



## The Potential Role of Transient Receptor Potential Vanilloid 1 Receptor in Vincristine Induced Neuropathic Pain

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### Abstract:

- **Background** :Vincristine is one of the most common chemotherapeutic drugs used to treat various types of malignancies Its major dose-limiting side effect is neurotoxicity that requires discontinuation of treatment.
- **Methods** : in rat model of vincristine induced neuropathic pain we tried to investigate the potential analgesic effect of SB-366791 ,the trpv1 receptor antagonist, on vincristine induced pain via behavioral tests as tail immersion & hot plate tests , also via measuring the level of some pain mediator and histopathological examination of sciatic nerve.
- **Results** : We found that vincristine induce thermal hyperalgesia assessed by behavioral and biochemical testes, whereas SB-366791 showed improvement in thermal hyperalgesia induced by vincristine and showed significant decrease in pain mediators like serum tumor necrosis factor alpha level and spinal nitric oxide content and also improvement in histopathological examination of sciatic nerve fiber.
- **Conclusion:** the result indicates that trpv1 receptor may have a potential role in vincristine induced neuropathic pain.

**Key words:** Vincristine, neuropathic pain, TRPV1

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### 1. Introduction

One of the most common chemotherapeutic drugs used to treat various types of malignancies is vincristine (Postma TJ et al,1993, Sandler SG, Tobin W, Henderson ES 1969). Its major dose-limiting side effect is

neurotoxicity that requires discontinuation of treatment and thus greatly affects the survival of cancer patients (Sandler SG, Tobin W, Henderson ES (1969), Weiden PL, Wright SE

1972). Casey EB, Jellife AM, Le Quesne PM, Millet YL (1973).

A promising way of producing analgesia at the level of the primary sensory neuron is the pharmacological modulation of the nociceptor function. There are many experimental data demonstrate that the transient receptor potential vanilloid-1 (TRPV1) receptor is a central molecular integrator of a variety of noxious stimuli and has a central function in the transmission of nociceptive information. It is a nonselective cation channel with significant permeability to calcium, protons, and large polyvalent cations (Holzer P 2008).

A new strategy in neuropathic pain relief is the Pharmacological modulation of TRPV1 represents. by silencing receptors where pain is generated TRPV1 antagonists relief pain rather than stopping the propagation of pain, as many traditional pain killers do (Van Abel M, et al 2005, Meyer MB, et al. 2006).

Clinical value of TRPV1 antagonists can be subdivided into two broad categories. The first category related to the role of TRPV1 to inflammatory and neuropathic pain that determines the efficacy of antagonists. The second category relates to the physiological roles of TRPV1 that might indicate potential adverse effects for antagonists (Arpad Szallasi1 et al, 2006).

Novel TRPV1 receptor antagonists as SB366791, a structurally novel inhibitor of rat and human TRPV1 receptor, developed by GlaxoSmithKline (Rami HK, et al 2004).

Various key mediators of neuropathic pain following peripheral nerve injury (Inoue K, Koizumi S, Tsuda M 2007, Marchand F et al, 2005, Ueda H (2006), White FA, Bhangoo SK, Miller RJ (2005a). There are some inflammatory and immune responses in the injured peripheral nervous system have an important role in the development and maintenance of neuropathic pain following peripheral nerve injury (Cui JG et al 2000, Scholz J & Woolf CJ 2002).

TNF- $\alpha$  plays a role in the mediation of neuropathic pain peripherally, chemotherapy produces peripheral neuropathy with massive

release of TNF- $\alpha$  in serum (Tonini G, et al 2002)

Nitric oxide (NO) is involved in synaptic transmission in both the central and peripheral nervous systems (Garthwaite J et al 1988, Kawamata T, Omote K 1999, Vincent SR (1994). Neuronal NOS (nNOS) is localized in the superficial dorsal horn of the spinal cord this leads to the notion that NO plays a role in nociceptive transmission was initially (Dun NJ et al, 1992, Saito S 1994, Terenghi G et al, 1993). Facilitated synaptic transmission in the spinal cord requires the release of NO, since NOS inhibitors reduce (Coderre TJ, Yashpal k (1994), Haley JE et al 1992, Malmberg AB, Yaksh TL 1993, Moore PK et al, 1993, Roche AK et al, 1996). Basing on these findings NO may be able to promote or reduce synaptic transmission of nociceptive stimuli in the spinal cord.

In this study we evaluate the potential analgesic effect of SB-36671 through acting on TRPV1 receptor in an animal model of Vincristine-induced painful peripheral neuropathy and thus show the potential role of TRPV1 in this neuropathic pain.

## 2. Materials and Methods

### Animals

Fifteen female Egyptian rats (140-160 g) were housed in groups of five under a 12-h light/dark cycle. Food and water were available. Experiments were carried out in accordance with NIH regulations for animal care and with the approval of the Institutional Animal Care and Use Committee of the University of California, San Francisco. All efforts were made to minimize the number of animals used and their suffering

### Drugs

*Vincristine-treated group:* Vincristine (Vincristine Pierre Fabre 1 mg/ml, Boulogne, France) was diluted in normal saline (NaCl 0.9%, Braun, Melsungen, Germany) just before administration to give a final

concentration between 50 and 100 mg/ml, depending on the animal weight and ensuring that volumes of less than 1 ml would be injected I.P.

*SB-366791 treated group:* SB-366791 was dissolved in 99% ethanol.

*Control groups:* Injected volumes of saline (NaCl 0.9%) were calculated according to the weight of the rat.

## **Experimental Procedures**

### ***Animals were classified into 3 groups***

*Vincristine-treated group:* Vincristine was administered I.P. every 2 days until five injections had been given 150 mg/kg (cumulative dose: 750 mg/kg). To avoid acute effects the injections were given after the behavioral tests were performed.

*SB-366791 treated group:* , the animals will be injected with vincristine dose (150µg/kg) ip once every 2 days with SB-366791 (500 µg/kg) IP once every 2 day till 7 doses beginning from 3<sup>rd</sup> vincristine injection time.

*Control group:* animals will be injected with volumes of normal saline according to body weight.

### **Behavioral Assessment**

Rats were habituated to handling investigator and the testing Procedures during the week prior to the experiment.

### **Tail Immersion Test**

The tail of the rat was immersed in cold water maintained at noxious (4°C) temperature, until the tail was withdrawn. The duration of immersion was recorded and a cut-off time of 15 s was used. Immersion in a cold (4°C) water bath. Scores were determined before the first, the third and the fifth injection and 1, 4, 8, 12, 16, 20 days after the last injection of vincristine (Necker P, Hellon R.F (1978).

### **Hot-plate test**

In this test, the animals were placed in a glass cylinder on a heated metal plate maintained at 55±1°C. The latency of nociceptive responses such as licking or shaking one of the paws or jumping was recorded as the reaction time (Woolfre HG, MacDonald AD 1944).

### **Biochemical studies**

Serum were separated by centrifugation after keeping the samples at 10°C for 30 minute and used for TNFα ELISA assay. After blood collection the animals were killed by cervical dislocation, spinal cord and sciatic nerve were excised. The amounts of TNF-α in serum were measured by ELISA (R&D Systems (Engelmann H, et al. (1990). Nitric oxide in spinal cord was determined spectrophotometrically using vanadium (III) reduction method (Miranda k, Espey M, Wink D (2001).

### **Statistical analysis**

The data are expressed as means SE, significance of differences between groups was assessed with Student's t-test (comparison of two groups) or an analysis of variance (ANOVA) where scheffe test was performed to compare between each two means if F value was significant. Significance was adopted at p<0.05 for interpretation of results of tests of significance.

### **Histology**

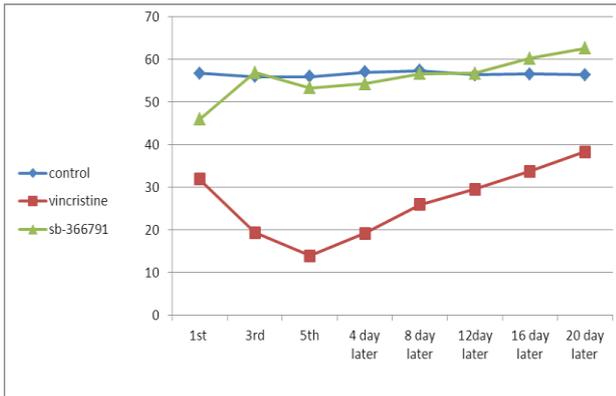
Samples of sciatic nerve were processed and paraffin embedded sections cut at 3-5 mm thickness on glass and charged slides for routine hematoxylin and eosin staining method for light microscopy examination.

## **3. Results**

### **Behavioral Examinations**

Figure (1) showed that whatever the temperature applied, the Saline treated group did not show modification in the withdrawal latency at anytime (p>0.05). Treatment of rats

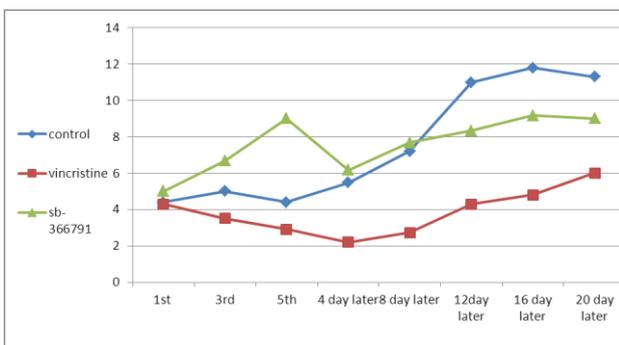
with Vincristine (150µg/kg) resulted in a significant decrease in the withdrawal latency compared to that of saline treated animals from the first vincristine injection time till the last one ( $p < 0.05$ ). Treatment of animals with SB-366791 (500µg/kg) resulted in a non significant increase in the withdrawal latency compared with before drug administration ( $p > 0.05$ ).



**Figure (1) tail immersion test**

**Hot plate test**

Figure (2) rats treated with normal saline did not show a significant modifications in the withdrawal latency, except a significant Treatment of rats with (Vincristine 150µg/kg) resulted in a significant decrease in the withdrawal latency compared to saline treated animals from the 5th vincristine injection time till the end of the study ( $p > 0.05$ ). Beginning from 12 day later there was a significant increase in the withdrawal latency till it was as nearly as the first injection time ( $p < 0.05$ ). Difference

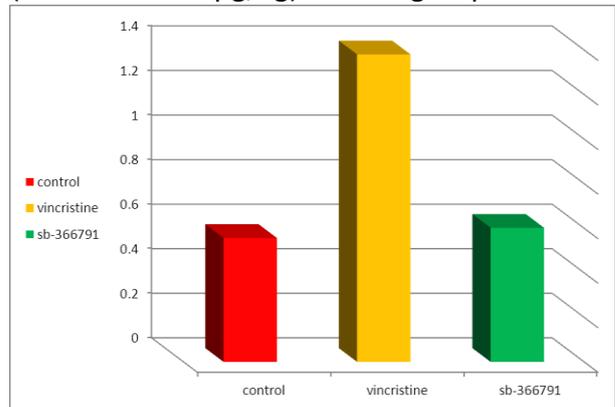


between the beginning and the end of the study ( $p = 0.0001$ ).

Treatment of rats with SB-366791 (500 µg/kg) resulted in at 3rd&5th vincristine injection time there was a non significant increase in latency ( $p > 0.05$ )

**Figure (2) hot plate test  
Serum TNF alpha**

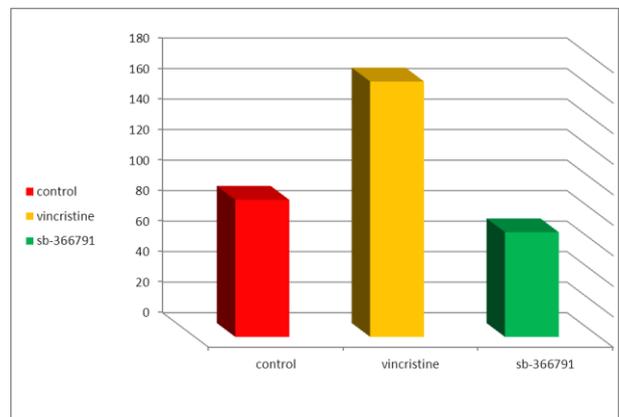
Figure (3) showed that treatment of rats with vincristine (150µg/kg) resulted in a significant increase ( $p = 0.000$ ) in serum TNFα level as compared to that of the control saline group. Treatment of rats with SB-366791 (500µg/kg) resulted in significant decrease ( $p < 0.05$ ) in TNFα serum level as compared to that of the (Vincristine 150µg/kg) treated group



**Figure (3) serum TNF α**

**Spinal cord tissue nitric oxide levels**

As shown in figure (4) treatment of rats with vincristine (150µg/kg) resulted in significant



increase ( $p < 0.05$ ) in NO content in spinal cord

tissue as compared to that of the control saline group.

Treatment of rats with SB-366791 (500µg/kg) resulted in a significant decrease ( $p < 0.05$ ) in NO content spinal cord tissue as compared to that of the Vincristine (150µg/kg) treated group.

#### Figure (4) spinal nitric oxide

##### Histological examination

As shown in figure (5) Microscopic examination of Vincristine (150µg/kg) treated rats showed changes in sciatic nerve fibers compared to concurrent control rats (fig.), showing degenerated and perineuronal mononuclear cell infiltrations and extensive (+++) nerve fibers Combined treatment of rats with (Vincristine (150µg/Kg) and SB-366791 (500µg /kg) showed sciatic nerve fibers section with only some swelling of the nerve fiber

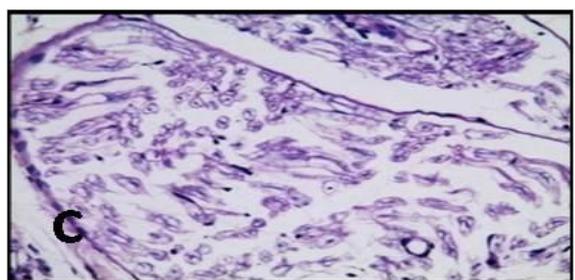
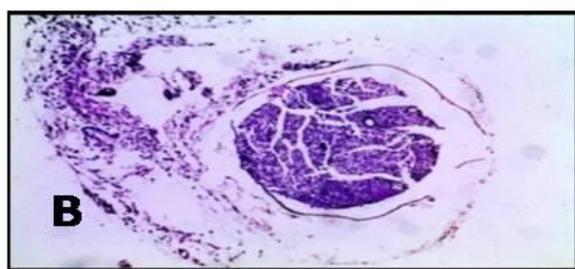
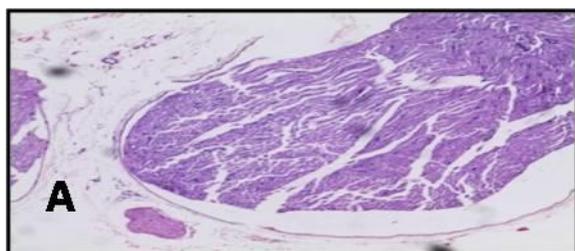


Figure (5) histopathological examination (a) control, (b) vincristine, (c) SB-366791

##### Discussion

In new animal model vincristine, antineoplastic agent, used to induce neuropathic pain. SB-366791 the new transient receptor potential vanilloid 1 (TRPV1) antagonist was used as analgesic for vincristine induced neuropathic pain.

In this study, we tried to investigate the potential role of TRPV1 receptor in vincristine induced neuropathic pain and subsequently the potential analgesic effect of SB-366791, the TRPV1 antagonist, on this neuropathic pain through behavioral, biochemical and histopathological studies.

The results showed that vincristine treatment of animals with vincristine resulted in significant decrease in withdrawal latency in both tail immersion test and hot plate test, while treatment of animals with SB-366791 resulted in non significant increase in withdrawal latencies in both testes. Biochemical studies showed that vincristine treatment resulted in significant increase in both serum tumor necrosis factor alpha ((248.2% of control) and spinal nitric oxide content (197.53% of control). while SB-366791 treatment showed significant decrease in both serum tumor necrosis factor alpha (38.461 % of vincristine) and spinal nitric oxide content (37.5% of vincristine). Histopathological examination of rat sciatic nerve showed that treatment of animals with vincristine (150µg/kg) resulted in extensive nerve fiber degeneration, while treatment of animals with SB-366791 (500µg/kg) resulted in only some swelling of nerve fibers compared with that of normal nerve fibers.

Our results were agree with (Nicolas Authier, Jean-Pierre Gillet et al, (2003)., who found that successive injections of Vincristine

(150µg/kg) induce thermal hyperalgesia and allodynia due to degeneration of sciatic nerve myelinated axons in the fine nerve fibers of the subcutaneous paw tissue. Also these results agree with (Angelika Varga, Jo'zsef Ne'meth et al, (2005) who found that SB366791 can be considered a more selective and potent antagonist of the TRPV1 receptor than capsaizepine in the rat, therefore, it is a suitable antagonist for testing the role of TRPV1 receptors in different experimental conditions.

All of these findings showed that transient receptor potential vanilloid 1 (TRPV1) may have a potential role in vincristine – induced neuropathic pain consequently the potential analgesic effect of TRPV1 antagonist on this induced pain.

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