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A comparative study of antinociceptive effects of Tapentadol, amitriptyline and combination of both in albino rats

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Abstract

Objective: The aim of the study was to investigate the possible antinociceptive effects of combination of Tapentadol and Amitriptyline in comparison to the single drugs.

Materials and Methods: After approval from Animal Ethics Committee, a total of twenty four (n = 24) healthy male albino rats were divided into four groups of six rats in each. Group I was control, Gr. II – tapentadol treated, Gr. III – amitriptyline treated and Gr. IV – treated with both tapentadol and amitriptyline. The test drugs administered thirty minutes prior to the procedure once at weekly intervals (on day 0, 7, 14, 21 and 28) for up to 28 days. Haffner's tail clip and formalin test were conducted to evaluate the antinociceptive effects.

Results: In both animal test models the antinociceptive effects observed from the combination of tapentadol and amitriptyline was greater than that produced by tapentadol and amitriptyline alone.

Conclusion: From the results obtained in this study, we can conclude that coadministration of tapentadol and amitriptyline showed much greater antinociceptive effects than either drug singly . Thus, in combined form they can potentiate each – others action via multifactorial mechanism.

Keywords: Tapentadol, Amitriptyline, Nociception, Haffner's tail-clip method, Formalin test

1. Introduction

Pain is an unpleasant sensationbut usually protective to the body and occurs whenever any tissues are being damaged. It causes the individuals to react to remove the painful stimulus. Although, many pathological conditions may results severe and chronic pain, tissue injury is the immediate cause of pain. This results in the local release of variety of chemical mediator which acts on the nerve terminals, either activating them or enhancing their sensitivity to other forms of stimulations.[1]

Thus, pain, is produced by the excitation of particular receptors, the nociceptors or of their afferent fibres, that respond to a broad spectrum of physical (heat, cold and pressure) or chemical noxious stimuli. In general, perception of noxious stimuli is termed as nociception. Nociception is not exactly same as pain, pain is a subjective experience and includes strong affective component which is lacking in nociception.[2]

Multimodal analgesia is currently recommended for effective pain control. It is achieved by combining different analgesics that act by different mechanisms, resulting in additive or synergistic analgesia.[3] The main goals of such combinations would be to improve analgesia and/or to reduce the adverse effects induced by each drugs when administered separately.

The opioid analgesic drugs remain the most effective therapy available for the treatment of moderate to severe pain. However, the side effect profile of opioids, which includes nausea / vomiting, sedation, constipation, and respiratory depression, should be considered when using large doses of these drugs. Using combinations of medications that elicit analgesic synergism could allow reduction in the required dosage of opioid and there by decreases side effects.[4]

The newer opioid analgesic, tapentadol has dual mechanism of action i.e., μ - opioid receptor agonist and nor - epinephrine (NE) – reuptake inhibition. [5] Its high efficacy has been demonstrated in a variety of animal models of acute and chronic, nociceptive, inflammatory, and neuropathic pain as well as in clinical studies

with moderate to severe pain arising from a number of different etiologies.[6]

It is believed that modulation of endogenous pain mechanisms through the serotonin and noradrenaline descending inhibitory pathways is a major mechanism of antinociceptive activity of antidepressants. Moreover, numerous studies have suggested that activation of opioid endogenous system, blocking of nor-adrenoreceptors, muscarinic and histaminic receptors, blocking conduction in ions channels, activation of adenosine antinociceptive system, as well as peripheral modulation of inflammatory and immune parameters might be involved in the antinociceptive action of antidepressants.[7]

In animal studies, antidepressants produce pain relief in acute nociceptive and neuropathic pain tests as well as in models of inflammation.[8,9] Studies have also shown an enhancement of antinociception from systemic opioids by intrathecal injection of amitriptyline.[10,11]

The purpose of this studywas to determine the antinociceptive effects of tapentadol, amitriptyline and their combination (Tap + Ami) in albinorats by using tail clip and formalin tests.

2. Materials and Methods

2.1 Animal

The experiment were performed on healthy adult male albino rats (n=24), of an average weight of 150 – 200 gms. Rats were divided into four groups consisting of six rats in each group and placed in separate adequately roomed cage. All cages were labelled properly according to their group division. Rats in each cage were also labelled with different colours by using permanent coloured pen marker to recognise the individual rat with their drug and doses given.

The rats were maintained under controlled room temperature (25˚± 2˚C) and light and dark cycle of 12:12hr. Animals were given standard laboratory diet including bread and crushed maize and soybeans with water *ad libitum*. Arrangement were made for regular cleaning of animal house and disposal of their excreta. They were left for ten days of acclimatization in the laboratory environment before starting the experiment. The whole experiment was conducted in accordance with ethical norms approved by Institutional Animal Ethics Committee (IAEC) Guidelines.

2.2 Details of Group

Groups	No of Rats	Drugs
Gr.A	6	1% gum acacia
Gr.B	6	tapentadol (4.5mg /kg)
Gr.C	6	amitriptyline (2.25mg/kg)
Gr.D	6	tapentadol (4.5mg/kg) and amitriptyline (2.25mg/kg)

2.3 Drugs and Doses

1) Tapentadol – Tab. Tapal ER 50 mg. from MSN Laboratory Pvt. Limited, Medak Distt., Andhra Pradesh, India.
2) Amitriptyline – Tab. Amitry 25 mg. from KC Laboratories, Ankleshwar, Gujarat, Indiia.
All the drugs were procured from the local market.

2.4 Dose Calculation

Dose of the drugs was calculated from the standard clinical human dose on the basis of surface area.[12] Surface area ratio of 200g rat for 70 kg man is 0.018. Thus human dose of drug (for a 70 kg person) multiplied by 0.018 gives the value of that drug for 200g of rat.[13] Then each animal was weighed and dose calculated according to their body weight.

2.5 Experiment Design

2.5.1 Method I

2.5.1.1 Haffner's Tail Clip Method [14]

An artery clip was placed at the root of the tail to apply noxious (mechanical) stimulus. A quick response of the animal was seen as biting the clip or tail, where clip was placed. The reaction time between application of the clip and response was noted by stopwatch. The test drugs was given orally with the help of gavage tube (18 G) to the rats 30 min before starting the procedure and responses assessed at 0, 15, 30 and 60 min. Reaction time of test animals, greater than the cut off time denotes a positive response indicating analgesic effect of the drug.

2.5.2 Method II

2.5.2.1 Formalin Test [15]

This test is highly sensitive for centrally acting analgesics like opioids. Noxious (chemical) stimuli was given by injecting 2.5% formalin (0.05 ml.) subcutaneously into dorsal surface of hind paw of the animal. Animal showed biphasic nociceptive behaviour.

First (early/acute) Phase: Starts immediately after injection of formalin and lasts for 3 – 5 min. This phase is due to direct stimulation of nociceptors causing C – fibre activation.

Second (late/chronic) Phase: Starts after 10 – 15 min of injection and lasts for 20 – 40 min. This phase appears due to

combination of an inflammatory reaction in the peripheral tissues and functional changes in the dorsal horn of spinal cord.

Animal responds by elevation of paw, flinching or excessive licking and biting of the paw. The characteristic nociceptive behaviour was recorded in video camera to avoid missing of any response. The time spent in licking and biting was noted in both phases, first at 0 - 5 min (phase I) and second at 20 - 40 min (phase II). The test drugs was given orally with the help of gavage tube (18 G) to the rats 30 min before injecting formalin (0.05ml) and the procedure was repeated . If both paws of the animal allowed to rest on the floor with no obvious nociceptive behaviour of the injected paw, was considered as positive analgesic response of the test drugs.

2.6 Statistical Analysis

Data entry was done on Microsoft EXCEL 2007 and data analyzed by using standard statistical software, 'SPSS version 20'. All values were expressed in Mean ± SD. The data were analysed by one - way Analysis of Variance (ANOVA) test to determine the significant variance between the mean values of treatment groups. Turkey's honestly significant difference (HSD) test was used for *post hoc* analysis of significant overall differences. P=<0.05 was considered statistically significant.

3. Results

3.1 Effects of oral administration of tapentadol and amitriptyline alone and their combination (Tap.+ Ami.) in comparison to control group in Haffner's tail clip test

The Tapentadol treated (Gr. B) rats showed significantly more antinociceptive action, the reaction time increased from, 5.74±0.50, 11.44±0.74, 17.16±0.67, and 23.19±0.83 (P<0.05) in comparison to control group (Gr. A), 2.42±0.35, 3.04 ± 0.23, 3.30 ± 0.62 and 3.35 ±0.43(P<0.05) at the time interval of 0 min, 15 min, 30 min and 60 min., respectively.

The Amitrptyline treated (Gr. C) rats showed significantly more antinociceptive action, the reaction time increased by , 3.68±0.13, 6.39±0.42, 8.83 ± 0.36 and 13.55±0.93 (P<0.05) in comparison to control group (Gr. A) , 2.42±0.35, 3.04 ± 0.23, 3.30 ± 0.62 and 3.35 ±0.43 (P<0.05) at the time interval of 0 min, 15 min, 30 min and 60 min., respectively.

The combination (tapentadol + amitriptyline) treated (Gr. D) rats showed significantly more antinociceptive action, the reaction time increased by7.33±0.40,14.02±0.14, 22.79±0.13 and 27.86±1.02 (P<0.05) in comparison to control group (Gr. A), 2.42±0.35, 3.04 ± 0.23, 3.30 ± 0.62 and 3.35 ±0.43 (P<0.05) at the time interval of 0 min, 15 min, 30 min and 60 min., respectively.

3.2. Effects of oral administration of tapentadol and amitriptyline alone and their combination (Tap.+ Ami.) in comparison to control group in Formalin test

Formalin (2.5%) , of 0.05 ml. injected with the help of 26 G sized needle on the dorsal surface of the hind paw of rats and following were observed :

The duration of licking / biting behaviour of pain was significantly reduced, by 12.01±0.07 and 48.36±0.14,(P<0.05)in group B (tapentadol treated) rats in comparison to control group (Gr. A) Rats, by 74.08±0.18 and 134.5±0.17, (P<0.05) in phase I and phase II, respectively.

In group C (amitriptyline treated) rats, the duration of licking / biting behaviour of pain was significantly reduced, by 60.72±0.18 and 96.78±0.17, (P<0.05) in comparison to control group (Gr. A) rats, 74.08±0.18 and 134.5±0.17, (P<0.05) ,in phase I and phase II, respectively.

The duration of licking biting behaviour of pain was significantly reduced, by, 9.34±0.05 and 41.80±0.09, (P<0.05) in group D (tapentadol + amitriptyline treated) rats in comparison to control group (Gr. A) of rats, i.e., 74.08±0.18 and 134.5±0.17, (P<0.05) in phase I and phase II, respectively.

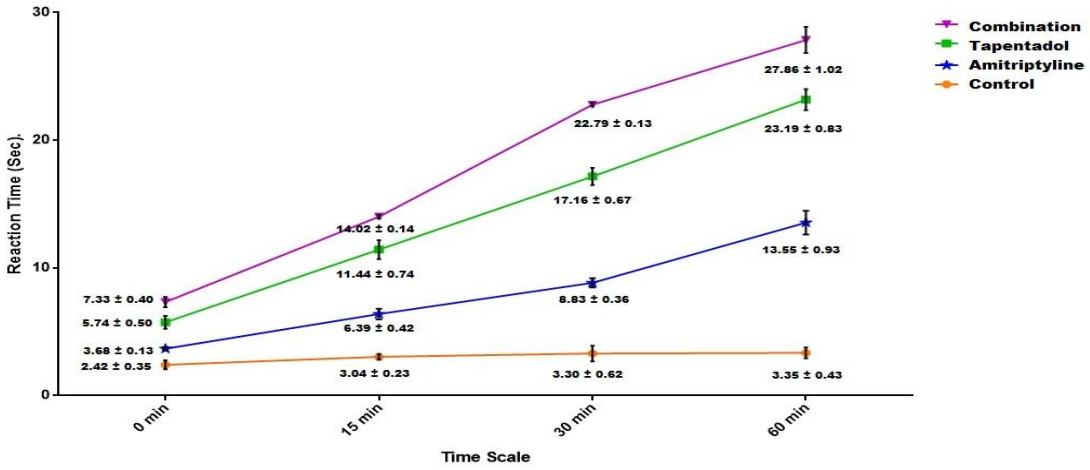

Figure 1: Comparison of Average Reaction Time (in sec.) of All groups by Tail Clip Method

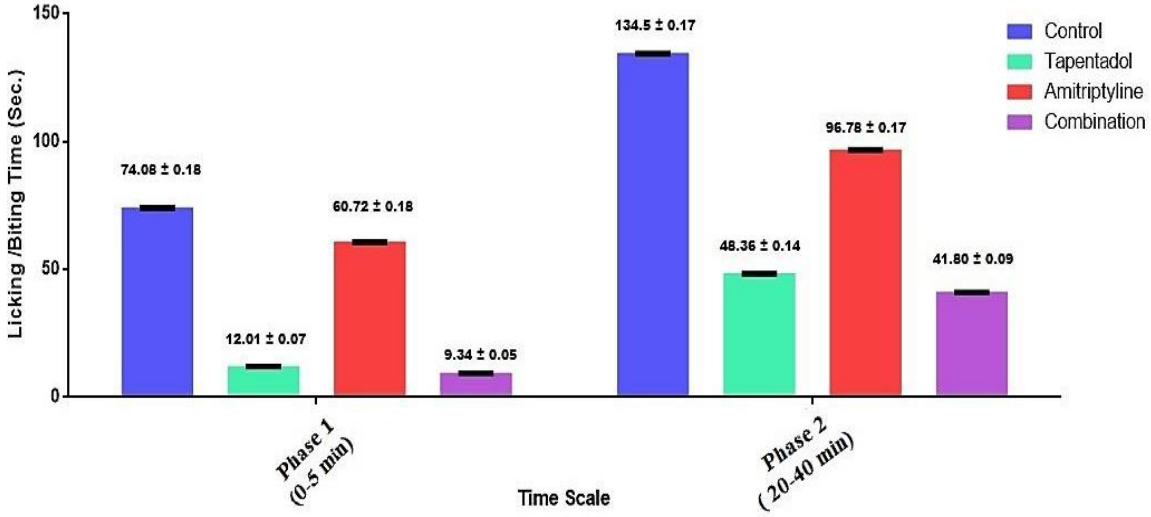

Figure 2: Comparison of Average Licking / Biting Time (in sec.) of Phase I and Phase II of Formalin Test among All Groups

4. Discussion

The current study showed that tapentadol and amitriptyline have significant antinociceptive effects in the experimental pain models. An enhanced antinociceptive effects was observed on combined administration of both the drugs in this study (Figure 1 & 2).

Tapentadol is the centrally acting opioid analgesic and possibly exerted its effect by dual analgesic effect of μ receptor activation and NE-reuptake inhibition . Tapentadol, acts in ascending pathways, responsible for pain perception and descending tracts that suppresses transmission of noxious stimuli. Analgesic effect of tapentadol has already been established in various animal models using thermal, mechanical [16] and chemical [17] stimuli.

In our study, administration of Amitriptyline exhibit its analgesic effect in tail – clip test as well as induces an inhibitory effect on the first phase and the second phase of the nociceptive response in the formalin test. Amitriptyline is mostly used for the treatment of persistent, chronic and neuropathic pain. But previous animal studies have been reported its action in acute pain conditions.[18]

It is believed that the modulation of endogenous pain mechanism through serotonin (5 – HT) and noradrenaline in descending inhibitory pathways is the major mechanism of antinociceptive activity of Amitriptyline. Amitriptyline has a large number of pharmacologic actions including blocking of adrenergic, cholinergic, and histaminergic receptors.[19] It has been shown that amitriptyline also has antagonistic effects on N – methyl – D - aspartate receptors, which are known to play an important role in inflammatory pain states.[20]

Tapentadol elicit antinociceptive effects by interacting with both peripheral and central opioid receptors as well as inhibitingnorepinephrinereuptake, which activate α2 receptors in the spinal cord and modulate transmission of noxious stimuli.[21] More recent studies have shown a local peripheral analgesic effect of amitriptyline that is mediated, by an interaction with endogenous adenosine.[22] Therefore, it is possible that combined action on peripheral and central sites may play important role in theanalgesic effect of systemic amitriptyline.

Philippe Luccarini, et al., 2004; performed similar type of study with morphine and amitriptyline. They also found that combined administration of morphine and amitriptyline produces synergistic action in comparison to single drugs.[23]

In summary**,** the present study shows that coadministration of tapentadol and amitriptyline have greater antinociceptive effects in the experimental pain models of rats. The enhancing antinociceptive effects maybe via central and peripheral mechanism, possibly due to pharmacodynamic interactions, in which concurrent activation of distinct systems may modulate a common pathway or one compound may enhance the affinity or coupling of the other.

5. Conclusion

Although wide range of analgesics are available for treating pain some time it become challenging to control pain with a single agent. Even the most efficacious opioid groups of analgesics are not devoid of serious dose related side effects. Combining analgesics of different pharmacological profile can produce greater pain inhibition and at the same time can reduce the side effects of individual drug. Therefore more elaborate studies are required to establish effective and

rational analgesic combination by exploring the pharmacokinetic and pharmacodynamic profiles of currently available analgesics.

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Conflict of interest

The authors declare no conflict of interest.

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