



### Araştırma/Research

## Is the Modulating Effect of Midazolam on Morphine Induced Antinociception to Time Dependent?

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### Abstract

**Aim:** The goal of this study was to investigate the effects of morphine, midazolam and midazolam-morphine on nociception. Benzodiazepines are known to influence opioid analgesia, and variations can be expected in the interaction between benzodiazepines and opioids at different times of the day.

**Method:** The effects of midazolam, morphine, and midazolam-morphine combination on nociception were evaluated with tail flick test in mice, with together the time-dependent changes. Midazolam has not any antinociceptive effect and it inhibited in a fashion dose-dependent the antinociceptive effect of morphine.

**Results:** Control group injected with saline displayed a significant diurnal rhythmicity in the tail flick test significantly. Saline control group's response latency at light hour (at 16.00 h,  $P < 0.05$ ) was shorter than that at late dark hour (at 04.00 h) and early morning hour (at 08.00 h). Morphine injected animals had shorter response latencies in light hours (at 12.00 and 16.00 h,  $P < 0.01$ ) than at late dark hour (at 04.00 h) and early morning hour (at 08.00 h). When midazolam was used in combination with morphine, the tail flick response latencies became significant shorter clearly at every experiment times ( $P < 0.001$ ), however this inhibition was slightly lower at 04.00 h than the other hours.

**Conclusion:** Our results indicate that midazolam antagonism on morphine antinociception have not diurnal rhythmicity, however it may only slight decreased at late dark hours of day.

**Keywords:** Morphine, midazolam, antagonism, antinociception, circadian rhythm, tail flick, mice.

### Yazışmadan Sorumlu Yazar

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## Midazolam'ın Morfin Antinosesepiyonundaki Düzenleyici Etkisi Zamana Bağlı mıdır?

### Öz

**Amaç:** Bu çalışmanın amacı, midazolam, morfin ve midazolam-morfin kombinasyonunun nosisepsiyon üzerindeki etkilerini araştırmaktır. Benzodiazepinlerin opioid analjezisini etkilediği bilinmektedir ve günün farklı zamanlarında benzodiazepinler ve opioidler arasındaki etkileşimlerde değişiklikler beklenebilir.

**Yöntem:** Midazolam, morfin ve midazolam-morfin kombinasyonunun nosisepsiyon üzerindeki etkileri farelerde kuyruk flick testi ile birlikte zamana bağlı değişiklikler olarak değerlendirildi.

**Bulgular:** Salin enjekte edilen kontrol grubu, kuyruk flick testinde anlamlı bir günlük ritmikliği gösterdi. Kontrol grubunun aydınlık saatlerindeki (16.00 saatte,  $P < 0.05$ ) gecikme süresi, karanlık saatlerde (04.00 saatte) ve sabah saatlerindeki (08.00'de) süreden daha kısa idi. Morfin, aydınlık saatlerdekine göre (12.00 ve 16.00 saat,  $P < 0.01$ ) karanlığın geç saatleri (04.00 saatte) ve sabah saatlerinde (saat 08.00'de) daha kısa tepki gecikme sürelerine sahipti. Midazolam morfin ile birlikte kullanıldığında, kuyruk hareket cevabı gecikme süreleri, her deney zamanında ( $P < 0.001$ ) açıkça belirgin şekilde kısaltıldı, ancak bu inhibisyon, diğer saatlere göre 04.00 saatte biraz daha düşüktü.

**Sonuç:** Midazolam'ın antinosesepitif bir etkisi yoktur ve morfinin antinosesepitif etkisini doza bağlı bir şekilde inhibe eder. Bulgular morfin antinosesepiyonundaki midazolam antagonizmasının günlük ritmik olmadığına işaret etse de, günün karanlık saatlerinde sadece hafif bir şekilde azalabilir.

**Anahtar Kelimeler:** Morfin, midazolam, antagonizm, antinosesepiyon, sirkadiyen ritim, kuyruk çekme testi, fare.

### Introduction

Benzodiazepine and opioid combinations are widely used in anesthesia practice for premedication, sedation, anesthesia and treatment of acute and chronic pain. The effect of opioids can be modulated by benzodiazepines including midazolam, but the reports of interaction between benzodiazepines and opioids in animals are as contradictory as the human studies. Benzodiazepine agonists can both decrease (1) or increase (2) or have no effect (3) on opioid antinociception. We have also observed that midazolam, a short acting benzodiazepine, had antagonistic effect on the analgesia of morphine in postoperative patients of our anesthesia clinic (unpublished observation). On the other hand, it is well known that there are the circadian variations in the basal pain sensitivity and, response of mice to opioid analgesic can change (4, 5). As the benzodiazepines are known to influence opioid analgesia, we can expect variations in the interaction between benzodiazepines and opioids at different times of the day. Thus, the present study in which pain thresholds male mice was designed to answer the following questions: 1) does midazolam have an antinociceptive or a hyperalgesic action when it is administered systemically alone?; 2) can midazolam decrease the antinociceptive effect of morphine?; 3) If midazolam has an antinociceptive or a hyperalgesic action and decrease the

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antinociceptive effect of morphine, are these effects going to change during the period of 24 hour? Pain thresholds, therefore, were evaluated with the tail-flick test in male mice.

## **Materials and Methods**

### *Animals*

Adult male Swiss-albino mice weighing 30-40 g, obtained from The Experimental Research Unite, Bülent Ecevit University, were used in this work. They were given food and water *ad libitum*. Mice were housed in groups of 15 animal per cage (cage diameter is 35 × 30 × 16 cm) for at least two weeks before the experiments. The lightening regimen was 12 h of light and 12 h of darkness (lights on 07.00-19.00) at a room temperature of 22 ± 2 °C with constant humidity (60 ± 5%). All experiments were performed during February-March period to avoid the seasonal variations. The study protocol was prepared in accordance with the proposals of the Committee for Research and Ethical Issues of IASP and approved by The Ethic Committee of Experimental Animal Research of Bülent Ecevit University.

### *Drugs and administration routes*

Two sets of experiments were performed. In the first set, responses of morphine and/or midazolam on nociception were evaluated by the dose-response relationship (n=15 animal for each group). The following dose-response relationships obtained at for the experiments which were carried on at 12:00 h were studied: 1) morphine (1, 2.5, 5, and 10 mg/kg) 2) morphine (1, 2.5, 5, and 10 mg/kg) plus midazolam (1.5 mg/kg) 3) morphine (2.5 mg/kg) plus midazolam (0.5, 1, 2, and 5 mg/kg). Therefore, for the basis of dose-response experiments in time-dependent studies, they were chosen doses of these drugs mentioned below.

In the second set, for time-dependent studies, mice were divided randomly into four groups containing 15 animals each as following: 1) saline control group (0.2 ml/animal saline) 2) morphine group (2.5 mg/kg morphine) 3) midazolam group (1.5 mg/kg midazolam), and 4) morphine-midazolam group (2.5 mg/kg morphine + 1.5 mg/kg midazolam). Each experimental group was tested by tail flick at particular times of day which were 08:00, 12:00, 16:00, 20:00, 00:00, and 04:00 h. In order to avoid the learning and tolerance development, each particular time experiment was carried out on different week (eg. 08:00 experiment on the 1<sup>st</sup> week, 12:00 experiment on the 2<sup>nd</sup> week, etc). Morphine hydrochloride (Sandoz, Basel, Switzerland) or midazolam (Roche, Switzerland) were diluted in saline and injected intraperitoneally (ip) in a final volume of 0.2 ml/animal. Tail flick test was performed 30 minutes after the injections,

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because the time-course of effect of midazolam on morphine analgesia was determined at previous study (7).

#### *Nociceptive Test*

In the tail flick (May TF 9508, Commat LTD, Ankara), the tail of each animal was placed on a rectangular metal plate under an illuminated lamp (190W from 10 cm), with the light beam focused approx. 3 cm from the tip of the tail. Beam intensity was adjusted to give a tail flick latency of 3.0-3.5 s in control animals. Two trials were carried out for each test and the time interval between trials was 10 s. Latency response was presented as average of these two trials. The end point was represented by the numbers of the seconds until the mouse flicks its tail out of the beam. To avoid tissue damage, the test was a cut off of terminated at 10 s if the animal did not respond. The antinociceptive response was expressed as tail flick latency.

#### *Statistics*

Data are express as mean  $\pm$  S.E.M. The results were evaluated statistically by the analysis of variance (ANOVA) and applied Bonferroni corrections to *P* values. The paired *t*-test was used when the analysis was restricted to two means. *P* < 0.05 was considered as significant.

## **Results**

#### *Dose-response relationship of midazolam and morphine on nociception at 12 h*

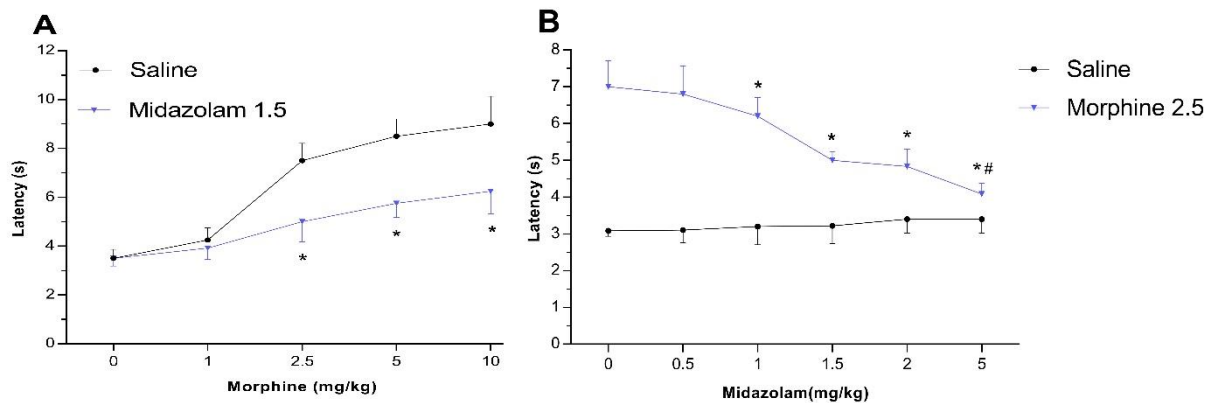
The antinociceptive effect of morphine is seen as a dose-dependent curve on Figure 1A. Tail flick latency was found to be 4.42, 7.18, 8.21 and 8.60 s at 1, 2.5, 5 and 10 mg / kg doses, respectively. Midazolam, in dose-dependent way, attenuated significantly the antinociceptive effect of morphine (at the dose of 2.5 mg/kg), (Figure 1B). When we administered midazolam at doses of 0.5, 1, 1.5, 2, and 5 mg/kg in conjunction with 2.5 mg/kg morphine, tail flick latencies were found as 6.83, 6.26, 5.14, 4.82, and 4.27 s, respectively. Midazolam doesn't have any significant antinociceptive or hyperalgesic effect at any doses. As illustrated Figure 1A, the dose of 1.5 mg / kg of midazolam significantly attenuated the antinociceptive effect of 1, 2.5, 5, and 10 mg / kg of morphine.

#### *Time-depended effects of midazolam on morphine antinociception*

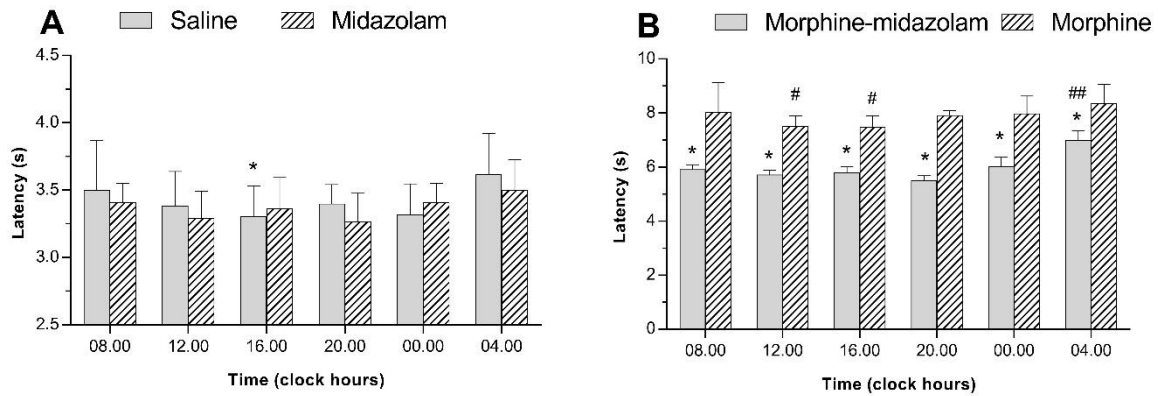
Figure 2 A shows the response latency changes of saline and midazolam injected animals. The saline injected control group displayed a diurnal rhythmicity of tail flick test. Response latencies of the control group were significantly shorter at 16.00 h than at those of

04.00 h and 08.00 h (Figure 2 A,  $P < 0.05$ ). Midazolam alone, had no significant effect on the response latencies when compared with the control group. In addition to this finding, no diurnal rhythmicity has been detected for midazolam. The response latencies of morphine group were significantly longer than those of midazolam and control groups at every experiment time ( $P < 0.001$ ). Morphine injected animals had shorter response latencies in light hours (at 12.00 and 16.00, Figure 2 B,  $P < 0.01$ ) than those at late dark and early morning hours (at 04.00 and 08.00 hrs).

Figure 2 B illustrates the significant differences between the response latencies of morphine and morphine–midazolam combination injected animals. When midazolam was used in combination with morphine, the tail flick response latencies became shorter at every experiment time when compared with morphine group (Figure 2 B,  $P < 0.001$ ). On the other hand, inhibition percent values of midazolam on the morphine antinociceptive effect were 53, 48, 46, 55, 49, and 30 at 08.00, 12.00, 16.00, 20.00, 00.00, and 04.00 hrs of day, respectively.



**Figure 1: Effects of morphine and midazolam, alone or combination, on nociception in tail flick test of mice.** Data are given as mean  $\pm$  S.D. ( $n=15$  in each group). (A) Dose-response to morphine in mice given either saline or midazolam 1.5 mg/kg. \* $P < 0.05$ : different from saline group. (B) Dose-response to midazolam in mice given either morphine 2.5 mg/kg or saline. \* $P < 0.05$ : different from zero midazolam group, # $P > 0.05$ : no different from saline group.



**Figure 2:** Circadian variations in tail flick response latency in mice. (A) Groups treated with midazolam and saline. \*Different from the values of 08.00, and 04.00. (B) Groups treated with morphine, and morphine + midazolam (Morphine-midazolam). \*Different from isochronal morphine injected group, #different from the values of 08.00, and 04.00, ## different from the values of the other morphine-midazolam groups. The light period from 07.00 to 19.00. Results are given as mean  $\pm$  S.D. ( $P < 0.05$ ,  $n=15$ ).

## Discussion

The data of this study denoted that the antinociception caused by morphine has circadian rhythmicity and midazolam: 1) does not have antinociceptive or hyperalgesic effects, 2) decreases the antinociceptive effect of morphine dose-dependently, and in addition, changes by midazolam antagonism on morphine antinociception not showed time-dependent manner, but it may only slight decreased at late dark hours of day. Interaction between opioids and benzodiazepines has been discussed in previous studies, including various nociception tests. Rosland et al. (1) reported that 1 mg / kg of diazepam in both tail flick and hot plate tests was sufficient to decrease the antinociceptive effect of morphine. In another study, midazolam, short-acting benzodiazepine, and diazepam, long-acting benzodiazepine, decreased the antinociceptive effect of morphine in the hot plate test, while only midazolam produced such an action in the tail flick test (6). In the light of these observations, we decided to carry out our experiments by the tail flick test. According to our tail flick experiments there is an antagonism between midazolam and morphine when they are administered systemically (Figure 1). This finding is consistent with previous studies (6,7). The biological and physiological functions of the mammalian organism may vary depending on time.

Mammalian organisms evidently exhibit time-dependent variations in many of their biological and physiological functions. When the organism is stimulated to the organism at different times with the same stimulant, the response of the organism to this stimulus varies (8).

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Pain perception is one of the physiologic functions and pathophysiologic processes that exhibit time-dependent variations. Several studies have documented the diurnal variations in basal pain sensitivity and analgesic effect of morphine (9,10). The present study is consistent with these studies by demonstrating longer response latencies in morphine and control groups in dark periods. The morphine-induced diurnal variations and the basal in the nociceptive thresholds can be explained by the rhythms of availability and/or activity of endogenous opioid peptides (11).

In this study we saw that midazolam, when injected alone, did not have any antinociceptive or hyperalgesic effects, but we antagonized morphine analgesia (see Figure 1). Opioids, which produce their antinociceptive effects, partially inhibits GABA-ergic inhibitory neurons at the midbrain level; so GABA-ergic receptors play an important role in pain control (12). The central effects of benzodiazepines appear to modulate opioid analgesia via GABAergic mechanism (13,14). Rattan et al. (15) suggest that midazolam binds to opioid receptors and displacement of the morphine may be a key component of antagonism mechanism. Midazolam has a supraspinal inhibitory effect and a spinal potentiating effect on morphine analgesia depending on the route of injection (16). The activation of the Dynorphin A-mediated antianalgesic system by midazolam, after formation of a benzodiazepine/opioid receptor complex in the brain, is also another mechanism (17).

The response latency of the morphine-midazolam group at late dark period was found to be longer (Figure 2 B) and the percent inhibition of midazolam on morphine antinociception was lower than those at the other periods. Rattan et al. (15) suggested that  $\beta$ -endorphinergic system are stimulates by morphine and the stimulatory effect of morphine on the  $\beta$ -endorphinergic system are abolished by a concomitant treatment with midazolam. One of the reasons for the decrease of the antagonistic effect of midazolam in dark period may be this stimulatory effect of morphine in morphine-midazolam group. The peak effects of morphine appeared in late dark period. This may be another explanation for the time-dependent change in morphine-midazolam group. Labrecque and Vanier (10) has suggested a relationship between the reaction to pain and the rhythmicity of plasma endorphin concentrations in animal experiments. Rattan et al. (15,18) have observed that chronic treatment of midazolam, which had some affinity to the kappa receptor, resulted in a decrease  $\beta$ -endorphine levels in plasma, hippocampus, striatum, and adrenals. In the light of their suggestions, midazolam probably decreases the  $\beta$ -endorphine levels and shortens the response latencies in mice, however the

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decrease of midazolam antagonism may be dependent on high levels of  $\beta$ -endorphine at the late dark period. In addition, we speculate that high levels of melatonin secreted by pineal gland in dark period may support the morphine antinociception and thus midazolam antagonisation may be decreased with synergistic effect of melatonin on morphine analgesia (19). Treatment with midazolam, like other short-acting benzodiazepines, has been found to induce changes in both behavioral and endocrine circadian rhythms (20). Even the sensitivity of the central nervous system to midazolam may show a circadian variation on humans by way of its pharmacokinetic and pharmacodynamic effects (21). The diurnal variations in protein binding, metabolic activity, systemic and intrinsic clearances of benzodiazepines could be the other mechanisms to explain the occurrence of this antagonism in rodents (22).

In the literatures of clinical investigations, there are conflict results about the interaction between midazolam and opioid to decrease the algesia of patients. In epidural terminal cancer pain therapy, midazolam does not improve morphine analgesia (23). Cao et al. speculate that midazolam inhibits morphine-induced analgesia tolerance associated with spinal nitric oxide involved mechanism (24). The analgesic performances of morphine and the combination of morphine with midazolam assessed by visual analogue scale (VAS) were observed as similar in children with a long-bone fracture (25).

Morphine-midazolam or morphine alone in patients with upper and lower extremity fractures at 15, 30, 45, 60, 120 and 180 minutes were evaluated. The results of this assessment showed that there is no significant difference between the two groups. So findings of that research show that morphine does not change the pain relief effect of morphine in combination with midazolam (26). Additionally, the experimental studies reveal conflict data about the combinative effect of midazolam and opioid (27-29).

Taken together, there is no experimental study about time-dependent modulation of midazolam on morphine analgesia, so further studies are needed to compare our results. We conclude that the antagonistic modulator effect of midazolam on morphine analgesia may partly decrease at late dark period. These findings may be useful in estimating the efficacy of the opioids and benzodiazepines prescribed for pain treatment or sedation and analgesia in ICU, however, we need to take into account that circadian rhythms of rodents and humans are different.



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