Antihyperalgesic and allodynic effect of granisetron-A P1 receptor agonist in vincristine induced neuropathy in mice

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ABSTRACT

Objective: Granisetron, GRA an adenosine receptor agonist with fewer side effects, could be of interest to reduce neuropathic pain following antineoplastic drug treatment. In the present study, we demonstrated that Granisetron inhibits hyperalgesia and allodynia in a new mouse model of neuropathy induced by Vincristine, and exerts its effect preferentially via supraspinal and spinal mechanisms. Method: Mice pretreated with Vincristine sulphate (100 µg/kg, i.v.) showed induction of neuropathic pain was administered with Granisetron (1 and 10mg/kg. i.p.) for five days. Neuropathic pain symptoms like hyperalgesia and allodynia (mechanical) were evaluated in all the group of animals and reduction in neuropathic pain in drug treated group was compared on 5th day with vehicle treated control group in order to confirm the effectiveness of drug against the symptoms. Key findings: P1 receptor agonist Granisetron at the dose level of 1 and 10mg/kg.i.p. was found to be significantly effective (P<0.05) against neuropathic pain. Conclusion: These results suggest that P1 receptor agonist Granisetron at the dose range used may provide a better insight in the development of the newer drug for the treatment of neuropathic pain.

Key words: Neuropathy, Adenosine, Allodynia, Granisetron GRA, Vincristine sulphate

INTRODUCTION

Chemotherapy-induced neuropathy are very uncomfortable symptoms for patients with cancer, which can be circumvented in most of them with drug combinations containing tricyclic antidepressants (TCAs) together with antiepileptic drugs such as amitriptyline and gabapentin. [1, 2]. In our country, antinociception medications may be responsible for a sizable fraction of the total costs related to chemotherapy [3]. To bridge animal and clinical pain research, animal experimental pain models are used with the intention of mimicking clinical pain conditions. These models may also provide predictability of the clinical efficacy in future clinical trials.

Adenosine and ATP exert multiple influences on pain transmission at peripheral and spinal sites; the last decade has seen the development of a particular interest in the role of purines in nociception. A number of reasons account for this focus and development of P receptor targets for conditions where ATP contributes to the pathophysiology of pain. To test this, we investigated whether intraperitoneal injection of GRA influences pain and hyperalgesia and allodynia in mice with chemotherapy induced neuropathy.

EXPERIMENTAL

Animals

The inbred male Swiss albino mice weighing 25-30g were procured from the animal housing facility, Vel’s University, Pallavaram, Chennai were maintained at 24±1°C and 55 ± 5% humidity with a 12-h light–dark cycle and divided into four groups consisting ten animals each were housed and had food and water ad libitum. All animals were habituated to the room and to the tests at least 1 week before starting the experiments. Animals used in this study were approved by the Institutional Animal Ethics Committee (Ref. No. 290/CPCEA/2009-PH/PCOL-02.)

Dose and Drug administration

Vincristine Sulfate (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline (NaCl 0.9% in distilled water) and stored at 4°C at a stock concentration of 1mg/ml and a single intra venous dose was administered to mice at the doses of 100µg/kg for induction of neuropathic pain [9] in all the group of animals and the control group received physiological saline (2ml/kg, i.p.). Pregabalin (Kenny Pharma Pvt. Ltd., Ankleshwar Gujarat, India) was dissolved in saline and stored at a stock concentration of 6mg/ml and was administered in i.p 30 min prior to start of testing. Granisetron, GRA (Granforce 1, Discovery Mankind, Okhla ind. Estate, New Delhi) was dissolved in saline and refrigerated at a stock concentration of 0.1 mg/ml and 0.3 mg/ml

Mechanical hyperalgesia[10].

Mechanical hyperalgesia was tested by using the pin prick test. Animals were placed on the elevated grid; a pin prick test was performed using a safety pin. The lateral plantar surface of the right hind paw was briefly stimulated at intensity sufficient to indent but not penetrate the skin. The duration of paw withdrawal was recorded, with an arbitrary minimal time of 0.5 (sec) and a maximal cut off 15 (sec).

Mechanical Allodynia (Touch Evoked Tactile Allodynia) [11].

Tactile allodynia was assessed by lightly stroking the injured leg with a paintbrush. Allodynia response was ranked as 0-No response; 1-Mild squeaking with effects depending on particular receptor subtypes activated and on the localization of the receptor [4].

Granisetron (GRA) is a highly selective 5-HT3 receptor antagonist used for treatment of emesis induced by chemotherapy [5]. It has been found to have a blocking effect on the 5-HT, receptor that is equivalent to, or better than that of ondansetron [6]. The side effects reported are headache and constipation [7]. In a recent study, it was reported that injection of GRA into the masseter muscle of healthy subjects reduced pain and abolished allodynia/hyperalgesia induced by injected 5-HT [8].
Locomotor Activity [12].

The locomotor activity can be easily measured using actophotometer which operates on photoelectric cells which are connected in a circuit with a counter. When a beam of light falling on photocell is cut off by the animal, a count is recorded. Mice were individually placed in the activity cage for 10 mins and the scores were recorded.

Data analysis

All results were expressed as mean ± SEM. The statistical significance of differences between groups was obtained by means of student paired t test, one way analysis of variance (ANOVA) followed by dunnet’s t test. P < 0.05 was considered statistically significant.

RESULTS

Development of signs of neuropathic pain

All the Vincristine treated animals developed qualitative signs indicative of neuropathic pain. This was clearly present on the 5th day after administration of vincristine at a dose of 100 µg/kg, i.v. Table 1, showed Vincristine treated group paw withdrawal latency was reduced on 5th day in comparison to basal reading of the same animals. The paw withdrawal latency of the animal was decreased and allodynia score on 5th day was significantly increased in comparison to baseline measurements indicating the development of hyperalgesia and allodynia.

Table 1: Development of hyperalgesia and allodynia and suppressive effect of Granisetron on the pain behavior

<table>
<thead>
<tr>
<th>S No</th>
<th>Parameters</th>
<th>Vehicle (10 ml/Kg)</th>
<th>Granisetron 1 mg/Kg</th>
<th>Granisetron 10 mg/Kg</th>
<th>Pregabalin (80 mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paw withdrawal latency</td>
<td>14.3±0.33</td>
<td>4.0±0.36</td>
<td>9.2±0.42</td>
<td>13.2±0.28</td>
</tr>
<tr>
<td>2</td>
<td>Allodynia Score</td>
<td>0.0±0.03</td>
<td>3.6±0.15</td>
<td>2.2±0.14</td>
<td>1.02±0.20</td>
</tr>
<tr>
<td>3</td>
<td>Fall of time</td>
<td>343.2±24</td>
<td>285.4±22</td>
<td>300.4±34</td>
<td>320.2±24</td>
</tr>
<tr>
<td>4</td>
<td>Count</td>
<td>310.8±8.2</td>
<td>255.2±16</td>
<td>270.8±16</td>
<td>356.6±3.0</td>
</tr>
</tbody>
</table>

Statistical significance test was done by paired t test and the values are mean± SEM of 10 animals per group. Comparison was made between baseline Vs 5th day of the all groups. ***P<0.001, **P<0.01, *P<0.05

Effect of GRA on Mechanical Hyperalgesia

In the vehicle treated animals, the response duration decreased at 5th day in comparison to baseline measurements (Table 1) from 14.3±0.33 to 4.0±0.36 on the 5th day. Whereas the other two groups treated with GRA at a dose of 1mg/kg and 10 mg/kg from 14.3±0.33 to 7.2±0.42 and 9.2±0.28 the response duration was significantly increased at both the dose levels. Thus administration of GRA at both dose levels shows the increased the paw withdrawal latency in comparison to the vehicle treated group (figure 1). The results are dose dependent and comparable with that of standard drug Pregabalin (P<0.001)

Effect of GRA on Mechanical allodynia

The baseline paw withdrawal frequencies (0.5 (sec) determined by mechanical stimulation with paint brush was enhanced at 5th day in vehicle treated mice compared to baseline responses of the same animals. Granisetron treated group at a dose level of 1mg/kg and 10mg/kg decreased the allodynia score from the value of 3.16±0.16 to 2.50±0.14 for 1mg/kg and 2.20±0.18 for 10mg/kg. The values are statistically significant and the results were comparable with that of standard drug Pregabalin 80 mg/kg, i.p (figure 2) (P<0.01)

Effect of GRA on motor coordination

The basal fall off time and the locomotion of animal was observed and it was found to be considerably decreased on the 5th day after administration vincristine this shows the indicative value for the development of motor impairment. Then followed by the administration of granisetron at both the doses found to be corrective in the curation of motor activity of the animal. For rotated motor coordination (P<0.01) figure 3. Locomotor activity using actophotometer (P<0.01) figure 4.

Effect of GRA on motor coordination in chemotherapy induced neuropathic pain model

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Figure 1: Effect of Granisetron on mechanical allodynia in chemotherapy induced neuropathic pain model

![Figure 1: Effect of Granisetron on mechanical allodynia in chemotherapy induced neuropathic pain model](image1.png)

Figure 2: Effect of Granisetron on mechanical allodynia in chemotherapy induced neuropathic pain model

![Figure 2: Effect of Granisetron on mechanical allodynia in chemotherapy induced neuropathic pain model](image2.png)

Figure 3: Effect of Granisetron on motor coordination in chemotherapy induced neuropathic pain model

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Figure 4: Effect of Granisetron on locomotion in chemotherapy induced neuropathic pain model

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DISCUSSION

Rodent models of neuropathy have been developed for three of the most widely used and most neurotoxic cancer drugs – Cisplatin, Paclitaxel and Vincristine. Models using any of these three drugs produce a quantifiable neuropathy. These models have all been used to determine the efficacy of neuroprotective agents [13] and to study the mechanisms of sensory dysfunction that underlie neuropathy [14]. However, the Vincristine and Paclitaxel models have several advantages over Cisplatin models. General health effects are an issue for Cisplatin models. Low cumulative doses of either Paclitaxel or Vincristine produce quantifiable changes in sensory thresholds [15].

The mice chemotherapy-induced neuropathic pain model utilized in the present study is one of many, diverse animal models that have been used to investigate the pharmacological attenuation of neuropathic pain [16]. No drug class is considered to be both a ‘safe and effective analgesic’ in the treatment of chemotherapy-induced pain.

Purines can exert complex effects on pain transmission, with prominent actions at both peripheral and spinal sites in preclinical models. The nature of the modulation of pain signalling depends very much upon the receptor subtype activated. In the periphery, adenosine A1 receptor activation produces pain suppression, while adenosine A2 receptors in such actions, but no data on adenosine A2 involvement. On the basis of these effects, adenosine A1 receptors show a significant potential for therapeutic development. At both peripheral and spinal sites, the manipulation of endogenous adenosine levels by inhibition of adenosine kinase can produce antinociception by activating adenosine A1 receptor mechanisms. These actions, combined with the peripheral anti-inflammatory effects mediated by adenosine A2 receptor activation, may make these agents particularly useful in inflammatory pain. There is an enhanced production of adenosine. There is also a particular interest in the efficacy of adenosine in neuropathic pain, as adenosine and its analogs appear to have unique properties in case studies and limited trials. Attention to the multiple effects of adenosine on the regulation of pain may well yield novel therapies for pain control.

The main finding of this experimental study was that intraperitoneal administration of granisetron at 1 mg and 10mg/kg for 5 days attenuates hyperalgesia and allodynia induced by chemotherapy induced neuropathy pain. In addition granisetron increases the pain threshold in animals.

Effects on evoked pain

Vincristine at the single intravenous dose of 100µg/kg has been used extensively to induce evoked pain: hyperalgesia and allodynia, since the quality of pain mimic the acute clinical peripheral pain caused during chemotherapy. In this study, single intravenous dose of vincristine induced painful peripheral neuropathy.

Vincristine administration resulted in the development of mechanical hyperalgesia as reflected by decrease in the duration of paw withdrawal response when compared with the basal reading in actophotometer. Mice treated with higher dose of GRA at 10mg/kg also showed a reduction in neuropathic pain as shown in figure no 1. This suggests the potential activity of GRA in reducing neuropathic pain. Mice treated with Pregabalin showed a significant reduction in mechanical hyperalgesia.

Vincristine administration is associated with reduction in the locomotor as reflected by decrease in the number of counts when compared with the basal reading in actophotometer. Measurements were done on the 5th day of measurement at both the doses. Mice treated with Pregabalin also showed significant increase in motor coordination.

Vincristine administration is associated with reduction in the locomotor as reflected by decrease in the number of counts when compared with the basal reading in actophotometer. Measurements were done on the 5th day of measurement. GRA showed a significant increase in fall off time mice treated with Pregabalin which is a standard drug for neuropathic pain also showed significant increase in motor coordination on the 5th day of measurement.

In parallel with the development of adenosine-based pharmaceuticals in this context must be the recognition of the role of endogenous adenosine in other therapeutic modalities and the potential need to control dietary caffeine intake in order to achieve optimal benefit from these regimens.

CONCLUSION

In summary, the mice vincristine model of chemotherapy-induced neuropathic pain, originally developed [17], and found to be a reliable tool for the study of mechanical allodynia. Interestingly, mechanical hyperalgesia and mechanical allodynia was significantly suppressed with Granisetron. Overall, the mice Vincristine model of chemotherapy-induced pain appears to be predictive of efficacy in clinical cases of neuropathic pain. However, the positive analgesic effect of Granisetron was surprising given that this common, over-the-counter drug has rarely been reported as having been tested for inflammatory pain in humans. This effect of Granisetron on neuropathic pain can be a better insight for the treatment of newer drug therapy for neuropathic pain.

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REFERENCE