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Analysis of the antinociceptive effect of pethidine combination with ketamine or paracetamol in tail-flick test in mice

Fare tail-flick testi ile ketamin veya parasetamol ile birlikte kullanılan petidinin antinosiseptif etkisinin incelenmesi

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Abstract

Aim. The opioid drugs, such as pethidine, are the most effective analgesic drugs used in the treatment of severe acute and chronic pain. In this study, we aimed to investigate the potential antinociceptive interaction of pethidine with ketamine or paracetamol in tail-flick test in mice. **Methods.** Fifty male BALB/c mice were divided randomly into 5 groups. In three of the groups, the time course of antinociceptive action was determined after using intraperitoneal pethidine (10-100 mg/kg), ketamine (1.25-5 mg/kg) or paracetamol (2.5-10 mg/kg) assessing tail flick latencies (TFLs). In the remaining two groups, combination of minimal effective dose of pethidine (10 mg/kg) with ketamine (2.5 mg/kg) or paracetamol (1.25 mg/kg) were assessed by TFLs. **Results.** Intraperitoneally administered pethidine revealed a dose dependent antinociception at 10-50 mg/kg doses but ketamine and paracetamol were ineffective at used doses. These ineffective doses of ketamine (2.5 mg/kg) or paracetamol (1.25 mg/kg) significantly potentiated the antinociceptive effect when combined with pethidine ($p<0.05$). **Conclusions.** This interaction between pethidine and ketamine or paracetamol gives us an advantage of reducing their doses and also their adverse effects. So these combinations may be a suitable choice for antinociception after further animal and clinical studies

Keywords: Pethidine, ketamine, paracetamol, analgesia, tail-flick test, mice

Özet

Amaç. Petidin gibi opioid ilaçlar, ciddi akut ve kronik ağrı tedavisinde kullanılan en etkili analjezik ilaçlardır. Bu araştırmada fare tail-flick testi ile ketamin veya parasetamol ile petidinin potansiyel antinosiseptif ilişkisi incelendi. **Yöntem**. Elli erkek BAL/c fare rastgele olarak beş gruba ayrıldı. Üç grupta intraperitoneal petidin (10-100 mg/kg), ketamin (1.25-5 mg/kg) ya da parasetamolün (2.5-10 mg/kg) antinosiseptif etki süresi tail flick latens (TFL) süreleri ölçülerek incelendi. Diğer iki grupta ketamine (2.5 mg/kg) veya parasetamol (1.25 mg/kg) ile birlikte petidinin (10 mg/kg) minimal etkin dozunun kombinasyonu TFL ile değerlendirildi. **Bulgular**. İntraperitoneal olarak uygulanan petidin doz bağımlı olarak 10-50 mg/kg'dan başlayarak ağrı kesici etki sağladı, fakat kullanılan dozlarda diğer ilaçlar etkisizdi. Ketamin (2.5 mg/kg) veya parasetamolün (1.25 mg/kg) bu etkisiz dozları, petidin ile kombine edildiğinde antinosiseptif etkiyi anlamlı olarak güçlendirdi ($p<0.05$). **Sonuçlar**. Petidin ile ketamin veya parasetamol arasındaki bu ilişki bu ilaçların dozlarını ve yan etkilerini azaltma avantajı vermektedir. Böylece bu kombinasyonlar, ileri hayvan ve klinik çalışmalardan sonra, analjezi için daha uygun bir seçenek olabilir.

Anahtar sözcükler: Petidin, ketamin, parasetamol, analjezi, tail-flick testi, fare

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Introduction

Ideal analgesia is attained with optimal pain relief and minimal risk for the patient. The opioid drugs such as pethidine are the most effective analgesic drugs used in the treatment of severe and chronic pain. However, side effects such as vomiting, pruritus, respiratory depression, tolerance, or dependence have restricted their clinical use [1]. Some studies have shown that the adverse effects of pethidine were dose related [2, 3].

The dissociative anesthetic agent ketamine is a noncompetitive blocker of the glutamate subtype of N-methyl-D-aspartate (NMDA) receptors which also exerts analgesic properties in rodents and humans [4, 5]. Ketamine has been used as an anesthetic, rather than an analgesic in clinical practice in the past but recently it has been introduced as an analgesic agent to be used in the management of chronic pain [6], neuropathic pain as well as of cancer pain [7, 8]. In acute pain management, ketamine is not effective when used as a sole analgesic, but it produces a synergistic effect with opioids [9, 10].

Paracetamol is often included with non-steroidal anti-inflammatory drugs (NSAIDs) in classifications of analgesics, even though it has differences in both action and side effect profile. Combinations of NSAIDs with potent analgesics are currently widely used in the management of pain [11]. A study has concluded that paracetamol might be regarded as an analgesic choice in pregnancy. In late pregnancy, paracetamol is certainly safer than other NSAIDs [12].

In this study, we aimed to investigate the potential antinociceptive interaction of pethidine with ketamine or paracetamol in the radiant heat tail-flick test in mice.

Methods

Animals

Fifty male BALB/c mice, each weighing 25-30 g, were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experiments were approved by the Animal Care Committee of our school. Mice were acclimatized in a 12-h light/dark cycle, pathogen-free conditions at 23°C with food and water available *ad libitum*. All experiments were conducted during the light period of the cycle (08.00-14.00).

Tail-flick tests

Antinociception was assessed using the radiant heat tail-flick test (May TF 0703 Tail-flick Unit, Commat, Ankara, Turkey). The radiant heat source was focused on the distal portion of the tail at 2-2.5 cm after administration of the study drugs. Before drug administration mice were divided into 5 groups and tail flick latencies (TFL) were obtained. In the first group, the time course of antinociceptive action was determined after using 10, 25 and 50 mg/kg intraperitoneal pethidine, and assessing TFL at 20, 40, 60, 120, 180, and 240 min. In the second and third groups, it was determined after using 1.25, 2.5 and 5 mg/kg intraperitoneal ketamine and 2.5, 5 and 10 mg/kg intraperitoneal paracetamol, and assessing TFL at 20, 40, and 60 min. In the last two groups, combinations of pethidine (10 mg/kg) with paracetamol (1.25 mg/kg) or ketamine (2.5 mg/kg) were assessed by TFL at 20, 40, 60, 120, 180, and 240 min. The person assessed TFLs did not know which drugs the mice had been administered.

Data analysis

ED50 value with 95% confidence intervals (CI) of pethidine was calculated with regression analysis of dose-response curves using a customized Visual Basic program

FlashCalc (Michael H. Ossipov, personal communication) at the time of the peak effect. The doses of ketamine and paracetamol used in this study did not show analgesic effect. So ED50 value and maximum effect time could not be calculated.

Base line TFLs typically ranged from 2 to 3 sec. The cut off latency was set at 10 sec to avoid tissue damage. Those animals, which did not respond after 10 seconds, were excluded from the study. Data were expressed as % analgesia that was calculated by using the following equation:

% analgesia=[(Trial TFL-Baseline TFL)/(Cutoff Time-Baseline TFL)]x100

Drugs

Chemicals used in the current experiments were pethidine from Gerot Pharmazeutika GmbH (Vienna, Austria), paracetamol from Bristol-Myers Squibb (New York, NY, USA), ketamine hydrochloride from Eczacıbası (Istanbul, Turkey). All the drugs were dissolved in distilled water and were freshly prepared on the day of the experiments.

Statistical analysis

A non-parametric method of statistical analysis was used. Statistical significance of more than two groups was evaluated by Kruskal-Wallis ANOVA test ($p<0.05$), followed by Dunnett's multiple test for individual comparisons.

Results

Experiment 1: % analgesic effects of pethidine, ketamine and paracetamol

At the beginning of the experiments, in order to determine EC50 values, increasing doses of pethidine (10-50 mg/kg), ketamine (1.25-5 mg/kg) and paracetamol (2.5-10 mg/kg) were injected to mice intraperitoneally. Although the doses of ketamine and paracetamol used in this study did not produce any antinociceptive effect, pethidine produced ED50 values in a dose dependent manner. ED50 value for pethidine was 20.12±1.54.

To determine time dependent antinociceptive effects of pethidine at the doses of 10, 25 and 50 mg/kg, TFLs were assessed at 5th, 20, 40, 60, 120, 180 and 240 min. Time versus effect curves revealed that antinociceptive effect of pethidine peaked at 60 min at all doses, and after 60 min decreased gradually and came to baseline level at 240 min (Figure 1).

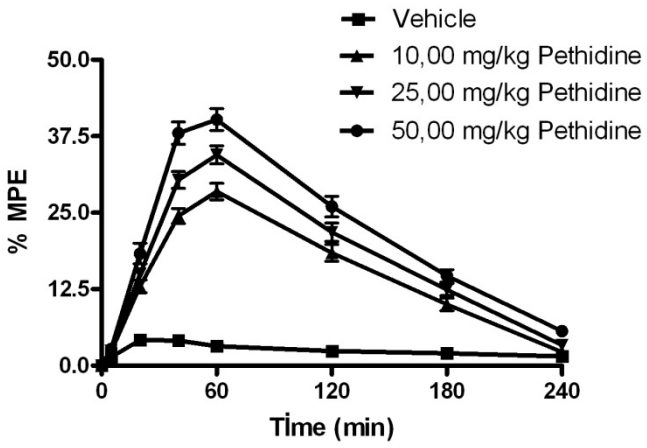

Figure 1. Time dependent % antinociceptive effect of pethidine.

To determine time dependent antinociceptive effects of ketamine at the doses of 1.25, 2.5, and 5 mg/kg, TFLs were assessed at 20, 40, and 60 min. Five mg/kg or lower doses of intraperitoneal ketamine did not produce any antinociceptive effect.

For time dependent antinociceptive effects of intraperitoneal paracetamol at doses of 2.5, 5 and 10 mg/kg, TFLs were assessed at 20, 40 and 60 min. Ten mg/kg or lower doses of paracetamol did not produce any antinociceptive effect.

Experiment 2: Combination of pethidine and ketamine

Ketamine (1.25 mg/kg) was administered 20 min before the pethidine (10 mg/kg) administration. Pethidine TFLs were assessed at 5, 20, 40, 60, 120, 180 and 240 min. Analgesic effect of pethidine in the presence of ketamine began at 5 min (% 11.99), and peaked at 60th min (% 50). The analgesic effect of pethidine-ketamine combination started to decrease gradually after 60 min, but remained quite high at 240th min (22.21%) (Figure 2).

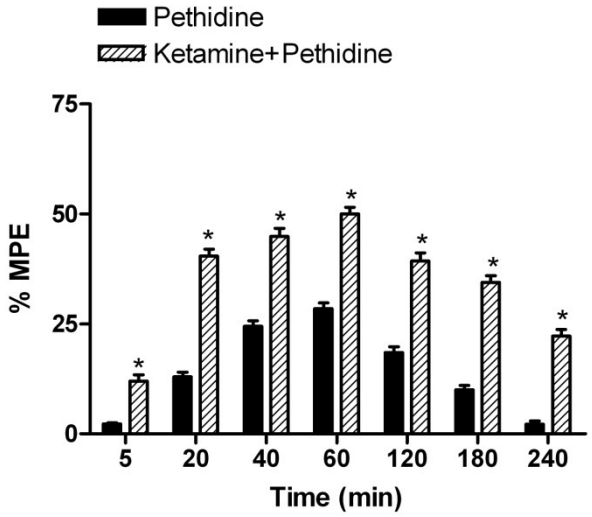

Figure 2. Time dependent analgesic effect of pethidine and ketamine combination. *Statistically different from pethidine group (P<0.05).

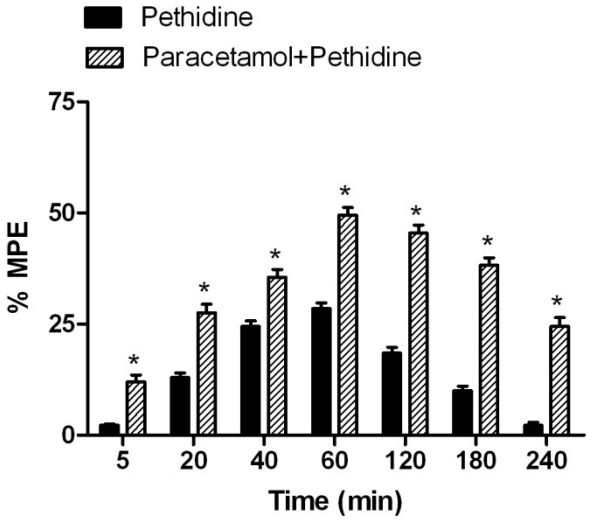

Figure 3. Time dependent % analgesic effect of pethidine and paracetamol combination. *Statistically different from pethidine group (P<0.05).

Experiment 3: Combination of pethidine and paracetamol:

Paracetamol (2.5 mg/kg) was administered 20 min before the pethidine (10 mg/kg) administration. Pethidine TFLs were assessed at 5, 20, 40, 60, 120, 180 and 240 min. Analgesic effects of pethidine in the presence of paracetamol began at 5 min (12.01%) and peaked at 60 min (%49.5). The analgesic effect of pethidine-paracetamol combination gradually decreased after 60 min but continued beyond 240 min (% 24.45) (Figure 3).

Discussion

A potential advantage of using combination therapy is that analgesic effects can be maximized while the incidence of adverse side effects is minimized [13]. Therefore, using combinations of medications that offer analgesic synergism should allow a reduction in required dosage and decrease the incidence of adverse effects [14].

Because of its lesser adverse effects and respiration depression compared with the same doses of morphine, pethidine is a preferred agent. Some studies have indicated some limitations of pethidine use and its complications because of its metabolite normeperidine, which is known as a central nervous system irritant, and which can cause behavioral changes such as bizarre feelings, delirium, and psychosis as well as central nervous system excitation symptoms such as nervousness, myoclonus, and seizures [15, 16]. It has also been suggested that the extent of postoperative nausea and vomiting was related to the dose of pethidine administered preoperatively [2, 3]. Therefore, it was thought that suitable combinations of pethidine with other agents could reduce its dose related adverse effects.

Many clinical studies have shown that ketamine is useful at subanaesthetic doses in the management of postoperative pain in humans [17, 18]. Ketamine exerts this effect via NMDA receptors, and the involvement of spinal cord NMDA receptors with the generation and maintenance of acute and chronic pain states in animal models has also been well established [19, 20]. In recent years, ketamine is used for acute or chronic cancer pain and postoperative pain at sub-hypnotic doses. The NMDA receptor has been reported to be involved in opioid tolerance. Adjuvant subcutaneous infusion treatment with (very) low-dose ketamine improves analgesia and at the same time appears to reduce opioid tolerance. It has also been suggested that ketamine could reduce opioid tolerance in the terminally ill cancer patients. Furthermore, low-dose subcutaneous ketamine infusion used as an adjuvant treatment to opioids has been stated to be an inexpensive, readily available, and non-invasive treatment [21]. Similar combination regimes may also be useful in other clinical settings, to enable long-term sedation with lower doses of opioids. There are other studies showed interaction between pethidine with ketamine, and the suggested mechanisms in this interaction include the activation of the monoaminergic descending inhibitory system, a blocking action on NMDA receptors, and a local anesthetic-like activity [9, 10].

Paracetamol is often included with non-steroidal anti-inflammatory drugs and is available in combination with weak opioid drugs (e.g. codeine, dihydrocodeine, and dextropropoxyphene) for the treatment of moderate pain [22, 23]. There is some evidence for synergy between opioids and NSAIDS in animal models of neuropathic and inflammatory pain [24], and synergistic interactions between ibuprofen and hydrocodone have been reported in a thermal model, the radiant heat tail-flick assay [25].

In the present study, we aimed to combine intraperitoneal pethidine with ketamine or paracetamol to utilize these combinations with different mechanisms of action and thereby enhance analgesic activity of pethidine with fewer side effects. We first defined the TFLs of different doses of intraperitoneally administered pethidine and then combined its minimal effective dose with ineffective doses of ketamine or paracetamol to observe their interaction in mice. These ineffective doses of ketamine or paracetamol strongly potentiated the antinociceptive effect of pethidine. This interaction can be called as

pharmacological potentiation that is a special form of synergism. In cases of potentiation, one of the two agents *per se* exerts no effect upon exposure; but upon exposures to combinations of the agents, the effect of the active agent is increased [26]. Ketamine or paracetamol may affect the metabolism of pethidine but we know that ketamine or paracetamol does not affect the activity of microsomal enzymes [25, 26], so these interactions cannot be via a metabolism effect. Instead, they may be involved in opioid receptor up-regulation, thereby enhancing the effect of pethidine. However, it is not possible to increase the amount of the receptor in such a short time and by only one time exposure. Thus, it could be that these agents may increase endogen endorphins release. If they had such an effect, they should also have an antinociceptive effect when they are administered alone. Therefore, it could be argued that this interaction may be seen by the enhanced pethidine receptor sensitivity in the presence of ketamine or paracetamol. Further pharmacological studies should be performed to better understanding of such interactions.

In conclusion, the data obtained suggest that the combination of pethidine with ketamine or paracetamol is much more effective than pethidine alone in antinociception. This interaction could enable us to reduce the required doses and adverse effects of these agents. Thus, it is our hope that combined usage of the agents studied might provide a suitable choice for analgesia after performing further animal and clinical studies.

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