COMPARISON OF THE SEDATIVE AND ANTINOCICEPTIVE EFFECTS OF MIDAZOLAM AND DIAZEPAM IN HORSES

(Comparação dos efeitos sedativos e/ou antinociceptivos do midazolam e diazepam em equínos)

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SUMMARY

The aim of this study was to evaluate the antinociceptive and sedative effects of midazolam and diazepam in 10 Thoroughbred mares. The sedative effects of midazolam (0.05, 0.1 and 0.15 mg/kg iv) and diazepam (0.05, 0.15 and 0.25mg/kg iv) were investigated by determination of the spontaneous locomotor activity (SLA) in automated behavior stalls and by measuring the head ptosis (HP). The antinociceptive effect was determined using a heat-projecting lamp by measuring the hoof withdrawal reflex latency (HWRL) and the skin twich reflex latency (STRL). The differences were evaluated by Tukey’s test (p < 0.05). A significant increase in SLA was observed only for diazepam at a dose of 0.05 mg/kg, in comparison to control (saline). Midazolam decreased the head height (p<0.05), leading to sedation between 5 and 75 min after drug administration. Diazepam showed a maximum antinociceptive effect (>10 sec) in all animals tested at a dose of 0.15 mg/kg, within 5 min. Midazolam produced a good sedative effect and diazepam a short acting antinociceptive effect.

KEY-WORDS: Midazolam, Diazepam, Behavior, Sedation, Antinociception

RESUMO

Pretendeu-se avaliar o efeito antinociceptivo do diazepam ou midazolam, em doses sedativas em 10 éguas PSI. Os efeitos sedativos do midazolam (0,05; 0,1; e 0,15 mg/kg IV) e diazepam (0,05; 0,15 e 0,25mg/kg IV) foram obtidos por meio da avaliação da Atividade Locomotora Espontânea (SLA) em baias comportamentais automatizadas e da Ptose da Cabeça (HP). Avaliou-se o efeito antinociceptivo utilizando-se como estímulo algésico uma lâmpada de projeção de calor e mensurando-se a latência para o Reflexo da Retirada do Membro (HWRL) e a Latência para o Reflexo do Frêmito Cutâneo (STRL). As médias foram avaliadas pelo teste de Tukey (p < 0,05). Observou-se aumento significante da SLA apenas para o diazepam na dose de 0,05 mg/kg, em comparação ao controle (salina). Com relação a HP, o midazolam provocou sedação entre 5 e 75 minutos após a administração do fármaco. Na avaliação da antinocicepção, o diazepam apresentou efeito máximo (>10 segundos) em todos os animais testados na dose de 0,15 mg/kg, no tempo 5 minutos. Nos demais tempos e na avaliação do midazolam, os resultados não mostraram diferença significante. Conclui-se que o midazolam demonstrou excelente efeito sedativo, nas doses testadas e o diazepam demonstrou efeito antinociceptivo bastante pronunciado, porém, fugaz.


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INTRODUCTION

The relief of pain, from injury and/or disease, is one of the main attributes of the veterinarian.

Private practitioners and anesthetists may consider that profound sedation provokes a certain degree of analgesia. This concept, although contested by various textbooks (MUIR & HUBBEL, 1995; THURMON et al., 1996), is plausible as depending on the method used to assess pain, confusion can occur in the differentiation between sedative and analgesic effects, (PETERSEN-FELIX et al. 1996; QUEIROZ-NETO et al. 1998). This observation is important because indicates that when an animal does not react to a pain stimulus, that does not always mean that the animal does not feel pain. It is possible that the method being utilized to assess analgesia is inadequate, with the response to the pain stimulus being masked by the sedative effect.

The complexity of the mechanisms involved in the perception of pain are reflected in the results of studies by various investigators, in relation to the effect of sub-hypnotic doses of general anesthetics on nociception. The hyperalgesic effect of low doses of barbiturates have been described in humans (DUUNDEE, 1960), and laboratory animals (NEAL, 1965) and for barbiturates and propofol in laboratory animals (EWEN et al., 1995). Conversely, other studies can be found at the same time evidencing the analgesic effects of these drugs at sedative doses (ANKERMOLLER et al., 1991; JEWETT et al., 1992).

PETERSEN-FELIX et al. (1996) reported that sedation could influence the nociceptive responses in some experimental animals used to test the analgesic efficacy of drugs. In this latter study the investigators tested in humans the effect of propofol and alfentanil in various pain models and observed that propofol produced the same increase in the threshold of nociceptive reflex caused only by electrical stimulation, and that alfentanil, nonetheless, showed a hyperalgesic effect when the nociceptive stimulus was mechanical stress. According to the authors, these results indicated that the increase in the threshold of nociceptive reflex could be caused by sedation and not by analgesia. As the antinociceptive reflex has an afferent sensitivity, and an efferent motor component, the difference between propofol and fentanyl could be explained by a greater depression of the motor component by propofol. According to MAIN et al. (1995), the methods utilized to determine the antinociceptive effect of drugs depend upon an animal’s reflexive or organized behavior response and yet these responses will almost certainly be influenced by drugs that produce aberrant motor function.

The study of the sensitive aspects of pain is difficult due its subjective and multidimensional nature, which challenge methods of quantitative measure of pain, or of antinociceptive effect. When the subject of a study cannot cooperate, as in the case of animals or children, the measure of latency of reflex to a nociceptive stimulus seems to be an adequate method.

Based on the pioneering work of HARDY et al. (1940), who developed a method to measure pain threshold using heat as pain stimulus provoked by a concentrated beam of light, a number of investigators have utilized the latency time for limb withdrawal, in response to pain stimulus, for the study of analgesic substances in various animal species. To minimize the influence of motor function on the latency to evade the pain stimulus, an alternative method was developed (QUEIROZ-NETO, 1997), adapted from KAMERLING et al. (1985a), which consists of directing a light beam at the region of the animal’s withers and measuring the latency time for shivering.

Benzodiazepines (BZP) are well known in veterinary practice, and diazepam is one of those most utilized. Benzodiazepines act by potentiating the inhibitory effects of gamma aminobutyric acid (GABA) in the brain and spinal cord (SPINOSA et al., 1999). Benzodiazepines were initially introduced for the treatment of anxiety in humans. Later this group of drugs was used as sedative, tranquilizing, anticonvulsant and muscle relaxant (GILMAN et al., 1990). Diazepam and midazolam produce muscular relaxation and are anticonvulsant, anxiolytic and hypnotic, by causing a depression in reticular formation and blocking postsynaptic spinal reflexes through potentiation of GABAergic transmission (MUIR & HUBBEL, 1995; COLDWELL et al., 1998; LAU et al., 1998; ANDERSEN et al., 2000, WIKINSKI et al., 2001).

Benzodiazepines belong to a class of substances with central action that have sedative, anxiolytic and muscle-relaxing effects (LAU et al., 1998; COLDWELL et al., 1998; ANDERSEN et al.; 2000, WIKINSKI et al., 2001). These effects are related to its action on the stimulation of GABAergic transmission, the principal inhibitory transmission of the brain in mammals (CARDINALI & GOLOMBEK, 1998). Benzodiazepines have an allosteric effect on GABAergic receptors, facilitating this transmission. Theses drugs exert their effect by binding to specific sites in the macromolecular complex of the GABA receptor system, potentiating chloride uptake into the axon with consequent hyperpolarization and inhibitory action (WIKINSKI et al., 2001).

In this study, the objective was to compare in Thoroughbred horses, the sedative effect of midazolam and diazepam, by means of assessing spontaneous locomotor activity (SLA) in a behavior stall and by head postis (HP), and determining the effect of these substances on nociception. The latency time for the limb flexor reflex (HWRL) was determined after the application of the pain...
stimulus to the region of the proximal phalange of the thoracic limb. The latency time for the shivering reflex (STRL) was determined after the infliction of the pain stimulus in the region of the withers.

**MATERIAL AND METHODS**

Ten Thoroughbred mares aging from 8 to 13 years old and weighing between 450 and 550 kg, were used. These animals belonged to the experimental herd of the Faculty of Agricultural and Veterinarian Sciences - UNESP.

Sedative and/or antinociceptive effects were assessed by utilizing midazolam¹ (0.05, 0.10 and 0.15 mg/kg iv), diazepam² (0.05, 0.15 and 0.25 mg/kg iv) or saline.

Sedation was assessed by measuring locomotor activity of the animals, utilizing the method described by Kamerling *et al.* (1988), at the following times: -45, -30, -15, 0 (time of injection), 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 270 and 300 min after injection of the drugs.

The determination of the antinociceptive effect, was carried out using a heat projection lamp designed according to KAMERLING *et al.*, 1985a; KAMERLING *et al.*, 1985b and constructed by the Department of Electrical Engineering, University of Kentucky, USA.

The rapid exposure to the hot spotlight was utilized as a pain stimulus, which was directed in independent experiments to the following sites:

- region of the lateral side of the proximal phalange of the thoracic limb to unleash the classic limb flexor reflex, which was quantified by the latency time for the Hoof Withdrawal Reflex Latency (HWRL), defined as the time elapsing between the application of the heat lamp and withdrawal of the limb.

- region of the horse’s withers to measure the Skin Twitch Reflex Latency (STRL), considering the time elapsed between the start of the stimulus and the quivering of the skin.

In both cases, the pain stimulus was interrupted when the time of exposure reached a maximum of 10 sec (cut-off time), to prevent injury to the tissues.

Both HWRL and STRL were measured at 30 min immediately before injection of saline (control) or BZD, and also at 5, 15, 30, 45, 60, 90, 120, 150 and 180 min after saline or BZD.

Analysis of variance was performed in a randomized design, to investigate differences in time and between groups. A level of significance (Tukey’s test) of 5 % was used.

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¹ Dormonid® - Roche, Rio de Janeiro.
² Diazepam® - Diazepam, União Química Farmacêutica S/A, São Paulo.
Table 1 - Mean values of head ptosis of thoroughbred mares in a behavioral stall after injection of different doses of midazolam (0.05, 0.1 and 0.15 mg/kg iv) or control. Results are mean ± SEM.

<table>
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<th>Tempo</th>
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<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>120</th>
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<th>210</th>
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<tbody>
<tr>
<td>Control</td>
<td>2.8</td>
<td>1.3</td>
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Mean followed by the same capital letter within each column did not differ at the 5% level of probability (Tukey test).

C.V. = Coefficient of Variation (percent).
F = Result of the F test of analysis of variance.
NS = Not significant.

Table 2 - Mean Spontaneous Locomotor Activity of Thoroughbred mares in a behavioral stall after injection of different doses of diazepam (0.05, 0.1 and 0.15 mg/kg iv) or saline (control). The results are reported as mean number of interruption of infrared light/minute ± SEM.

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<th>Tempo</th>
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<tbody>
<tr>
<td>Diazepam</td>
<td>0.05 mg/kg iv</td>
<td>2.1</td>
<td>2.1</td>
<td>2.0</td>
<td>1.9</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
<td>1.5</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.1</td>
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<td>0.8</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
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<td>n = 10</td>
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F = Result of the F test of analysis of variance.
NS = Not significant.

**DISCUSSION**

MARQUES (1996) studying the effect of 0.2 mg/kg iv of midazolam in fillies, observed that eight minutes after administration, the animals became calm, however alert to the ambient stimulations. After 15 minutes the animals showed HP, moderate degree of ataxia, without recumbence and muscular relaxation. In the present study a 2 times lower dose produced sedation in horses assessed by HP, with the effect occurring in the first 5 min after injection. In regard to locomotion, there was no notable alteration other than motor incoordination at the doses tested. COLDWELL et al. (1998) showed that in humans midazolam, given as a continuous infusion (0.75 µg/kg/min/70 min), decreases the peak of muscular force (compared to the control group) 5 min after drug administration. In the present study, midazolam although not statistically significant, increased SLA and significantly decreased head height. Diazepam increased SLA at 0.05 mg/kg iv, with a mean value approximately 3 times greater than that.
of the control group, and had no effect on HP. Another relevant observation in these animals was muscular fasciculation and the constant change of the bear limb. This observation can be related to the decrease of muscle tone as described by COLDWELL et al. (1998). This suggests that the head ptosis is better for assessing sedation than SLA, as SLA can involve ataxia, whith a constant change of the support limbs, resulting in a greater interruption of the light beams that quantify SLA.

GRIEBEL et al. (1999) in a study with rats observed that diazepam discretely increased mean locomotor activity using doses 10 times greater than here. In the same manner, WIKINSKI et al. (2001), observed that rats treated with 0.1 and 1.0 mg/kg ip diazepam increased locomotor activity in an open-field test when compared to the control group. Diazepam increased SLA at the lowest dose tested (0.05mg/kg iv). The animals were less excited with higher doses. A biphasic effect was also observed in rats (LAU et al., 1998) in mice (LOPEZ et al., 1988).

Various textbooks also point out a paradoxical excitatory effect for benzodiazepines, where ataxia, euphoria and irritability, among other adverse effects, occur (GILMAN et al., 1990, SPINOSA et al., 1999). An inverse dose-dependent effect on SLA was observed for diazepam. A greater increase in SLA was obtained at a lower dose, and as greater doses were administered the effect was progressively smaller. This finding although difficult to interpret can be explained in various ways in relation to the pharmacokinetics of different benzodiazepines, in which stimulatory and sedative effects can be associated with low and high plasma drug concentrations, respectively. Therefore, high concentrations unleash both effects, the excitatory effect being meanwhile surpassed by the sedative one (LAU et al. 1999).

It can also be speculated that the benzodiazepine could be acting on receptors with different affinities, or otherwise on receptors formed by different subunits composition. According to this, the study of KORPI et al. (1999) showed that mice without genetically expressing α2-GABAergic type receptors were shown to be more sensitive to the Rota-Rod test after diazepam treatment, than normal mice.

Several experimental and clinical investigations pointed out the role of GABAergic transmission on antinociceptive processes (DICKENSON et al., 1997) and the analgesic effect of benzodiazepines (YANG et al., 1979; PANG et al., 2001; DEL ROSARIO, 2001). On the other hand, other studies suggest other effects than analgesic (JOHNSTON, 1996; MEHTA & TICKU, 1999). DICKENSON et al. (1997) attribute this diversity of effects to the large variety of experimental protocols, nociception models and routes of drug administration.

The results presented in this study do not show an antinociceptive effect for midazolam. This finding is not in concordance with results obtained by KONTINEN & DICKENSON (2000) who observed antinociception in a model of acute pain caused by peripheral GABAergic stimulation by midazolam. Otherwise, diazepam increased HWRL and STRL, showing an antinociceptive effect. PANG et al. (2001) also reported an antinociceptive effect after the administration of diazepam in mice at a dose of 1.0 mg/kg. The results presented here show that even with doses 10 times lower antinociception was observed.

Some animals showed signs of coprophagia after the administration of midazolam, which was not observed after the administration of saline or diazepam. HIGGS & COOPER (1996), reported that midazolam acts on the parabrachial nucleus, increasing the consumption of food in animals already fed. The action of midazolam on the parabrachial nucleus did not affect locomotor activity. However, according to these authors, at low doses, hyperlocomotor activity and sedation was observed, in contrast to this study. The authors suggested that GABA_A receptors are localized in the parabrachial nucleus (HIGGS & COOPER, 1996).

This investigation draw a picture of the effects of BZD on locomotor behavior, sedation and antinociception in horses. Midazolam demonstrated having a significant sedative effect without action on nociception. Diazepam otherwise, didn’t show any sedative effect, but was distinguished in the antinociceptive action.

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