

## THE EFFECT OF KETAMINE, DICLOFENAC AND THEIR COMBINATION ON FOUR MODELS OF INDUCED PAIN IN MICE

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### Abstract

Adjuvant analgesics are drugs that have weak or nonexistent analgesic action when administered alone, but can enhance analgesic action when co-administered with known analgesic agents. Ketamine, an anesthetic drug, is an adjuvant analgesic drug. Its use in doses lower than therapeutic doses might be important in the management of certain types of pain as neuropathic pain.

The present study was performed to investigate the effects of subanesthetic doses of ketamine in four animal models of induced pain and to compare its effects with diclofenac sodium. It is also intended to test the effectiveness of combining both drugs together in these animal models of pain.

All experiments were performed on albino mice. Mice were evaluated for their responsiveness to noxious stimuli using four tests: tail-flick test, hot-plate test, formalin test and acetic acid-induced writhing test. These effects were measured before and one hour after intraperitoneal drug administration. In some experiments, they were followed for 6 and 24 hours.

We found that Ketamine, in subanesthetic doses, resulted in a significant analgesic effect in all the four models of pain. It increased pain latencies in tail-flick test by 78% compared to pre-administration time, and by 95% in hot plate test. It also decreased the number of lickings and bitings in formalin test by 41.9% and the number of abdominal writhing by 73.5%. These analgesic effects represented around 60% of diclofenac effect in heat-induced pain models, but it is similar to diclofenac in the other two models. The enhancement of diclofenac analgesic effect by ketamine ranged from 13.6% to 46% in the four tests.

It is concluded that Ketamine in subanesthetic doses has a significant analgesic effect comparable to diclofenac. It can enhance diclofenac effect by a margin not exceeding 50% of diclofenac effect. Much smaller doses of ketamine are required to be tested in the future.

### Introduction

Co-analgesics are defined as drugs that have primary indications other than pain but may be analgesic in selected circumstances<sup>1</sup>. They can also be defined as drugs that have weak or non-existent analgesic action when administered alone, but can enhance analgesic actions when co-administered with known analgesic agents<sup>2</sup>. This combination increases analgesia without increasing the dose of analgesics, and therefore, can reduce the incidence of adverse effects<sup>3</sup>. The combined

treatment of the two types of drugs at doses much lower than therapeutic doses may be of great value in pain therapy<sup>4</sup>.

Ketamine is an N-methyl-D-aspartate receptor, NMDA, antagonist. It is used as a general anesthetic and found effective in certain cases of neuropathic pain<sup>5</sup>. It reduced opiate requirement when used for post-operative analgesia in sub-anesthetic doses<sup>6</sup>.

Ketamine may be a good co-analgesic for severe pain caused by terminal

cancer<sup>7</sup>. Adding ketamine to tramadol improved analgesia and patient comfort and decreased the amount of tramadol required for post-operative pain management after major abdominal surgery<sup>8</sup>. It also enhanced the analgesic effect of morphine, thus reducing the dose of morphine<sup>9</sup>. In other studies, ketamine by intrathecal route potentiated analgesia obtained with morphine, clonidine and lidocaine<sup>10,11</sup>. It is said to provide safe and effective analgesia in acute musculoskeletal trauma<sup>12</sup>.

The interaction between NSAIDs and ketamine showed contradictory results. Ketoprofen with ketamine synergistically depressed NMDA-mediated nociceptive spinal transmission of the neonatal rats *in vitro*<sup>13</sup>. On the other hand, diclofenac was not found to have additive effect to ketamine and remifentanyl on postoperative analgesia in women undergoing laparoscopic surgery<sup>14</sup>.

The present study is, therefore, intended to investigate the analgesic

effect of sub-anaesthetic dose of ketamine when used alone in four models representing different types of painful stimuli in mice. It is also to be investigated in combination with the non-steroidal anti-inflammatory drug; diclofenac sodium, a commonly employed post-operative analgesic and in musculoskeletal pain, and also in some visceral painful conditions such as renal colic.

## Materials and Methods

### Animals

All experiments were performed on albino-mice 8-12 weeks old, weighing approximately 20 to 25g and housed in special plastic cages at an environmental temperature of 24±2°C. The animals had free access to food and water.

### Groups

Four groups, 6 mice each (3 males and 3 Females), were used; one control group (group1) and three treatment groups (Group 2, 3 and 4).

Groups	Volume of drug administered intraperitoneally
Group 1	0.2ml of normal saline (NS)
Group 2	0.1ml (NS) + 0.1ml diclofenac sodium (3mg/kg)
Group 3	0.1ml (NS) + 0.1ml ketamine (2.5mg/kg)
Group 4	0.1ml Diclofenac + 0.1ml ketamine

### Pain assessment methods

Mice were evaluated for their responsiveness to noxious stimuli, using four methods of pain assessment: tail-flick test, hot-plate test, formalin test and acetic acid-induced writhing test.

#### Tail-flick test

Each animal was restrained by hands and the tip of its tail (the last 2 cm) was dipped in a water-bath heated at 48±0.2°C. The time for tail curling or flicking was measured as the latency of the test. Each animal was subjected to three trials separated by a minimum interval of 3 minutes. The average of three measurements was considered as

the latency of tail-flick test for each mouse<sup>15</sup>.

#### Hot-plate test

An animal was placed on a metal plate maintained at 52±0.2°C and the latency of nociceptive responses such as licking or flicking of the hind limb or jumping was measured according to the method described by Kanaan et al<sup>15</sup>.

Only mice that showed the nociceptive response within 18 seconds were used for the experiments. The latency of nociceptive responses in these animals was expressed as the hot-plate latency. A cut-off time of 45 seconds was selected to prevent tissue damage.

The obtained values for each group were compared with the baseline measurements established before administration of the drugs, using each animal as its own control to account for any possible variation in the nociceptive thresholds produced by stress due to frequent manipulation.

#### **Formalin test**

The nociceptive response in the formalin test was performed using the method described by Mahmoudi et al<sup>16</sup>.

Before the test, the animals were allowed to adapt to the test environment for 30 minutes before drug injection. They were placed in transparent plastic cages. Twenty microliters of 5% formalin was injected subcutaneously into the planter region of one of the hind paws using a micro-syringe. The number of nociceptive responses such as licking and/or biting of the injected paw were recorded over a 30 minute observation period.

#### **Writhing test**

All tested drugs were administered 60 minutes before I.P. administration of acetic acid 0.7% (1ml/kg). Animals were individually housed in a glass container and a mirror was arranged at an angle of 45° under the container.

Antinociception was recorded by counting the number of writhes immediately after injection of acetic acid and during 30 minutes thereafter. A writhe is indicated by abdominal constriction and stretching of at least one hind limb<sup>17,18</sup>.

#### **Statistical analysis**

The results were expressed as mean±SD; the data were analyzed statistically by one-way analysis of variance (ANOVA), paired and independent t-test, using SPSS (computer package program version 9), P<0.05 was considered to be the lowest limit of significance.

#### **Results**

Ketamine (2.5mg/kg) given intraperitoneally resulted in an analgesic effect

ranging from around 78% increase in tail-flick time to 95% increase in hot-plate time in comparison to pre-injection measurements. This is compared to around 127% and 168% for diclofenac sodium in the two tests respectively.

The combination of the two drugs resulted in higher and statistically significant analgesic effect compared to each drug given alone (152% compared to 127% and 78% in tail-flick test, and 246% compared to 168% and 95% in hot-plate test for the combination, diclofenac sodium and ketamine respectively, Table I and II).

The maximum analgesic effect occurred about two hours after injection and faded at four hours for the two drugs given individually. The analgesic effect continued longer (for 6 hours) for the two drugs given in combination (Figure 1).

In writhing test, ketamine also reduced the number of abdominal writhings in 30 minutes by 73.5% with respect to control group compared to 72% by diclofenac sodium. In addition, it reduced the number of lickings and bitings of the affected paw in formalin test by 42% with respect to control group compared to 44% by diclofenac sodium (table III).

The combination of the two drugs produced higher effect in both writhing and formalin tests compared to each drug used alone (81.8% and 59.7% in writhing and formalin tests respectively)(table III).

When the effect of ketamine was measured as the ratio of percent change caused by ketamine to the percent change caused by diclofenac sodium in the four models of pain, it can be seen that the effect of ketamine was either similar to, or produced less analgesic effect than diclofenac depending on the model used. However, when it is used in combination with diclofenac, it enhanced the analgesic effect of

diclofenac by 33% in heat-induced somatic pain (tail-flick and hot-plate tests) and by 37% in chemically induced

somatic pain (formalin test), while only by 14% in visceral-type of pain (writhing test).

**Table I: Effect of ketamine, diclofenac sodium, or their combination on tail-flick test in mice**

	Control (normal saline) N=6	Diclofenac sodium (3mg/kg) n=6	Ketamine (2.5mg/kg) n=6	Diclofenac sodium + ketamine n=6
Before I.P. injection	5.29±0.71	5.1±0.28	5.29±0.39	5.11±0.39
One hour after I.P. injection	4.99±0.48	11.57±1.56	9.40±1.22	12.89±2.69
Percent change with respect to pre-injection time	5.7%	126.9%	77.7%	152.3%
Statistical significance	not significant	P<0.001	P<0.001	P<0.001

Data are expressed as mean±SD of tail-flick time in seconds.

**Table II: Effect of ketamine, diclofenac sodium, or their combination on hot-plate test in mice**

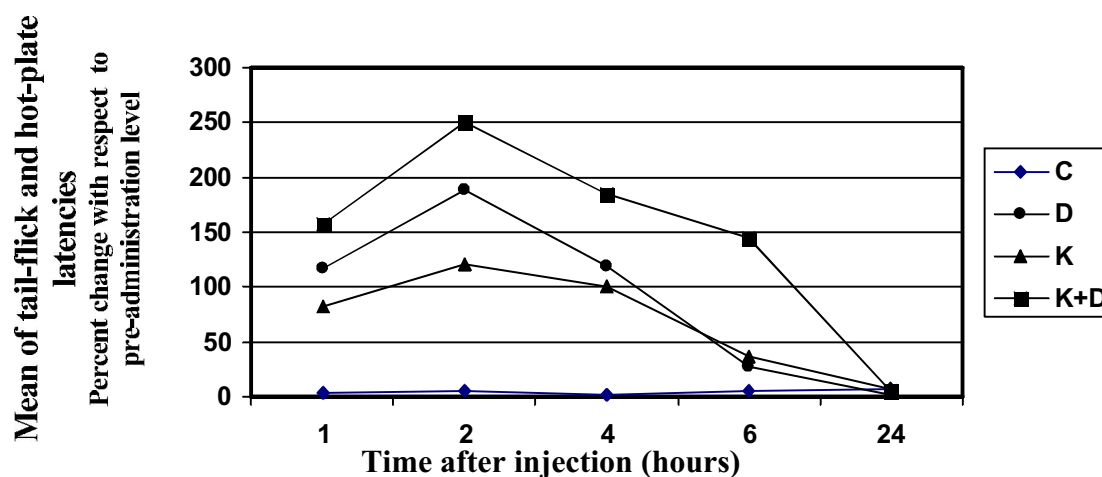
	Control (normal saline) n=6	Diclofenac sodium (3mg/kg) n=6	Ketamine (2.5mg/kg) n=6	Diclofenac sodium + ketamine n=6
Before I.P. injection	4.55±0.52	4.88±0.4	5.11±0.6	4.75±0.6
One hour after I.P. injection	4.92±0.54	13.10±1.46	9.98±1.15	16.44±0.91
Percent change with respect to pre-injection time	8.1%	168.4%	95.3%	246.1%
Statistical significance	not significant	P<0.001	P<0.001	P<0.001

Data are expressed as mean±SD of hot-plate time in seconds.

**Table III: Effect of ketamine, diclofenac sodium, or their combination on acetic acid-induced writhing test and on formalin test in mice**

Drug	Dose	n	Writhing test	Formalin test
			Number of abdominal writhings in 30 minutes	Number of lickings or bitings of the affected paw
Control	Normal saline	6	45.83±10.20	20.66±2.42
Diclofenac sodium	3mg/kg	6	12.66±1.76*** (-72%)	11.66±1.21*** (-43.6%)
Ketamine	2.5mg/kg	6	12.16±1.16*** (-73.5%)	12±1.41*** (-41.9%)
Diclofenac + ketamine	3+2.5mg/kg	6	8.33±0.81*** (-81.8%)	8.33±0.81*** (-59.7%)

Data are expressed as mean±SD of n=6 with percentage of change from control. Significant difference with respect to control: \*\*\*: P<0.001



**Figure 1:** Effect of ketamine, 2.5mg/kg (K), diclofenac sodium, 3mg/kg (D) or their combination (K+D) on tail-flick and hot-plate latencies (grouped together) before and 1,2,4,6 and 24 hours after drug administration in mice with respect to pre-injection latency and in comparison to control.

## Discussion

Pain is a common problem, and often associated with inflammatory conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are clinically effective because they alleviate pain and inflammation and restore patients to more normal daily function<sup>19</sup>. Thus, the NSAID: diclofenac sodium was selected in the present study as a standard drug to be compared and co-administered with ketamine.

Ketamine may be a good co-analgesic drug for severe pain. It reduced opiate requirement when used for postoperative analgesia in sub-anesthetic doses<sup>20</sup>. Experimental studies in animals have suggested that a combination of morphine with N-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine may have additive or synergistic analgesic effects<sup>21,22</sup>. This synergistic interaction tends to confirm the interest of using this type of combination in the clinical context, and also to study other types of combinations e.g. with NSAIDs; the aim of the present study.

In the present study, ketamine in sub-anesthetic dose, produced a significant analgesic effect in all the four tests

used. This effect is lower than diclofenac in heat-induced pain models, representing around 60% that of diclofenac, and similar to it in writhing and formalin tests.

The mechanism of ketamine action seems to be a central one. Intravenous ketamine produced dose-dependent inhibition of the spinal cord neuronal responses evoked by urinary bladder distention in rats. This neurophysiologic evidence supports a spinally mediated analgesic effect of ketamine in this model of urinary bladder nociception, an effect likely to be caused by N-methyl-D-aspartate receptor antagonism<sup>23</sup>.

Pain reduction was, also, significantly correlated with ketamine induced changes in hallucinatory behavior and excitement. Behavioral results indicate that ketamine induced failure of neural integration between cortical and sub-cortical regions results in psychotic symptoms and alters pain perception in neuropathic pain<sup>24</sup>.

Administration of ketamine with other drugs e.g. paracetamol has been investigated previously<sup>25</sup>. Co-administration of a non-active dose of paracetamol (10µg/kg) together with low dose of

dextromethorphan (0.1 µg/kg) and ketamine (10 and 100 µg/kg), resulted in potentiation of the nociceptive effect in comparison with their effects after individual administration<sup>25</sup>. In the present study, co-administration of the NSAID: diclofenac with ketamine resulted in enhancement of diclofenac

effect. ketamine enhanced the analgesic effect of diclofenac by an average of 33% in heat-induced pain models, 13.6% in writhing test and by 37% in formalin test. Therefore, Ketamine in sub-anesthetic doses has its own analgesic effect and can enhance the effect of diclofenac if administered together.

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