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Original Article

Pharmacology Section

Comparison of the Effects of Zonisamide and Flupirtine on Paclitaxel Induced Peripheral Neuropathy in Rats

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ABSTRACT

Introduction: Many peripheral and central causes lead to neuropathic pain disorders. Effective treatment of neuropathic pain is not completely manageable and hence, it becomes necessary to evaluate the application of Zonisamide as a sodium channel and T-type calcium (Ca^{+}) current inhibitors and Flupirtine by activation of potassium (K) channel through N-methyl-D-aspartate (NMDA) receptor inhibition in the treatment of neuropathic pain.

Aim: To analyze the analgesic effect of Zonisamide and Flupirtine in paclitaxel induced neuropathic pain model in rats by hot plate and cold allodynia test.

Materials and Methods: Total of six groups of animals, each with six rats were given with single intraperitoneal (i.p.) injection of 1 mg/kg of paclitaxel on four alternate days (day 0, 2, 4, and 6). Drugs Zonisamide (50 mg/kg daily for group 2, 100 mg/kg daily for group 3, Flupirtine (10 mg/kg, daily for group 4, 20 mg/kg daily for group 5) and Gabapentin daily for group 6 were administered in the dosages per group from days 0 to 7. Nociceptive tests were done for all animals on days 0, 7, 14, 21, 28 to assess the pain threshold. Student's t-test was used to analyze the statistical significance.

Results: In our study, on the 21st day of testing we observed that 100 mg/kg dosage of Zonisamide group has shown a significant increase in reaction time suggesting analgesic effect. Prominent increase in the reaction time was also observed that on day 14 of testing, both the Gabapentin and Flupirtine groups showed an earlier analgesic effects when compared with the Zonisamide group.

Conclusion: Zonisamide and Flupirtine showed anti-nociceptive activity in the Paclitaxel model of peripheral neuropathy compared with the standard treatment of Gabapentin.

Keywords: Analgesic action, Effect of ion channels, Neuropathic pain, Reaction time

INTRODUCTION

Neuropathic pain is a disabling condition affecting about 7-8% of the population [1]. The Health Related quality of life for individuals with neuropathic pain is rated low compared to those with coronary artery disease, diabetes mellitus and recent myocardial infarction [2]. Opioid analgesics have a role in the treatment of neuropathic pain, but they have been questioned in regard to their safety profile. This is especially important in treatment of the elderly who have an increased prevalence of neuropathic pain [3,4]. In a country like India, with an estimated 62 million people living with diabetes mellitus, treatment of neuropathic pain effectively is important [5]. The first line treatment for neuropathic pain includes Gabapentin, Anticonvulsants, Topical Lidocaine and Capsaicin - they have achieved a total treatment satisfaction of only 50% according to one study done in diabetic patients [6]. Thus, it becomes necessary to evaluate the application of other drugs in the treatment of neuropathic pain.

Flupirtine is a centrally acting, non-opioid, NMDA receptor antagonist that causes analgesic effect by opening Kv7 potassium channels. It is effective in alleviating several types of pain especially chronic musculoskeletal pain and post-surgical pain [7]. It has been demonstrated that Flupirtine has significant analgesic property in several animal pain models including in diabetic neuropathic pain model, carrageenan-induced hyperalgesia and ciguatoxin induced pain in rats [8,9].

Zonisamide is an antiepileptic drug that has been tried previously for the treatment of chronic pain with inconclusive results [10]. It has been evaluated in the Streptozotocin induced Peripheral Neuropathy model previously and has shown to have an effect in reducing the pain induced [11]. In the published literature, it was found that Flupirtine provided significant pain relief in patients with small fiber neuropathy within a week when the other first line drugs like gabapentin and pregabalin have failed. Furthermore, studies have shown it to be useful in neuropathic pain only in conjunction with opioids and there isn't any literature evidence with head-head comparison of the drug with the currently available first line drugs [12]. In a recent review in Cochrane library on Zonisamide in neuropathic pain, there was only one study done on 25 patients with painful diabetic neuropathy (13 in Zonisamide vs 12 in placebo) which showed that three out of 11 responders had ≥50% reduction in pain intensity which may be due to one of its diverse mechanisms of action. There was no study directly comparing the effect the drug with that of the first line drugs [13].

Since, the data available regarding the modulation of pain in the Paclitaxel-induced neuropathic pain in rat model, by Zonisamide and Flupirtine is insufficient this study evaluated Zonisamide and Flupirtine for their pain relieving effects in the Paclitaxel Model of Peripheral Neuropathy in rats.

MATERIALS AND METHODS

This experimental study was carried out after obtaining ethical clearance from the Institutional Animal Ethics Committee of PSG Institute of Medical Sciences and Research, Coimbatore, India during May 2014 to July 2014.

All the animals were procured from the Animal House at the Institution. All the groups were housed in different cages with the male and female rats separated. They were allowed to acclimatize to the ambient temperature, following with they were tamed before each injection to facilitate easy handling.

Paclitaxel Model of Peripheral Neuropathy in Rats

Paclitaxel induced peripheral neuropathy in rats has been evaluated as

a robust model for studying the antinociceptive effects of various drugs [14, 15]. This model involves administration of single intraperitoneal (i.p.) injection of 1 mg/kg of paclitaxel on four alternate days (0, 2, 4, and 6). The volume of injection is kept constant at 1 mL/kg. This model typically presents sensory neuropathy on the 14th day.

Test Groups

This consisted of six groups of animals, each with 6 wistar rats (weight 200-250 g). Three male and three female in each group.

Group-1: Paclitaxel alone

Group-2: Paclitaxel + Zonisamide 50 mg/kg

Group-3: Paclitaxel + Zonisamide 100 mg/kg

Group-4: Paclitaxel + Flupirtine 10 mg/kg

Group-5: Paclitaxel + Flupirtine 20 mg/kg

Group-6: Paclitaxel + Gabapentin

Nociceptive tests were done for all animals on days 0, 7, 14, 21, 28 to assess the pain threshold.

The dosages of drugs were chosen based on the previous published literature in similar rat models [8,9]. In these studies the dosages considered included 50 mg/kg, 100 mg/kg, and 150 mg/kg of orally administered Zonisamide. These were found to be the optimum dosages in which there was a significant analgesic effect obtained.

Drugs Zonisamide, Flupirtine and Gabapentin were administered everyday orally in the dosages as per the group from days 0 to 7.

Outcome Parameters

Hot plate test: The hot plate test involves placing the rat in a transparent glass cylinder on a preheated plate at 55°C. After 60 minutes of corresponding test drugs administration, per group rats were placed on hot plate to measure the latency period with cutoff time 60 seconds was allowed to each animal to prevent injury. The time duration of latency is the time interval between placing the rat on the hot plate and the time for the rat to perform two behavioural components, namely, paw licking and jumping. The average latency time period in different groups was 17-21 seconds [Table/Fig-1]. Both of these responses are supra-spinal integrated responses [16,17].

Cold allodynia test: This was performed by immersing the tip of the tail in cold water at four degrees. The time taken for a withdrawal reflex is the time of latency considered. A cut of latency of 20 seconds was kept to avoid any injury [18,19]. Both the tests were done for all the animals on days 0, 7, 14, 21, and 28.

STATISTICAL ANALYSIS

Data obtained from the study were entered in SPSS software version 19.0. Student's t-test was done to analyze the statistical significance.

RESULTS

Paclitaxel-induced neuropathy was seen on day seven by Hot plate test and on day 14 by cold allodynia test. Decrease in latency was observed in hot plate and cold allodynia tests and it was persisted till 28 days in the control group. No other abnormal behavioural response was seen.

Hot Plate Test

Latency period was significantly delayed for the paclitaxel group on Day 7 (p=0.01) and Day 14 (p=0.01) whereas Gabapentin treated group, both Flupirtine treated groups 10 mg/kg (p=0.02) showed significant delay in latency time on day 14. However, Zonisamide showed significant delay (p=0.02) in latency time only for the dose 100 mg/kg on the day 21 compared to Gabapentin and control groups [Table/Fig-1].

Cold Allodynia Test

The latency time for a reaction is reduced in the paclitaxel group significantly from day 14 (p=0.01 and still reduction seen on day 28 (p=0.02) when compared to Day 0. Also, there was a significant delay in reaction time in the 100 mg/kg Zonisamide group (p=0.02) on Day 14, Gabapentin group (p=0.01) and both the 10 mg/kg (p=0.01), 20 mg/kg (p=0.01) of Flupirtine groups and showed delay in latency time for a reaction in on day 14 [Table/Fig-1].

Day	Paclitaxel alone (Control) group			
	Cold allodynia test		Hot plate Test	
	Avg. Time (sec)	p-value	Avg. Time (sec)	p-value
Day 28	25.67	0.02*	10.74	0.01*
Day 21	26.59	0.02*	11.04	0.01*
Day 14	23.35	0.01*	11.44	0.01 *
Day 7	31.33	0.12*	13.97	0.01*
Day 0	36.76	NA	18.92	NA
	Paclitaxel + Zonisamide 50 mg/Kg			
Day 28	31.16	0.16#	13.27	0.16#
Day 21	31.59	0.03#	11.75	0.46#
Day 14	32.46	0.01#	13.96	0.13#
Day 7	34.53	0.31#	12.53	0.24#
Day 0	38.50	NA	21.63	NA
	Paclitaxel + Zonisamide 100 mg/Kg			
Day 28	36.92	0.02#	19.96	0.01#
Day 21	37.32	0.02#	18.84	0.02#
Day 14	31.40	0.02#	16.69	0.03#
Day 7	30.32	0.50#	12.11	0.19#
Day 0	38.77	NA	18.60	NA
	Paclitaxel + Flupirtine 10mg/kg			
Day 28	34.13	0.02#	18.83	0.01#
Day 21	36.71	0.03#	19.68	0.01#
Day 14	37.53	0.01#	19.12	0.02#
Day 7	36.37	0.16#	13.04	0.47#
Day 0	37.26	NA	19.88	NA
	Paclitaxel + Flupirtine 20mg/kg			
Day 28	38.05	0.02#	19.72	0.01#
Day 21	39.84	0.01#	21.74	0.01#
Day 14	36.63	0.01#	20.10	0.01#
Day 7	36.82	0.10#	13.27	0.47#
Day 0	41.78	NA	17.86	NA
	Paclitaxel + Gabapentin			
Day 28	31.05	0.07#	15.88	0.01#
Day 21	32.23	0.05#	16.73	0.01#
Day 14	32.91	0.01#	16.72	0.01#
Day 7	34.15	0.30#	13.99	0.98#
Day 0	37.15	NA	18.27	NA

[Table/Fig-1]: p-value of both Cold allodynia test and Hot plate test.
*p-value compared to Day 0, (NA-Not applicable), #p-value (0.01) compared to paclitaxel group(significant p value p≤0.05).

DISCUSSION

The Paclitaxel induced peripheral neuropathy model in rats has shown to be an established indicator which mimics neuropathic pain in humans. It has been used to evaluate the neuropathic effect of other anticonvulsants [5].

The results show that neuropathic pain was induced by the 14th day from the starting of Paclitaxel in group 1, identified by the decrease in latency time. This is in keeping with the Paclitaxel model of

peripheral neuropathy. On examining the difference in groups, we find that the Gabapentin group showed delayed latency period from day 14, i.e., less neuropathic pain. This cements the proven potent analgesic effect of Gabapentin [10].

In our study, on the 21st day of testing we observed that the group with the 100 mg/kg dosage of Zonisamide has shown a significant increase in reaction time suggesting analgesic effect.

Similar prominent increase in the reaction time was observed for the Gabapentin and both the Flupirtine groups earlier, on day 14 of testing, showing that Gabapentin and Flupirtine act producing earlier analgesic effects when compared to the Zonisamide group. Thus, in our study both Zonisamide and Flupirtine produce analgesic effects comparable to the established Gabapentin.

Zonisamide, an anticonvulsant, has different mechanism of action, one of which includes inhibition of the T-type Calcium channels [20,21]. These channels have been studied and they have been implicated in the modulation of chronic pain [22]. They are emerging targets for treatment of neuropathic pain [23]. Lercanidipine, an antihypertensive, also acts via modulation of the T-type Ca^{++} channels in the body. This drug has been shown to be effective in treating neuropathy in this Paclitaxel model [19]. Previous studies have shown that Zonisamide has a potential ability to treat neuropathic pain [24]. There is literature on evaluation of the analgesic property of Zonisamide on chronic pain models [25]. On the other hand, a recently published Cochrane analysis has shown that Zonisamide has not been efficacious enough to advocate routine use in the treatment of peripheral neuropathy [10].

Flupirtine is a K(V) seven channel activator with GABA-A receptor modulating action [26]. It has central nervous system activity causing analgesic effects [27]. Flupirtine has not undergone extensive clinical trials but there is evidence of analgesic activity as shown in previously evaluated animal pain models [28]. One study has demonstrated its analgesic activity on prostate bone metastasis in rats [29]. Flupirtine has also been studied previously in combination with an Opioid in Streptozotocin Induced Peripheral neuropathy model in rats showing analgesic properties [9]. There had been no published reports of the analgesic effects being evaluated in the Paclitaxel model.

Gabapentin has been evaluated extensively in the treatment of peripheral neuropathy and has been placed as one of the first line drugs used in the treatment of peripheral neuropathy [30].

Zonisamide and Flupirtine showing anti-nociceptive activity in the Paclitaxel model of peripheral neuropathy, evaluated alongside a treatment standard of Gabapentin, provide robust evidence of their analgesic activity.

Gabapentin is used as a first line drug in patients with neuropathic pain. Since, many of these patients have comorbidities it becomes vital to choose a drug that has minimal adverse reactions [31]. Gabapentin is associated with adverse reactions such as sedation, nausea and vomiting, which makes it cumbersome to use in debilitated patients [32]. The other drugs which have been used in neuropathic pain including opioids and sedatives have significant adverse effects [33].

Flupirtine has a unique mechanism of action and studies have shown that it causes a anti-nociceptive action without producing sedation [9]. Flupirtine has also shown to have neuroprotective and muscle relaxing properties [34,35]. Further since Zonisamide and Flupirtine are non-opioid drugs and tolerance does not develop in most cases they have minimal abuse potential [36,37]. Flupirtine has also shown shown to be effective in refractory neuropathic pain [38].

LIMITATION

In this study only two methods (cold allodynia, hot plate) of evaluation were used. A nerve conduction study would have given better results.

Further only two doses of Zonisamide have been tested. Other dosages to evaluate the most effective dosage have to be evaluated.

CONCLUSION

Zonisamide and Flupirtine, thus can be used as adjunctive drugs and reduce the dose related side effects of conventional therapy. They also work by a different, novel mechanism of action when used for the treatment of neuropathic pain, making them a potential choice for complementary therapy.

Thus, further studies evaluating the antinociceptive property of Zonisamide and Flupirtine in humans may further their usage in pain pathways.

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