Effects of Naloxone and Flumazenil on Antinociceptive Action of Acetaminophen in Rats

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ABSTRACT

BACKGROUND: Studies of acetaminophen suggest that multiple nociceptive pathways are involved in the drug's analgesic action.

OBJECTIVE: The purpose of this study was to determine whether naloxone and flumazenil were able to modify or antagonize the antinociceptive effect of acetaminophen in rats.

METHODS: Adult albino Wistar rats were used in the study and randomly allocated to 1 of 4 groups. The acetaminophen group (A group) was administered IP saline and then 300 mg/kg IP acetaminophen 5 minutes thereafter. The acetaminophen + naloxone group (AN group) was pretreated with 1 mg/kg IP naloxone, followed by 300 mg/kg IP acetaminophen 5 minutes later. The acetaminophen + flumazenil group (AF group) was pretreated with 1 mg/kg IP flumazenil, followed by 300 mg/kg IP acetaminophen 5 minutes later. The control group received 2.5 mL IP saline, followed by an additional 2.5 mL IP injection of saline 5 minutes later. The paw-withdrawal latency period of the rats was assessed by an investigator blinded to treatment using the hot-plate test at 30, 45, 60, and 90 minutes after administration of acetaminophen.

RESULTS: Thirty-two rats were evenly randomized by envelope method into 4 groups of 8 rats each. Baseline values for the A, AN, AF, and control groups were not significantly different (9.1 [2.3], 10.5 [2.7], 9.8 [3.0], and 8.9 [1.4] sec, respectively). In the AF group, flumazenil appeared to antagonize the analgesic effect exerted by the acetaminophen in the hot-plate test (30 min, 10.3 [3.7] sec; 45 min, 11.7 [5.1] sec; 60 min, 12.1 [5.1] sec; and 90 min, 12.2 [4.9] sec) and values were not significantly different from those obtained in the control group (30 min, 9.8 [2.2] sec; 45 min, 9.0 [1.6] sec; 60 min, 9.2 [1.6] sec; and 90 min, 8.5 [2.0] sec). In the AN group, naloxone did not significantly affect the values observed in the hot-plate test (30 min, 18.0 [4.5] sec; 45 min, 21.5 [7.8] sec; 60 min, 20.5 [5.9] sec; and 90 min, 22.3 [7.4] sec) and values at all time points were not significantly different from those obtained in the

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A group (30 min, 17.8 [7.6] sec; 45 min, 20.9 [6.9] sec; 60 min, 21.5 [7.3] sec; and 90 min, 23.8 [8.6] sec). All postbaseline values in the A and AN groups were significantly increased versus baseline and versus the control group values (all, $P < 0.05$). All postbaseline values in the A group were significantly greater than those in the AF group (all, $P < 0.05$).

CONCLUSION: Flumazenil antagonized the analgesic effect exerted by acetaminophen, while naloxone had no significant effect on acetaminophen's antinociceptive action in this pain model in rats. (*Curr Ther Res Clin Exp*. 2010;71:111–117) © 2010 Excerpta Medica Inc.

KEY WORDS: antinociceptive action, acetaminophen (paracetamol), flumazenil, naloxone.

INTRODUCTION

Acetaminophen's mechanism of action is primarily believed to be via central cyclooxygenase inhibition.[1] Studies designed to prove the antinociceptive action mechanism of acetaminophen have suggested that multiple nociceptive pathways may be involved in the analgesic action.[1–5]

Gamma-aminobutyric acid (GABA) is a significant inhibitor neurotransmitter in the central nervous system.[6] GABA receptors are located at both peripheral and central sites and, in particular, they are present in the dorsal horn of the spinal cord.[7] GABA receptors act by means of adhesion to chloride channels located in subsynaptic membranes in the spinal system, generating a GABA receptor-chloride channel complex. GABA receptor agonists create a conformational difference in the chloride channels.[6]

Based on pharmacologic experiments, 3 types of opioid receptors were postulated. They were named μ for morphine type, κ for the ketocyclazocine type, and σ for the SKF10047 (N-allylnormetazocine) type. In addition, a high-affinity receptor for enkephalins found in the mouse was deferens, and named as a delta (δ) receptor.[8]

Flumazenil, as a derivate of 1,4-imidazodiazepine, is an antagonist of benzodiazepines with partial agonist properties.[6] Benzodiazepine receptors are associated with pain transmission in the dorsal horn of the spinal cord.[9] Flumazenil has been reported to reverse all of the dose-related effects of benzodiazepines (anticonvulsive, sedative, anxiolytic, myorelaxant, and amnesic).[6] It acts by means of competitive adherence to synaptic GABA receptors. However, it does not have any effects on peripheral GABA'ergic (renal, cardiac, hepatic, testicular, adrenal, etc) receptors.[10]

Naloxone is a semisynthetic opioid competitive antagonist. Naloxone antagonizes the analgesic actions as well as the other effects of morphine such as respiratory depression by means of binding to the μ, δ, κ, and σ central receptors of morphine and similar analgesics.[8,11]

This study was conducted to gain insight into the mechanism of the analgesic action of acetaminophen and the influences of naloxone and flumazenil on this action. Accordingly, the purpose of this study was to find out whether naloxone and flumazenil were able to modify or prevent the antinociceptive effect of acetaminophen in the hot-plate test in rats.

MATERIALS AND METHODS

Experiment Animals

This study was performed in adult albino Wistar rats at the Experimental and Clinical Research Center of Erciyes University Medical Faculty, Kayseri, Turkey. The ethical guidelines for investigation of experimental pain in conscious animals were followed in all tests, and the procedures were carried out according to the EEC ethical regulations for animal research (European Economic Community council 86/609; D.L. 27/01/1992, No. 116). The rats were housed in plastic cages, 8 per cage, with free access to rat food (Aytekinler, Konya, Turkey) and tap water, and maintained on a 12-hour dark/light cycle (light on at 7:00 AM) under controlled environmental conditions (temperature, 20°C; humidity, 40%–50%).

Hot-Plate Test

The rats that were used in the experiment were randomly assigned by envelope method to 1 of 4 groups. The hot plate consisted of an electrically heated surface with temperature readout kept at a constant temperature of 52°C and double checked with surface measurements. "Cut-off time" was adjusted to 40 seconds. The latencies for paw withdrawal (or jumping) of the rats were assessed with the hot-plate test at 30, 45, 60, and 90 minutes after acetaminophen administration by the same investigator blinded to the treatment groups. To ensure blinding, the investigator who assessed paw withdrawal latency was different from the investigator who administered treatment.

Experiment Groups and Drug Doses Used

All administration of medication and normal saline was conducted intraperitoneally. Rats in the acetaminophen group (group A) were administered IP saline followed by 300 mg/kg IP acetaminophen[*] 5 minutes thereafter. In the acetaminophen + naloxone group (group AN), pretreatment with 1 mg/kg IP naloxone (1 mg/mL; Sigma-Aldrich, St. Louis, Missouri) was followed by 300 mg/kg of acetaminophen 5 minutes later. The acetaminophen + flumazenil group (group AF) was pretreated with 1 mg/kg IP flumazenil[†] (0.5 mg/5 mL) followed by 300 mg/kg IP acetaminophen 5 minutes later. The control group was administered 2.5 mL IP saline followed by a second administration of 2.5 mL saline 5 minutes later.

Statistical Analysis

Statistical analysis of the study was performed using SPSS software version 15.0 (SPSS Inc., Chicago, Illinois). The primary end point of the study was paw withdrawal latency. The obtained data was reported as mean (SD). A 1-way ANOVA was performed for between-group comparisons. Statistical significance was determined by Tukey test. Repeated measures ANOVA test was performed for in-group comparisons. $P < 0.05$ was considered statistically significant.

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[*]Trademark: Perfalgan® (Bristol-Myers Squibb, Rueil Malmaison, France).
[†]Trademark: Anexate® (Roche Products, Basel, Switzerland).

RESULTS

Thirty two rats (weight range, 210–250 g) were evenly allocated to 4 groups of 8 rats each. Baseline values for the A, AN, AF, and control groups were not significantly different (9.1 [2.3], 10.5 [2.7], 9.8 [3.0], and 8.9 [1.4] sec, respectively) (**Table**). In the AF group, flumazenil appeared to antagonize the analgesic effect exerted by the acetaminophen in the hot-plate test (30 min, 10.3 [3.7] sec; 45 min, 11.7 [5.1] sec; 60 min, 12.1 [5.1] sec; and 90 min, 12.2 [4.9] sec) and values were not significantly different from those obtained in the control group (30 min, 9.8 [2.2] sec; 45 min, 9.0 [1.6] sec; 60 min, 9.2 [1.6] sec; and 90 min, 8.5 [2.0] sec). In the AN group, naloxone did not significantly affect the values observed in the hot-plate test (30 min, 18.0 [4.5] sec; 45 min, 21.5 [7.8] sec; 60 min, 20.5 [5.9] sec; and 90 min, 22.3 [7.4] sec) and values at all time points were not significantly different from those obtained in the A group (30 min, 17.8 [7.6]; 45 min, 20.9 [6.9]; 60 min, 21.5 [7.3]; and 90 min, 23.8 [8.6] sec). All postbaseline values in the A and AN groups were significantly increased versus baseline and versus the control group values (all, $P < 0.05$). All postbaseline values in the A group were significantly greater than those in the AF group (all, $P < 0.05$).

Table. Paw latency period in a hot-plate test in rats administered acetaminophen (A), acetaminophen + naloxone (AN), acetaminophen + flumazenil (AF), or saline (control) (N = 32). Data are mean (SD) seconds.*

Time, min	Group A (n = 8)	Group AN (n = 8)	Group AF (n = 8)	Control Group (n = 8)
Baseline	9.1 (2.3)	10.5 (2.7)	9.8 (3.0)	8.9 (1.4)
30	17.8 (7.6) (0.019)[†] (0.03)[‡]	18.0 (4.5) (0.008)[†] (0.016)[‡]	10.3 (3.7) (0.045)[§]	9.8 (2.2)
45	20.9 (6.9) (0.001)[†] (0.002)[‡]	21.5 (7.8) (0.01)[†] (0.001)[‡]	11.7 (5.1) (0.02)[§]	9.0 (1.6)
60	21.5 (7.3) (0.001)[†] (0.001)[‡]	20.5 (5.9) (0.004)[†] (0.002)[‡]	12.1 (5.1) (0.01)[§]	9.2 (1.6)
90	23.8 (8.6) (0.002)[†] (<0.001)[‡]	22.3 (7.4) (0.003)[†] (0.001)[‡]	12.2 (4.9) (0.005)[§]	8.5 (2.0)

*There were no statistically significant differences between the AF and control groups; there were no statistically significant differences between the A and AN groups.

[†] Versus baseline.

[‡] Versus the control group.

[§] Versus the A group.

DISCUSSION

Studies suggest that acetaminophen may have multiple mechanisms of antinociceptive action.[1,12,13] The present study suggests that the antinociceptive action of acetaminophen is antagonized by the benzodiazepine-GABA receptor antagonist, flumazenil.

It has been reported that the antinociceptive action of high- (400 mg/kg) and low-dose (100 mg/kg) acetaminophen is antagonized by naloxone, which is a nonselective opioid receptor antagonist, and that the antinociceptive action caused by morphine is potentialized with low-dose acetaminophen and this effect is dependent upon an interaction between opioidergic and serotoninergic systems.[14,15] In addition, the fact that naloxone blocks the enhancement of antinociceptive action was associated with acetaminophen using receptors identical to that of naloxone.[14–16] Naloxone has been reported to antagonize the antinociceptive actions of some nonopioid drugs (such as acetaminophen) in certain experiments, and observed to have certain activities as a GABA antagonist.[17,18]

In the scope of the present study, opioid receptors were blocked with naloxone and then acetaminophen was administered at a dose of 300 mg/kg. The antinociceptive action of acetaminophen was not antagonized by pretreatment with naloxone. Sandrini et al[15] administered morphine (2, 3, or 5 mg/kg SC) and acetaminophen (50 or 100 mg/kg IP) to male Wistar rats that were pretreated with naloxone (1 mg/kg IP) in their study. As a result, they found that the enhancement in antinociceptive action made by acetaminophen was blocked by naloxone. However, this reduction could be in association with the decrease of antinociceptive action of morphine via opioid-receptor blockage. Pini et al[18] administered naloxone 30 minutes prior to the administration of acetaminophen to assess whether or not the antinociceptive action was antagonized in rats. However, in the present study, naloxone was administered 5 minutes before the administration of acetaminophen to avoid the possible reduction of the receptor blockage after 60 minutes. Throughout this study, in which the antagonization of the antinociceptive action was assessed at 30 minutes after acetaminophen administration, the antinociceptive action generated by acetaminophen was not antagonized by naloxone at any measurement point. These results suggest that the mechanism of action for acetaminophen does not involve opioid receptors.

Gear et al,[19] in a double-blind, placebo-controlled study, reported that flumazenil enhances the analgesic action of postoperative morphine in patients with dental pain who are administered preoperative benzodiazepine and that benzodiazepines antagonize the opioid analgesia via $GABA_A$ receptors. According to the study performed by Holtman et al,[20] flumazenil–morphine interaction on analgesia was tested after intraperitoneal and intrathecal routes of administration in female rats; IP flumazenil (0.5 mg/kg) alone did not produce analgesia and analgesia was enhanced by the concurrent administration of IP flumazenil (0.5 mg/kg) with morphine (2 mg/kg). In contrast, analgesic action was not enhanced by morphine administered intrathecally. These data suggest that morphine enhances analgesic efficacy by benzodiazepine receptors at sites other than the spinal cord.

Studies indicate that GABA receptors are involved in spinal nociception.[19,20] By performing the present study we take into consideration the possibility that the mani-

festation of the interaction of acetaminophen with GABA receptors, which have been proven to have significant effects on the transmission of nociception and its regulation,[21] will be a new explanation for the mechanism of antinociceptive effects of acetaminophen. Therefore, we have aimed at achieving a competitive antagonism at the receptor level by administering flumazenil acting as GABA receptor antagonist, prior to the administration of acetaminophen. This study found that the antinociceptive action of acetaminophen in the AF group was antagonized by flumazenil. The antagonism of the antinociceptive action was induced in the 30th minute following acetaminophen administration and it was maintained up to the 90th minute at all measurement points. There was not any statistically significant difference observed at any of the intermediate measurement points in the AF group.

This was a small experimental study in a pain model in rats; therefore, the results cannot be extrapolated to clinical use. Further studies are needed to evaluate the interaction between acetaminophen and flumazenil activation pathways.

CONCLUSION

Flumazenil significantly antagonized the analgesic effect exerted by acetaminophen, but naloxone had no significant effect on acetaminophen's antinociceptive action in this pain model in rats.

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