 \pm 0.7	17.4 \pm 0.4
Ketanserin 1 mg	5.2 \pm 0.4	18.7 \pm 0.9
Ketanserin 2 mg	6.4 \pm 0.5	20.2 \pm 1.2
Saline	4.5 \pm 0.7	17.5 \pm 0.8
Phenoxybenzamine 5 mg	6.7 \pm 0.3	16.8 \pm 0.7
Phenoxybenzamine 10 mg	8.3 \pm 0.7*	16.2 \pm 0.8

MPE = maximum possible effect; * $p < 0.05$ different from respective saline control groups. [†] $p < 0.05$, [‡] $p < 0.01$ different from midazolam control groups.

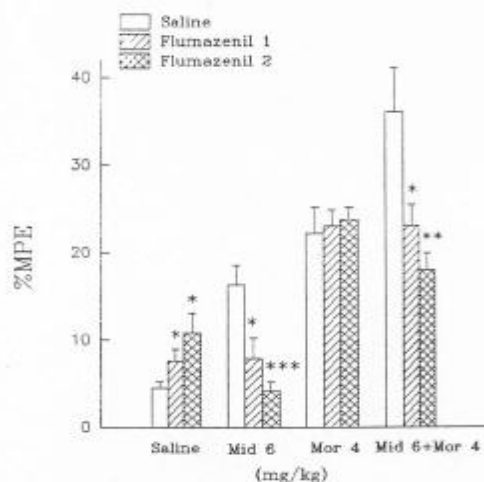


Figure 5. Change in antinociceptive effect of morphine, midazolam, or morphine plus midazolam due to flumazenil injection 15 min before midazolam (6 mg/kg or saline IP) or 20 min prior to morphine (4 mg/kg or saline IP) administration. Antinociception was recorded for 60 min after morphine/midazolam administration. Each point is the mean \pm SEM of %MPE for seven mice. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.01$ vs saline.

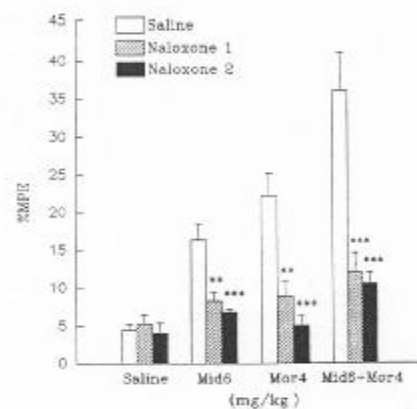


Figure 6. Effect of naloxone on antinociceptive effect of morphine, midazolam, or morphine plus midazolam. Naloxone was injected 15 min before midazolam (6 mg/kg or saline IP) or 20 min prior to morphine (4 mg/kg or saline SC) administration. Antinociception was recorded for 60 min after morphine administration. Each point is the mean \pm SEM of %MPE for seven mice. ** $p < 0.01$, *** $p < 0.001$ vs saline.

demonstrating that the opioid system may be involved in some responses to benzodiazepines, such as the anxiolytic effect.²⁵ Considerable evidence now exists for the involvement of the GABA-A receptor in benzodiazepine effects.²⁶⁻²⁸ For example, there is an association between the GABA and serotonergic systems.²⁹ Whether the analgesic effect of midazolam is partly due to such associations remains to be clarified. Our results also showed that methysergide, a serotonin (5-HT) receptor antagonist, increased the response induced by midazolam. However, the 5-HT₂ receptor antagonist ketanserin³⁰ did not alter the effect of midazolam in the present study, which indicates that at least the 5-HT₂ receptor mechanism is not involved. Other receptor antagonists, atropine and phenoxybenzamine, did not alter the midazolam effect. While the highest dose of propranolol tended to increase midazolam antinociception, they did not affect the midazolam response statistically significant. Thus, the involvement of cholinergic and adrenoceptor mechanisms can be excluded.

In conclusion, there are clinical reports indicating that the benzodiazepines are able to potentiate opioid response in gastrointestinal examination,¹¹ postoperative analgesia,¹² dental

procedures,¹³ acute muscle spasm and lancinating neuropathic pain.¹⁴ However, study did not show any such a response to these combinations.

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