

Augmentation of Analgesic Effect of Ibuprofen by Alprazolam in Experimental Model of Pain

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Summary

The reports of analgesic effects of benzodiazepines are inconsistent. There is evidence of a hyperalgesic effect induced by activation of supraspinal GABA_A receptors and an antinociceptive effect induced by activation of receptors located in the spinal cord (dorsal horns). The aim of the study was to discover whether the systemic administration of a benzodiazepine agent alprazolam increases the systemic analgesic efficacy of non-opioid analgesic ibuprofen. Experimental studies combining these agents have not yet been published. We used three experimental methods – writhing test (with acetic acid), tail-flick test and plantar test to assess analgesic action. The drugs were administered orally. Augmentation of the analgesic effect of ibuprofen by alprazolam was proved for the writhing test at a dose of 30 mg/kg of ibuprofen and alprazolam 1 mg/kg. The reaction time of the combination was significantly prolonged in comparison with ibuprofen alone. The results of the tail-flick test and plantar test were negative. The effect of ibuprofen was not enhanced by alprazolam in tests of acute thermal pain. Our results have demonstrated that the analgesic action of ibuprofen is only weakly enhanced by alprazolam.

Key words

Alprazolam • Benzodiazepines • Ibuprofen • γ -aminobutyric acid (GABA) • Pain • Writhing • Tail-flick

Introduction

Reports on the effects of benzodiazepines on pain are hitherto controversial. Both antinociceptive effects (Zambotti 1991, Bianchi 1993, Pick 1997, Tatsuo 1999) and hyperalgesic effects (Niv 1988, Zambotti 1991, Luger 1994, Tatsuo 1999) have been reported. Some studies have also shown an absence of any effects (Tejwani 1993, Zambotti 1991) in various pain tests.

Previous studies reported that doses of benzodiazepines (especially of midazolam) although themselves with no effect on nociception can potentiate

and prolong morphine-induced analgesia (Luger 1995). However, no experimental studies have assessed (Yanez 1990) whether benzodiazepines enhance the analgesic action of non-opioid analgetics.

The aim of our study was to discover whether a benzodiazepine agent alprazolam increases the analgesic efficacy of a non-opioid analgesic ibuprofen. Experimental results combining these agents have not yet been published. We found two pilot clinical trials in the literature (39 and 78 patients) with a combination of ibuprofen + diazepam and ibuprofen + alprazolam in the fibrositis/fibromyalgia syndrome and chronic orofacial

muscle pain. The combination therapy was more effective than the single agents alone (Russell 1991, Singer and Dionne 1997).

We tested the hypothesis whether alprazolam enhances the analgesic effect of ibuprofen. If alprazolam acts on spinal GABA_A receptors and ibuprofen is acting at the level of peripheral and central production of prostaglandines, they may negatively modulate transmission of nociceptive stimuli through a different but a complementary mechanism, then their combination could result in a synergic effect.

Methods

Animals

Male NMRI mice weighing 24-30 g (VUFB Konarovice, Czech Republic) were used in the present project. Food was withheld for 16 h before beginning of the experiments, but animals had free access to drinking. The animals were adapted to the laboratory environment for at least 1 h before being used, and the ethical standards guidelines were followed. The duration of the experiments was as short as possible, the number of animals involved was kept to a minimum, and the animals were killed immediately after the recording period by the administration of an anesthetic overdose.

All studies and procedures were approved by the Committee of Ethics for Animal Experiments at the Third Faculty of Medicine, Charles University.

Measurement of analgesic activity:

Writhing

The writhing tests were carried out as described previously by Millan (1994). The mice were assigned into treated groups which received the vehicle or drug. At the times indicated, acetic acid (0.7 %, 0.1 ml/10 g) was injected into the peritoneal cavity (i.p.). The number of writhes (i.e. abdominal constriction followed by dorsiflexion and stretching of hind limbs) occurring during a 20 min period beginning after acetic acid administration was measured. The results are expressed as the number of writhes during the 20-min period. Up to 3 animals were observed simultaneously by one observer.

Plantar test

The plantar test is a model of acute thermal pain. For heat, a light beam was focused on the animal's paw and the latency before paw withdrawal was determined. The standardized plantar test apparatus (Ugo-Basile, Comerio, Italy) was used.

Tail-flick

A radiant heat tail-flick algometer was used for measuring the response latencies according to the method described previously (Santos *et al.* 1999).

All animals were selected 24 h before the test on the basis of their reactivity and those mice that remained in the apparatus for up to 8 s were discarded. A latency period (cut-off) of 20 s was defined as complete analgesia.

Drugs

The following drugs were used: ibuprofen and alprazolam. All drugs were dissolved in sterile water and administered in a volume of 10 ml/kg. Control groups received an equal amount of the appropriate vehicle.

Selection of the alprazolam dose was based on our previous experience (Krsiak and Sulcova 1990). Doses higher than 1 mg/kg can produce sedative effects, a possible source of bias in the analgesic evaluation.

Data Analysis

Results are presented as mean values \pm S.E.M and were examined by one-way ANOVA (followed by Tukey's test) and ANOVA on ranks. Significance was accepted at the 0.05 level. The dose-response curve was obtained by linear regression analysis.

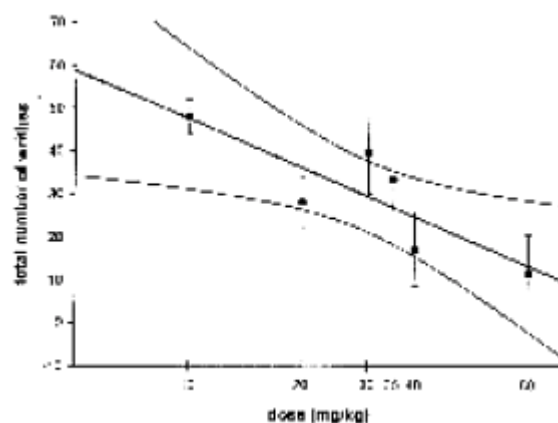
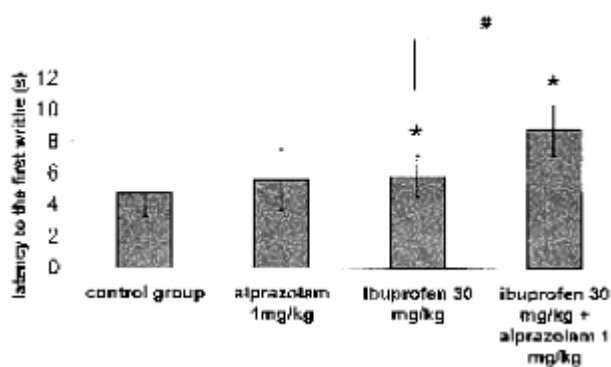


Fig. 1. Dose-response curve in mice for the antinociceptive activity after administration of ibuprofen. Antinociceptive activity was assessed by quantifying (total number of writhes) the writhing response produced by i.p. acetic acid during 20 min. A writhe was defined as the posture of flattened abdomen, depressed back and stretching of hind limbs. Each point represents data from six animals per group \pm S.E.M.

Results

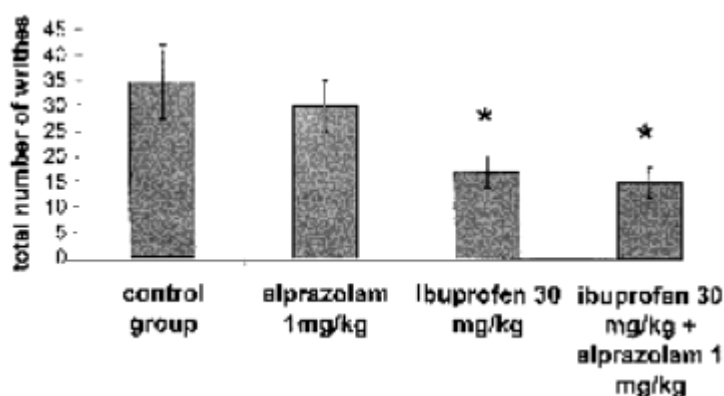
Ibuprofen, dose-response relationship

Figure 1 shows the relationship between ibuprofen oral dose and the antinociceptive effect (number of writhes). According to the dose-response curves we established doses for the combination with alprazolam. The selected doses were 10 and 30 mg/kg respectively.



This effect was not seen in response to the 10 mg/kg dose of ibuprofen and to the combination of ibuprofen 10 mg/kg plus alprazolam 1 mg/kg. The dose 10 mg/kg did not prolong the time to the first writhing response and alprazolam did not enhance the analgesic efficacy of ibuprofen (not shown in the figure).

Furthermore, we followed a second parameter of the writhing test, i.e. the total number of writhes during



We also found that ibuprofen (10 mg/kg) alone or in combination with alprazolam (1 mg/kg) did not suppress the writhing response significantly (not shown in the figure)

Writhing test

As shown in Fig. 2, the latency time to the first writhing response was significantly prolonged in the 30 mg dose of ibuprofen plus 1 mg/kg of alprazolam in comparison with ibuprofen alone. Alprazolam alone had no analgesic effect in the 1 mg/kg dose.

Fig. 2. Comparison of ibuprofen 30 mg/kg and alprazolam 1 mg/kg versus ibuprofen 30 mg/kg + alprazolam 1 mg/kg in the latency to the first writhing response. Antinociceptive activity was assessed by quantifying the writhing response produced by i.p. acetic acid. A writhe was defined as the posture of flattened abdomen, depressed back and stretching of hind limbs. Each column represents data from twelve animals per group \pm S.E.M. *, # Statistically significant at $p < 0.05$.

20 min (Fig. 3) and we did not observe any enhancement of the analgesic effect of ibuprofen by alprazolam. Figure 3 shows that a combination of ibuprofen (30 mg/kg) plus alprazolam (1 mg/kg) was as effective as of 30 mg/kg ibuprofen alone. Neither was there an analgesic effect of alprazolam (1 mg/kg) on this parameter of the writhing test.

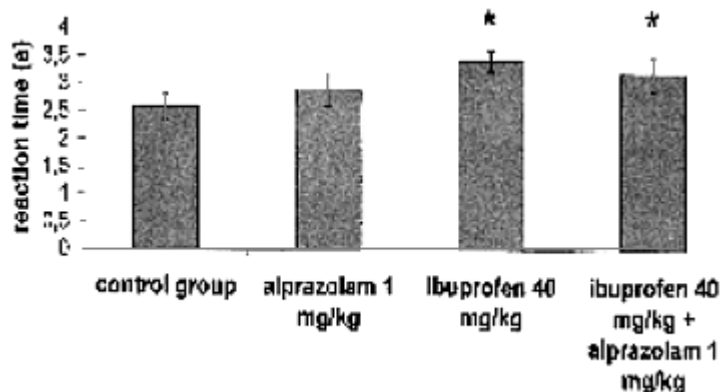
Fig. 3. Comparison of ibuprofen 30 mg/kg and alprazolam 1 mg/kg versus ibuprofen 30 mg/kg + alprazolam 1 mg/kg in the total number of writhes during 20 min. Antinociceptive activity was assessed by quantifying the writhing response produced by i.p. acetic acid. A writhe was defined as the posture of flattened abdomen, depressed back and stretching of hind limbs. Each column represents data from twelve animals per group \pm S.E.M. * Statistically significant at $p < 0.05$.

Plantar test

As far as the plantar test is concerned, we did not observe any enhancement of the analgesic effect of ibuprofen (at a dose of 40 mg/kg) by alprazolam (at a dose of 1 mg/kg) according to a general analysis of all the

paws tested. Neither ibuprofen alone nor in combination with alprazolam produced an appreciable analgesic action in comparison with the control group. Only a separate analysis of the left front paw exhibited a significantly

Fig. 4. Comparison of ibuprofen 40 mg/kg and alprazolam 1 mg/kg versus ibuprofen 40 mg/kg + alprazolam 1 mg/kg in the reaction time during the thermal stimulation of left front paw (plantar test). Reaction time is defined as the withdrawal time away from the light beam. Each column represents data from twelve animals per group \pm S.E.M.* Statistically significant at $p < 0.05$.



prolonged latency in the ibuprofen group, but when combined with alprazolam no further enhancement was observed (Fig. 4).

Tail-flick test

In the tail-flick test, no analgesic effect (expressed as significant prolongation of the tail-flick latency) was noted in the group of animals treated by ibuprofen, not even at a dose of 100 mg/kg. Addition of alprazolam in the dose of 1 mg/kg did not further potentiate the analgesic action of ibuprofen.

Discussion

In the present study, orally administered alprazolam exhibited only weak potentiation of the antinociceptive action of ibuprofen in one parameter of the writhing test.

The purpose of this study was to ascertain a possible synergic effect of ibuprofen and alprazolam. Ibuprofen is a non-steroidal antiinflammatory drug whose analgesic mechanism of action depends on inhibition of cyclooxygenase-producing prostaglandines. Some authors have proposed an additional central mechanism of action of non-steroidal origin *via* inhibition of cyclooxygenase in the spinal cord (Bjorkman 1995). A possible antinociceptive effect of benzodiazepines involve enhancing GABA action on GABA_A receptors in the spinal cord dorsal horns (Luger *et al.* 1994).

Some authors have claimed that benzodiazepines are hyperalgesic drugs (Tatsuo 1997), whereas other authors have found them to act as analgetic drugs (Luger 1994), and yet others have reported that they have no effect on the pain threshold (Tejwani 1993). It has also been reported that benzodiazepines both potentiate and attenuate morphine analgesia (Rattan 1991, Gear 1997). However, a detailed analysis of the available data has

shown that it is necessary to take the doses, route of administration and the pain model into account (Tatsuo 1999).

One important aspect concerns the question whether the antinociceptive effect of orally administered alprazolam is mediated at the spinal or supraspinal level. It was suggested that benzodiazepines exert opposite effects according to the site of administration (Luger 1995, Tatsuo 1999). In the spinal cord, benzodiazepines produce profound inhibition of the afferent transmission of nociceptive information by either a pre- or postsynaptic mechanism. Supraspinally, the activity of bulbospinal pain modulatory pathways appears to be suppressed by benzodiazepines acting at GABA_A receptors.

Another important point pertains to the model of pain used in assessing analgesic actions of benzodiazepines. In our study we applied one model of acute tissue injury (acetic acid writhing) and two models of acute thermal pain (tail-flick and plantar test). It seems that the GABAergic system is more involved in pain states originating from nerve injury (neuropathic pain). Reduced spinal GABAergic tone has been suggested just after nerve injury (Ibuki 1997). Kontinen showed that systemic administration of midazolam reduced firing of C-fibres in the spinal nerve ligation model (Kontinen and Dickenson 2000).

The negative results of the tail-flick and plantar test may be due to the lower sensitivity of spinal reflexes related to the analgesic tests (tail-flick, hot-plate, plantar test) to non-opioid analgetics (Hunnskaar 1986). These models are more appropriate for assessing analgesic activity of strong opioid analgetics. We consider that the

value of these results is not comparable with the writhing test, which has been successfully been employed in assessing the analgetic efficacy of non-opioid analgesics.

The involvement of γ -aminobutyric acid (GABA) in processing nociceptive information at the spinal cord level is well-established (Dickenson 1997). The highest density of GABA_A receptors is present in lamina II of the dorsal horns, a region which plays a pivotal role in the transmission of nociceptive information (Faull and Villiger 1986). The GABA_A receptor is a pentameric ligand-gated chloride ion channel with binding sites for GABA and separate modulatory binding sites for barbiturates, benzodiazepines, propofol and neurosteroids (Sieghart 1995).

There may be several subtypes of GABA_A receptors within the spinal cord since there is a wide

variety of mRNA in this region, which are coded for a variety of subunits of the receptor (Persohn 1992, Nadeson 1996). Thus, the analgesic effect of benzodiazepines in the spinal cord may be influenced by the expression of various GABA subtypes.

In summary, our results show that the amplification of the analgesic effect of oral ibuprofen by oral alprazolam in models of acute pain is only limited and the role of benzodiazepines in acute pain transmission needs further evaluation.

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References

- BIANCHI M, MANTEGAZZA P, TAMMISO R, ZONTA N, ZAMBOTTI F: Peripherally administered benzodiazepines increase morphine-induced analgesia in the rat. Effect of RO 15-3505 and FG 7142. *Arch Int Pharmacodyn Ther* **322**: 5-13, 1993.
- BJORKMAN R: Central antinociceptive effects of non-steroidal anti-inflammatory drugs and paracetamol. Experimental studies in the rat. *Acta Anaesthesiol Scand Suppl* **103**: 1-44, 1995.
- DICKENSON AH, CHAPMAN V, GREEN GM: The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. *Gen Pharmacol* **28**: 633-638, 1997.
- FAULL RL, VILLIGER JW: Benzodiazepine receptors in the human spinal cord: a detailed anatomical and pharmacological study. *Neuroscience* **17**: 791-802, 1986.
- GEAR RW, MIASKOWSKI C, HELLER PH, PAUL SM, GORDON NC, LEVINE JD: Benzodiazepine mediated antagonism of opioid analgesia. *Pain* **71**: 25-29, 1997.
- HUNSKAAR S, BERGE OG, HOLE K: A modified hot-plate test sensitive to mild analgesics. *Behav Brain Res* **21**: 101-108, 1986.
- IBUKI T, HAMA AT, WANG XT, PAPPAS GD, SAGEN J: Loss of GABA-immunoreactivity in the spinal dorsal horn of rats with peripheral nerve injury and promotion of recovery by adrenal medullary grafts. *Neuroscience* **76**: 845-858, 1997.
- KONTINEN VK, DICKENSON AH: Effects of midazolam in the spinal nerve ligation model of neuropathic pain in rats. *Pain* **85**: 425-431, 2000.
- KRSIAK M, SULCOVA A: Differential effects of six structurally related benzodiazepines on some ethological measures of timidity, aggression and locomotion in mice. *Psychopharmacology (Berl)* **101**: 396-402, 1990.
- LUGER TJ, HAYASHI T, LORENZ IH, HILL HF: Mechanisms of the influence of midazolam on morphine antinociception at spinal and supraspinal levels in rats. *Eur J Pharmacol*. **271**: 421-431, 1994.
- LUGER TJ, HAYASHI T, WEISS CG, HILL HF: The spinal potentiating effect and the supraspinal inhibitory effect of midazolam on opioid-induced analgesia in rats. *Eur J Pharmacol*. **275**: 153-162, 1995.
- MILLAN MJ: Serotonin and pain: evidence that activation of 5-HT_{1A} receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. *Pain* **58**: 45-61, 1994.
- NADESON R, GUO Z, PORTER V, GENT JP, GOODCHILD CS: Gamma-aminobutyric acid A receptors and spinally mediated antinociception in rats. *J Pharmacol Exp Ther* **278**: 620-626, 1996.
- NIV D, DAVIDOVICH S, GELLER E, URCA G: Analgesic and hyperalgesic effects of midazolam: dependence on route of administration. *Anesth Analg* **67**: 1169-1173, 1988.
- PERSOHN E, MALHERBE P, RICHARDS JG: Comparative molecular neuroanatomy of cloned GABA_A receptor subunits in the rat CNS. *J Comp Neurol* **326**: 193-216, 1992.

- PICK CG: Antinociceptive interaction between alprazolam and opioids. *Brain Res Bull.* **42**: 239-243, 1997.
- RATTAN AK, McDONALD JS, TEJWANI GA: Differential effects of intrathecal midazolam on morphine-induced antinociception in the rat: role of spinal opioid receptors. *Anesth Analg.* **73**: 124-131, 1991.
- RUSSELL IJ, FLETCHER EM, MICHALEK JE, MCBROOM PC, HESTER GG: Treatment of primary fibrositis/fibromyalgia syndrome with ibuprofen and alprazolam. A double-blind, placebo-controlled study. *Arthritis Rheum.* **34**: 552-560, 1991.
- SANTOS AR, MIGUEL OG, YUNES RA, CALIXTO JB: Antinociceptive properties of the new alkaloid, cis-8, 10-di-N-propyllobelidiol hydrochloride dihydrate isolated from *Siphocampylus verticillatus*: evidence for the mechanism of action. *J Pharmacol Exp Ther* **289**: 417-426, 1999.
- SIEGHART W: Structure and pharmacology of gamma-aminobutyric acid A receptor subtypes. *Pharmacol Rev.* **47**: 181-234, 1995.
- SINGER E, DIONNE R: A controlled evaluation of ibuprofen and diazepam for chronic orofacial muscle pain. *J Orofac Pain* **11**: 139-146, 1997.
- TATSUO MA, SALGADO JV, YOKORO CM, DUARTE ID, FRANCISCHI JN: Midazolam-induced hyperalgesia in rats: modulation via GABA(A) receptors at supraspinal level. *Eur J Pharmacol.* **370**: 9-15, 1999.
- TATSUO MA, YOKORO CM, SALGADO JV, PESQUERO SM, SANTANA MA, FRANCISCHI JN: Hyperalgesic effect induced by barbiturates, midazolam and ethanol: pharmacological evidence for GABA-A receptor involvement. *Braz J Med Biol Res* **30**: 251-256, 1997.
- TEJWANI GA, RATTAN AK, SRIBANDITMONGKOL P, SHEU MJ, ZUNIGA J, McDONALD JS: Inhibition of morphine-induced tolerance and dependence by a benzodiazepine receptor agonist midazolam in the rat. *Anesth Analg.* **76**: 1052-1060, 1993.
- YANEZ A, SABBE MB, STEVENS CW, YAKSH TL: Interaction of midazolam and morphine in the spinal cord of the rat. *Neuropharmacology* **29**: 359-364, 1990.
- ZAMBOTTI F, ZONTA N, TAMMISO R, CONCI F, HAFNER B, ZECCA L, FERRARIO P, MANTEGAZZA P: Effects of diazepam on nociception in rats. *Naunyn Schmiedebergs Arch Pharmacol.* **344** : 84-89, 1991.

Reprint requests

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