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Potential role of Tamoxifen as a secondary analgesic for chemotherapy induced neuropathic pain

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ABSTRACT

Pharmacological modulation of TRPV1 receptor is a promising way of producing analgesia in neuropathic pain models. in the present study we investigated the effect of Tamoxifen, PCK inhibitor ,on thermal hyperalgesia in rats after sciatic nerve injury induced by vincristine. Vincristine administration decreased the withdrawal latencies assessed by tail immersion and hot plate tests ,also it increased the level of inflammatory mediators , spinal NO and serum TNFα levels. Tamoxifen ,administered orally every 2 days one hour before vincristine injection till 7 times, resulted in the increase in withdrawal latencies compared to that of vincristine, also showed a decrease in spinal NO and serum TNFα levels. these findings suggested that TRPV1 receptor has a potential role in vincristine induced painful neuropathy and open the door for tamoxifen to be used as secondary analgesic in painful neuropathies.

INTRODUCTION

Those agents that have a pain-relieving property as a secondary nature of their clinical effects are called secondary analgesics . They can be used as analgesic agents solely in treatment of various types of pain states (Shiton, 1997).

Persons treated for cancer have a common syndrome known as neuropathic pain. Its underlying mechanisms are poorly understood. so, treatment isn't often adequate, and patients suffer so much. "pain initiated or caused by a primary lesion or dysfunction in the nervous system" (Portenoy, 2000) is a simple definition of neuropathic pain. One of the most common chemotherapeutic drugs used to treat various types of malignancies is vincristine (Sandler *et al.*, 1969; Postma *et al.*, 1993). Its major dose-limiting side effect is neurotoxicity that requires discontinuation of treatment and thus greatly affects the survival of cancer patients (Sandler *et al.*, 1969; Weiden and Wright, 1972; Casey *et al.*, 1973).

A promising way of producing analgesia at the level of the primary sensory neuron is the pharmacological modulation of the nociceptor function remains .There are a continuously increasing experimental evidences 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. It is the most polymodal TRP channel, it is activated by many stimuli, including heat, vanilloids, voltage, lipids, and protons/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 et al., 2005; Meyr *et al.*2006).

At the Subcellular level, TRPV1 is sequestered in intracellular compartments where it exist in a homomeric complex, probably as a tetramer (Garcia-sanz et al, 2004). When depolarized, neurons begin trafficking TRPV1 to the membrane where this receptor is activated by its agonists, desensitized and then recycled to the intracellular compartments (Morenilla-palao et al, 2004). Generally phosphorylation by protein kinases, protein kinase A (PKA) and protein kinase C (PKC), are vital for both sensitization

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and re-sensitization of TRPV1 involve (Mohapatra and Nau, 2005). Among the PKC isozymes, PKCε is more important because phosphorylation at Ser800 of TRPV1 by PKC contributes to the development of inflammatory hyperalgesia (Mandadi et al, 2006). This observation identifies Ser800 as a potential therapeutic target for compounds that block the development of hyperalgesia by preventing TRPV1 phosphorylation by PKC.

Tamoxifen (TAM), is a mixed estrogen agonist and antagonist, has been widely used in the treatment of advanced and adjuvant estrogen receptor positive breast cancer because of its antiestrogenic effects in breast tissue. Tamoxifen, has been shown to interact with protein kinase Cζ (PKCζ) (Lavie *et al.,* 1998).

Recent animal models showed that various key mediators of neuropathic pain following peripheral nerve injury (Inoue *et al.*, 2007; Marchand *et al.*, 2005; Ueda, 2006; White *et al.*, 2005a). Some inflammatory and immune responses in the injured peripheral nervous system (PNS) reported to play an important role in the development and maintenance of neuropathic pain following peripheral nerve injury (Cui *et al.*, 2000; Scholz and Woolf, 2007). TNF-α plays a role in the mediation of neuropathic pain peripherally. Clinically, chemotherapy produces peripheral neuropathy with massive release of TNF-α in serum (Tonini, 2002) and TNF-α that is used as anticancer treatment clinically leads to peripheral neuropathy (Drory, 1998).

Synaptic transmission in both the central and peripheral nervous systems thought to involve nitric oxide (NO) (Garthwaite *et al.*, 1988; Kawamata and Omote, 1999; Vincent, 1994). localization of neuronal NOS (nNOS) in the superficial dorsal horn of the spinal cord leads to the notion that NO plays a role in nociceptive transmission was initially (Dun *et al.*, 1992; Saito *et al.*, 1994; Terenghi *et al.*, 1993). The release of NO is required for facilitated synaptic transmission in the spinal cord, since NOS inhibitors reduce nociception (Coderre and Yashpal, 1994; Haley *et al.*, 1992; Malmberg and Yaksh, 1993; Moore *et al.*, 1993; Roche *et al.*, 1996). Basing on these findings, it was suggested that 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 Tamoxifen through acting on TRPV1 receptor in an animal model of VCR-induced painful peripheral neuropathy.

MATERIALS AND METHODS

Animals

Fifteen female Egyptian rats (140-160 g).Rats 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 Fabre1 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.

Tamoxifen treated group: Tamoxifen were dissolved in D.W. at 37º C.

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. Tamoxifen treated group:, the animals will be injected with vincristine dose (150µg/kg) ip once every

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

Behavioral Assessment

2 days till 5 injections with Tamoxifen (1mg/kg) orally once every 2 days one hour before each vincristine injection till 7 times. 24) 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 and Hellon,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 and Macdonald(1944).

Biochemical studies

Serum were separated by centrifugation after keeping the samples at 10ºc 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 (Miranada *et al*, 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.

RESULTS

Behavioral Examinations

Tail Immersion Test

Figure (2) 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 rats with (Vincristine (150µg/kg + Tamoxifen 1mg/kg) resulted in first non significant increase at 3rd vincristine injection time , but at the time of 5th injection the withdrawal latency was as nearly as before tamoxifen administration. but at 4 day later time there was anon significant increase ($p>0.05$)

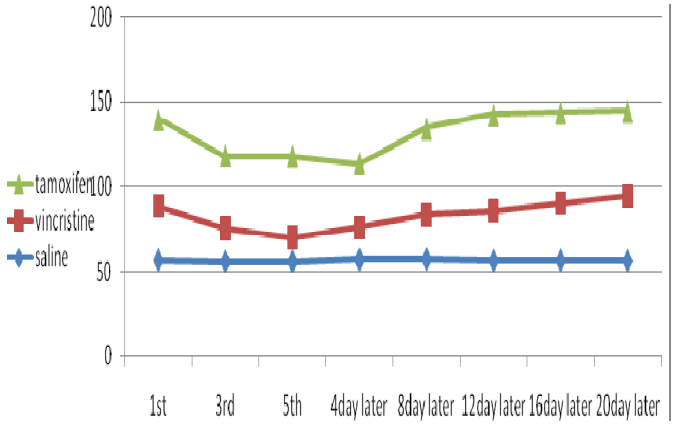

Fig. 2: Mean withdrawal latency in seconds by tail immersion test among the control (saline),vincristine(150µg/kg),and tamoxifen(1mg/kg) treated groups at different times of assessment.

Hot plate test

Figure (3) 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 (vincristine (150µg/kg +Tamoxifen 1mg/kg) resulted in a non significant increase in latency at 3rd vincristine injection time was observed , but at 5th injection a significant increase observed, then at 4 day later there was a non significant increase observed

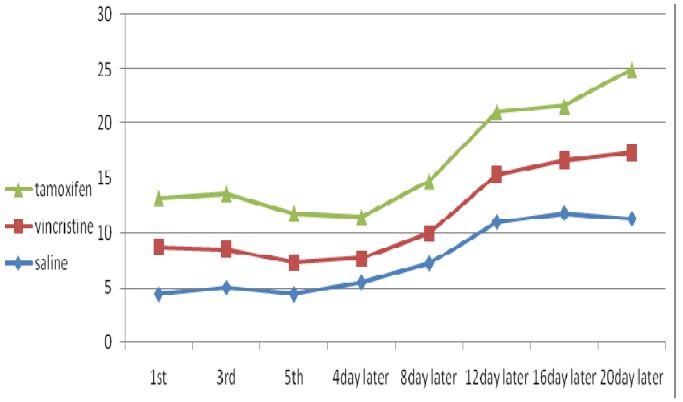

Fig. 3: Mean withdrawal latency in seconds by tail immersion test among the control (saline),vincristine (150µg/kg),and tamoxifen(1mg/kg) treated groups at different times of assessment

Serum TNF alpha

Figure (4) 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 (vincristine150µg/kg + Tamoxifen 1mg/kg) resulted in significant decrease ($p<0.05$) in serum TNFα level as compared to that of the (vincristine 150µg/kg) treated group.

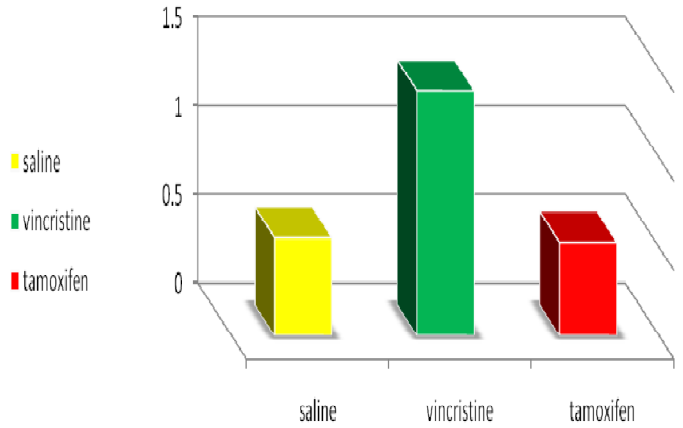

Fig. 4: Mean serum TNF alpha level of saline group, vincristine(150 µg/kg),and tamoxifen(1mg/kg) treated groups.

Spinal nitric oxide levels

As shown in figure (5) treatment of rats with vincristine (150µg/kg) resulted in significant increase($p=0.013$) in NO content in hind paw tissue as compared to that of the control saline group. Treatment of rats with (vincristine (150µg/kg + tamoxifen 1mg/kg) resulted in non significant difference($p>0.05$) in NO

content in hind paw tissue as compared to that of the (vincristine 150µg/kg) treated group.

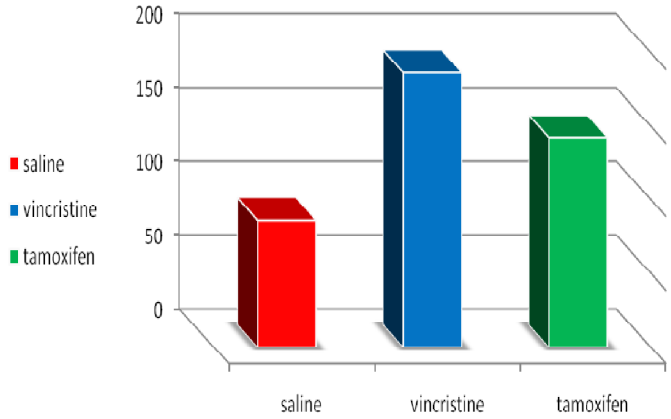

Fig. 5: Mean spinal NO content of saline group, vincristine(150 µg/kg),and tamoxifen(1mg/kg) treated groups.

Histological examination

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 degeneration (fig),concerning sciatic nerve fibers examination of rats treated with (**Vincristine** (150µg/Kg)+ **Tamoxifen** (1mg/kg)) showing mild (+) ballony degeneration(fig.6

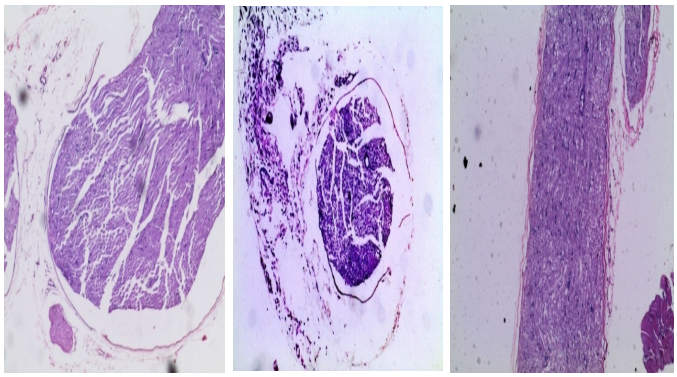

Fig. 6: (a) sciatic nerve section from normal control group showing apparently normal nerve fibers.(b) sciatic nerve section from (Vincristine (150µg/kg) showing extensive degeneration .(c)sciatic nerve section from (Vincristine (150µg/kg)+ Tamoxifen (1mg/kg)) treated group showing bullony degenerated nerve fibers.

DISCUSSION

This study describes a new model of treatment of peripheral nociceptive neuropathy produced after toxic injury of the peripheral nervous system by the antineoplastic agent, vincristine. In our study we tried to investigate the potential role of TRPV 1 receptor in vincristine induced neuropathic pain, and consequently the role of non competitive receptor antagonist as an analgesic of the induced pain. Tamoxifen, a synthetic anti-estrogen has been shown to interact with protein kinase Cε (PKCε) (Lavie *et al.*, 1998).

Our finding revealed that tamoxifen may have an analgesic effect on vincristine induced neuropathic pain .behavioral studies showed that peripheral nerve injury induced by vincristine leading to significant decrease in withdrawal latency in both tail immersion and hot plate testes ,these findings agrees with results of (Nicolas *et al.*,2003) showing that the maximum decrease in withdrawal latency was at 3rd and 5th vincristine injection time .Tamoxifen administration resulted in a non significant decrease in withdrawal latency at 3rd and 5th vincristine injection times.

Upregulated neuroinflammatory cytokines, during tissue damage, are involved in the pathogenesis of neuropathic pain syndromes (Marchand F.et al 2005). For example, the expression of tumor-necrosis-factor-α (TNFα) is increase in dorsal root ganglion (DRG) neurons after nerve injury (Schafers m.et al 2003) .our study results showed that peripheral nerve injury induced by vincristine resulted in significant increase ($p<0.05$)in serum TNFα level whereas tamoxifen treatment resulted in significant decrease in serum TNFα level this is suggested to be due to acting on TRPV1 receptor by inhibition of PKC and consequently inhibition of TRPV1. In the same way tamoxifen administration resulted in non significant ($p>0.05$) in spinal nitric oxide content.

All of these findings were confirmed by histological studies that showed that vincristine administration resulted in extensive(+++) sciatic nerve degeneration with perineuronal mononuclear cell infiltration whereas tamoxifen treatment resulted in mild nerve degeneration (+)

CONCLUSION

Transient receptor potential vanilloid 1 (TRPV1) may have a potential role in vincristine induced neuropathic pain, consequently tamoxifen the protein kinase C inhibitor may act as a secondary analgesic in chemotherapy induced painful neuropathy.

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