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EXPERIMENTAL EVALUATION OF ANALGESIC ACTIVITY OF FLUPIRTINE WITH OPIOIDS ON SWISS ALBINO MICE

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ABSTRACT

There is a need for new effective analgesic drugs with fewer side effects and minimum drug abuse liability. Flupirtine is a centrally acting, non-opioid analgesic agent with unique pharmacological properties. Materials and Methods: Tail flick (thermal method) and tail clip (physical method) were used as in-vivo model. Flupirtine (10 and 20 mg/kg i.p.) was administered as test drug in mice and compared with Pentazocine and tramadol (10 mg/kg i.p.) as standard drugs. The analgesic activity was studied by recording the reaction time after administration of the drug at frequent intervals up to 90 minutes. The results were analyzed by ANOVA and Bonferroni's test. P value < 0.05 was considered as significant. Results: Administration of flupirtine showed significant increase in reaction time as compared to control. Flupirtine inhibited the nociceptive responses induced by thermal and mechanical stimuli in rodents. Conclusion: Flupirtine has comparable analgesic activity with opioids in this experimental model of pain.

Keywords :- Analgesia, Flupirtine, Pentazocine, Tramadol.

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INTRODUCTION

Pain is not a uniform sensation, as illustrated by its many common descriptions, e.g. sharp, dull, aching, burning, shooting, cramping, stabbing and throbbing. Pain is a subjective sensation which cannot be measured objectively, and its intensity is not always a direct reflection of the nociceptive inputs provoking it. Nociceptive inputs which are easily ignored by one individual in one situation may be unbearable for other individual in similar situation. Analgesia is absence of pain in response to stimulation which would normally be painful. Analgesics are group of drugs used to relieve pain. Analgesic drugs act in various ways on peripheral and central nervous systems; they include the non-steroidal anti-inflammatory drugs (NSAIDs) and opioids. Over the last two decades research in this field has been focused largely on identifying safer NSAIDs resulting in introduction of COX-2 inhibitors and safer opioids. Pain has been a human problem since the beginning of time but the last decade has seen an explosion of information about the transmitters, receptors and channels involved in the transmission and modulation of noxious stimuli generated in peripheral tissues. This has led to the identification of a number of potential new targets for analgesic therapy.[1] Advances in understanding the pharmacology of pain and

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analgesia that have resulted from the application of techniques in molecular biology and the development of selective ligands for the various receptor classes involved in nociceptive transmission. Now it has been well established that pain is an extremely complex and dynamic process involving multiple, interrelated neurotransmitter /neuromodulator systems. There are numerous animal models available for clinical pain states such as inflammation and neuropathies, and the experiments using these models have shown that several transmitter systems having minor actions in acute pain can play important role in persistent pains.[2]

In the present study flupirtine, a centrally acting non-narcotic analgesic has been selected and its efficacy has been compared with two other potent analgesics, pentazocine and tramadol. Flupirtine is indirect NDMA receptor antagonist and is the first representative of a pharmacological class of selective neuronal potassium channel opener.[3] The study has been carried out on animal using albino mice. For analgesic study, tail flick (thermal method) and tail clip (physical method) were employed. The present study was undertaken to explore the possibility of use of flupirtine as a potent analgesic, when compared with pentazocine and tramadol.

AIM AND OBJECTIVES

Aim: To evaluate and compare the analgesic activity of Flupirtine with Opioids in mice.

Objectives

1. To evaluate the analgesic activity of Flupirtine.
2. To compare the analgesic activity of flupirtine with Pentazocine.
3. To compare the analgesic activity of flupirtine with Tramadol.

MATERIALS AND METHODS

Ethical Considerations

The study was commenced after Institutional Animal Ethics Committee (IAEC) approval was granted and was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

Study drugs and chemicals

The test drugs Flupirtine and the standard drugs Pentazocine and Tramadol were used. The drugs were purchased from local pharmacy and were manufactured by following manufacturers–

- Flupirtine by Lupin Pharmaceutical Ltd., Jammu,
- Pentazocine by Taj Pharmaceuticals Ltd (Taj Drug World), India.
- Tramadol by Abbott Healthcare Pvt. Ltd. Mumbai.

Experimental animals

Inclusion Criteria

- Albino mice of either sex weighing between 20-25 g.
- Age 3-4 months.
- Healthy with normal behavior and activity.

Exclusion Criteria

- Mice <20 g or >25 g and aged <3 months or >4 months.
- Pregnant mice or those who have recently delivered.
- Animals previously used in other experiments.

Animal housing: They were housed in polypropylene cages under standard laboratory conditions in a well ventilated room and fed standard pellet diet. They had free access to diet and water except at the time of experiment. They were placed in clean, neatly labeled cages, each containing 3 mice. The floor of the cages was stack with grain husk which were replaced every second day. The animals were inspected frequently to rule out infections. In each cage the animals were identified by appropriate markings.

Animal feed

Food: Animals were fed with commercially available 'Nutrimex Std-1020'. The nutrition provided by the pellet feed was as follows:

Energy: 3620 kcal/kg, Crude protein: 22.15%, Ash: 5.11%, Sand silica: 1.15%

Water: Drinking tap water supplied by local Municipal Corporation was provided to the mice through the feeding bottles with stainless steel nozzle in each cage.

Both food and water were replenished once daily in the morning, and were available to the mice ad libitum.

STUDY DESIGN

Study Groups: The mice were placed in 5 groups (G1-G5) containing 6 mice each. (30 mice in total)

- G1- were the vehicle control group received only Distilled water.
- G2 - were the standard control group received Pentazocine (10 mg/kg i.p.)
- G3- were the standard control group received Tramadol (10 mg/kg i.p.)
- G4- were the test group received test drug Flupirtine (10mg/kg i.p.)
- G5 - were the test group received test drug Flupirtine (20 mg/kg i.p.)

METHODS

Analgesic activity of Flupirtine (10 mg/kg, 20 mg/kg) was evaluated in graded dose and compared with Pentazocine and Tramadol by

1. Radiant heat method (Thermal method)[4]

The method of **Amour et al.,** as modified by Bass and Sander Brooke was used. In this method, pain

was induced by using radiant heat from the wire (heated electrically) in an apparatus called analgesiometer. The temperature of nichrome wire was kept constant at 55±0.5°C. A cut off time of 15 seconds is set to prevent the injury to the animal. For the assessment of tail flick response mice were kept in cylindrical holder in such way that its tail came out through the cut hole in the shutter at the rear end of the holder. The animals were kept in holders and the tail was kept on the platform provided. A constant current were passed to heat the wire and the time for tail flick response were noted. For this study mice were selected, who show tail flick response within 6 seconds. The animals which have shown readings more than 6 seconds were discarded. Drugs were administered half an hour before the actual test. The test drugs were given IP to the animals immediately after basal readings. The animals were subjected to tail flick apparatus 30 minutes after administration of drugs. Further readings were recorded at 60 minutes and 90 minutes.

2. Tail clip method (physical method) [4]

The method of **Bianchi et al.,** was used .Pain was produced by mechanical pressure on the tail by artery clip, the tips of which was covered with rubber tubing. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. It is applied 2 cm away from the base of tail .The mice making an attempt to remove the clip within 10 seconds was selected . The animal quickly responds to these noxious stimuli by biting the clip or the tail near the location of the clip. The reaction time of each mouse was then determined 30 min post-treatment for administration. A post-treatment cut-off time of 30 second was used.

Statistical analysis

Results are presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Bonferroni's test post hoc test for comparison between groups. For all the test s "p" value of 0.05 or less was considered for statistical significance.

RESULTS

Table 1. Effect of different drugs on tail flick response in mice

Parameter	Control (n=6) M ± SEM (second)	Pentazocine 10mg/kg (n=6) M ± SEM (second)	Tramadol 10mg/kg (n=6) M ± SEM (second)	Flupirtine at 10mg/kg (n=6) M ± SEM (second)	Flupirtine at 20mg/kg (n=6) M ±SEM (second)
Pre-treatment	4.75±.527	5.15±.318	5.20±.529	5.30±.438	5.17±..482
At 30min.	5.13±.263	6.15±.319	8.25±.481	7.23±.535	8.67±..335
At 60min.	5.33±.443	8.17±.401	9.78±.581	11.07±.605*	12.10±.510*#
At 90min.	4.99±.528	7.58±.212	9.30±.606	11.80±.523*#	11.92±.436*#

Data were presented as mean ± SEM. Analysis was done using one-way ANOVA followed by post hoc Bonferroni's test. The * depicts comparison with pentazocine, # depicts comparison with tramadol, * P<0.05, # P<0.05.

Table 2. Effect of different drugs on tail clip-induced pain in mice

Parameter	Control (n=6) M ± SEM (second)	Pentazocine 10mg/kg (n=6) M ± SEM (second)	Tramadol10mg/kg (n=6) M ± SEM (second)	Flupirtine at 10mg/kg (n=6) M ± SEM (second)	Flupirtine at 20mg/kg (n=6) M ± SEM (second)
Pre-treatment	4.23±.459	4.22±.533	4.47±.468	3.98±.601	4.11±.317
Post-treatment	4.31±.433	8.10±.562	9.33±.662	10.10±.535	12.55±.401*#

Data were presented as mean ± SEM. Analysis was done using one-way ANOVA followed by post hoc Bonferroni's test. The * depicts comparison with pentazocine, # depicts comparison with tramadol. * P<0.05, # P<0.05.

DISCUSSION

Radiant heat method (Thermal method)

Radiant heat method is known to evaluate centrally acting analgesics. In standard group with pentazocine, the mean reaction time increased from 5.15±.318 to 6.15±.319, 8.17±.401 and 7.58±.212 at 30, 60 and 90 second respectively. In standard group with tramadol, the mean reaction time increased from 5.20±.529 to 8.25±.481, 9.78±.581 and 9.30±.606 at 30, 60 and 90 second respectively. The mean reaction time also increased in test groups treated with flupirtine (10 mg/kg), maximum at 90 minute, from 5.30±.438 to 11.80±.523. The maximum reaction time was 11.92±.436 seen with 20 mg/kg at 90 minute after post administration within test group.

As per statistical analysis, the mice treated with both flupirtine 10 mg/kg and 20 mg/kg have shown a significant (p< 0.05) increase in the reaction time compared to the control groups. The mice treated with flupirtine 10 mg/kg have shown a significant (p < 0.05)

increase in the reaction time compared to pentazocine and tramadol at 90 minute. The mice treated with flupirtine 20 mg/kg have shown a significant ($p < 0.05$) increase in the reaction time compared to pentazocine and tramadol at 60 & 90 minute.

Tail clip method

In standard group with pentazocine and tramadol, the mean reaction time increases, from 4.22±.533 to 8.10±.562 and 4.47±.468 to 9.33±.662 respectively. The mean reaction time also increased in test groups treated with flupirtine (10 mg/kg), from 3.98±.601 to 10.10±.535. The maximum reaction time was 12.55±.401 seen with 20 mg/kg after post administration within test group. As per statistical analysis, the mice treated with flupirtine 20 mg/kg have shown a significant ($p< 0.05$) increase in the reaction time compared to the control groups.

Nickel B. *et al*. compared the analgesic activity of flupirtine with pentazocine, morphine and codeine. The maximal antinociceptive activity of flupirtine was observed 30 min after dosing and analgesia lasted for at least 75 min.[5,6] In a previous study done by Kolosov *et al*. prostate cancer cells were injected into the right tibia of male Wistar rats, leading to development of hyperalgesia to noxious heat. Hyperalgesia was assessed by measurement of paw flick latency (PFL) to application of radiant heat. Both morphine (ED50 = 0.74 mg/kg) & flupirtine (ED50 = 3.32 mg/kg) caused dose-related anti-hyperalgesia. There was a synergistic interaction between flupirtine and morphine, as suggested by significant decreases in ED50 of both morphine (0.74 to 0.08 mg/kg) and flupirtine (3.32 to 0.31 mg/kg). [7] In another study done by Goodchild CS *et al*. (2008) complete reversal of carrageenan-induced hyperalgesia was caused by 10 mg/kg flupirtine (i.p.) in combination with 0.4 mg/kg morphine (i.p.).[8]

CONCLUSION

The results obtained during this study suggest antinociceptive activity of flupirtine as a novel analgesic agent. Its atypical mechanism of analgesic action suggests that it might be effective against pain where traditional analgesics may not be effective.

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