Effects of Benfotiamine and Methylcobalamin on Paclitaxel induced Peripheral neuropathy

Kawa F. Dizaye
Chro Y. Qadir

Hawler Medical University, Iraq

Correspondence:
Dr. Kawa Dizaye. Professor of Pharmacology,
Head of Dept of Pharmacology.
Hawler Medical University, Iraq
Email: dr_kawadizaye@yahoo.com

ABSTRACT

Background: Reports indicate that paclitaxel causes a dose-limiting distal and symmetrical sensorimotor peripheral neuropathy. This study was designed to evaluate the protective effects of benfotiamine and methylcobalamin on prevention of paclitaxel induced peripheral neuropathy.

Methods: Twenty four rats and twenty four mice were involved in this study. Each animal group was allocated to two main experimental groups [control group (n=6) and paclitaxel model group (n=18)]. The paclitaxel model group in rats was subdivided into 3 subgroups [paclitaxel group (6mg/kg i.p.) for 4 weeks, paclitaxel + benfotiamine (100mg/kg orally, daily for 8 weeks) and paclitaxel + methylcobalamin (500µg/kg i.p., twice weekly for 8 weeks)]. Whereas the paclitaxel model group of mice was subdivided into 3 sub groups [paclitaxel group (6mg/kg i.p. for 4 weeks), paclitaxel + benfotiamine (100mg/kg orally, daily for 6 weeks) and paclitaxel + methylcobalamin (500µg/kg orally, daily for 6 weeks)]. Electrophysiological and histological investigations, as well as a number of classical behavioural tests of nociception were performed.

Results: Paclitaxel administration produced significant increase in latency, but decrease in amplitude and conduction velocity in peripheral motor nerves in rats. Degenerative changes of sciatic nerve were observed in rats. The paw withdrawal latency for heat hyperalgesia and the tail withdrawal latency for cold (allodynia and hyperalgesia) in mice were significantly reduced. Benfotiamine administration significantly ameliorated all electrophysiological changes induced by paclitaxel in peripheral motor nerves. Moreover benfotiamine decreased histological changes in rat's sciatic nerve. In mice benfotiamine administration significantly ameliorated the reduced withdrawal latencies for cold and hot. Methylcobalamin administration together with paclitaxel attenuates the reduction in conduction velocity in rats but had no effect on the reduced amplitude. Methylcobalamin reduced degenerative changes in Schwann cells but had no effect on reduced myelin thickness. While in mice daily methylcobalamin administration significantly reduced the decreased withdrawal latencies for cold and hot.

Conclusion: Benfotiamine 100mg/kg was very efficient in prevention of sensorimotor neuropathy induced by paclitaxel, whereas the suggested methylcobalamin (500µg/kg) twice weekly did not sufficiently prevent peripheral motor nerve destruction induced by paclitaxel, while the administration of high dose methylcobalamin every day is efficient in removal of thermal nociception induced during paclitaxel treatment.

Key words: Benfotiamine; Methylcobalamin; Paclitaxel; peripheral neuropathy

Introduction

Approximately 1.5 million new diagnoses of cancer were anticipated in 2009 in the United States (1). Improved medical treatments and advances in technology have allowed many people with cancer to increase their lifespan; however, these life-saving interventions come with many potential risks. Chemotherapy induced peripheral neuropathy (CIPN) is a debilitating and disabling condition that affects approximately 3% to 7% of patients who are treated with a single agent, and more than 38% of patients being treated with a combination of drugs(2).

Peripheral neuropathy is a common and potential dose-limiting complication of cancer chemotherapy. Involvement of the peripheral nervous system may be in the form of purely sensory and painful neuropathy, which occurs after therapy with cisplatin, oxaliplatin and carboplatin, or mixed sensory-motor neuropathy which may be accompanied by dysfunction of the autonomic nervous system, that results after therapy with vincristine, taxanes, suramin and other drugs (3, 4).

Paclitaxel is one of the most effective and commonly used anti-neoplastic drugs originally derived from the bark of the western yew tree, Taxus brevifolia, with activity against several tumors including ovarian cancer not responsive to primary treatment methods, metastatic breast cancer, Kaposi's sarcoma, bladder, testicular, lung, and head and neck cancers (5,6).

Paclitaxel-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients.

Studies have shown that paclitaxel administration inhibits the usual regenerative response of axons and Schwann cells to nerve crush injuries in rodent models (7).

There have been several in vivo and in vitro experimental studies of taxane neurotoxicity. Cultured sensory neurons show proliferation and aggregation of neurotubules; application of nerve growth factor inhibits this effect (8).

Painful peripheral neuropathy occurs with other agents in the taxane class, as well as with chemotherapeutics in the vinca alkaloid and platinum-complex classes. The cause of the neuropathy and of the pain syndrome is unknown.

This study was designed to evaluate the neuroprotective effects of benfotiamine and methylcobalamin in paclitaxel induced peripheral neuropathy.

Materials and Methods

Animals

The experiments were performed on 24 male albino rats and 24 male albino mice. The rats were used for both nerve conduction studies and nerve biopsy, whereas the mice were used to detect the effect of each drug on heat nociception stimuli.

Before experiment, the animals were kept in the animal house of the college of Medicine / Hawler Medical University. They were housed in groups of six per cage, on sawdust, maintained on a 12h-12h light-dark cycle. They were given food rich in nutrient and tap water. Room temperature was maintained at 25 C°.

Anaesthesia

The rats were anaesthetized by a combination of Ketamine and xylazine which were injected intra-peritoneally at a dose of 35 mg/kg, and 5mg/kg body weight respectively (9). After six minutes a state of anesthesia was reached. They were placed on a heated table to maintain their body temperature at around 37 °C.

Induction of peripheral neuropathy

Peripheral neuropathy was induced by paclitaxel-induced peripheral neuropathy model. In the paclitaxel-induced peripheral neuropathy model, paclitaxel (6 mg/kg) was injected intraperitoneally once a week for 4 weeks - Days 0, 7, 14, and 21- (10, 11).

All experiments were conducted according to the guidelines of the Hawler Medical University Research Ethics Committee for Research Ethics Committee Approval.

Experimental design

The rats were divided into two groups. The first group consisted of 6 rats and served as a control group (injected with 0.5 ml sterile saline intraperitoneally). The second group consisted of eighteen rats which received paclitaxel (6 mg/kg) injection intraperitoneally (i.p.) once a week for 4 weeks) and served as a Paclitaxel model. The second group was subdivided into three subgroups of six rats each (first subgroup served as a positive control that received paclitaxel (6 mg/kg) injection, second subgroup received benfotiamine 100 mg /kg orally, daily for eight weeks and the third subgroup received methylcobalamin 500 µg /kg, intraperitoneally twice weekly for eight weeks).

The mice were divided into two groups. The first group consisted of 6 mice and served as a control group (injected with 0.5 ml sterile saline intraperitoneally). The second group consisted of eighteen rats that received paclitaxel (6 mg/kg) injection i.p. once a week for 4 weeks) and served as a Paclitaxel model. The second group was subdivided into three subgroups of six mice each (first subgroup served as a positive control received paclitaxel (6 mg/kg) i.p. injection, second subgroup received benfotiamine 100 mg /kg orally, daily for six weeks and the third subgroup received methylcobalamin 500 µg /kg, orally, daily for six weeks).

Motor nerve conduction studies

Electrophysiological measurements were conducted at Hawler Teaching Hospital/Neurophysiology Unit. The data were analyzed using (Nicolet, Madison, WI, USA) software program.

Experimental animal nerve conduction studies were done by using the invasive techniques, with needle electrodes (12, 13).

Latency and Amplitude were measured. Motor Nerve Conduction Velocity (MCV) was calculated by dividing the distance between the stimulation point and recording electrode by the motor latency.

Motor nerve conduction studies (MNCS) were determined 30 to 35days after last dose paclitaxel. Nerve conduction studies were performed using standard equipment (Nicolet, Madison, WI, USA) on anaesthetized rats.

Tail-immersion test

Antinociception was evaluated by measuring response latencies in cold water tail-immersion (tailflick) assay (14, 15, and 16).

Response latencies were measured as the period of time the animal took to respond to the thermal stimuli. The temperature of cold water (4±1°C) for cold hyperalgesia and (10±1°C) for cold allodynia. Water was maintained at the right temperature by the addition of ice cubes. The duration of tail immersion was manually recorded (1 sec. precision), with a cut-off time of 20 sec.

Tail-flick test was performed by gently holding the mouse in a terry cloth towel and immersing between 2 and 3 cm from the tip of the tail into the water, and the response was defined as the removal of the tail from the cold water (17).

The paw hot plate test for hot hyperalgesia

This test consists of introducing a mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that was heated by the electrical current (18). The plate was heated to a constant temperature (50±1°C) the response produced was in the form of two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. To determine latencies, the time was recorded from start of introducing a mouse to the occurrence of the first avoidance response, with a cut-off time of 20 seconds.

Statistical analysis

All data are expressed as means ± standard error of means (M ± SEM) and Statistical analysis was carried out using statistically available software (SPSS Version 11.5). Data analysis was made using one-way analysis of variables (ANOVA). Comparisons between groups were done using Duncan test and unpaired student t-test. $P< 0.05$ was considered as statistically significant.

Results

Effects of paclitaxel, methylcobalamin and benfotiamine on the Motor Nerve Conduction Studies (MNCS).

Effects on latency of sciatic nerve in rats

The mean latency of sciatic nerve in the control group was 0.92 ms ± SE 0.086 (Table 1); it was increased significantly in paclitaxel receiving group rats to 1.74 ms ± SE 0.087 (Table 1 - next page). In Benfotiamine and paclitaxel receiving group the mean latency was 0.94 ms ± SE 0.074 (Table 1), While in the methylcobalamin and paclitaxel receiving group the mean latency was 1.2 ms ± SE 0.094 (Table 1).

Effects on amplitude of sciatic nerve in rats

The mean amplitude of sciatic nerve in the control group was 27.5 mv ± SE 2.1 (Table 1). It was reduced significantly in the paclitaxel receiving group rats to 12.02 mv ± SE 0.92 (Table 1). The mean amplitude in the benfotiamine and paclitaxel receiving group was 26.66 mv ± SE 1.22 (Table 1). While the mean amplitude in methylcobalamin and paclitaxel receiving group was reduced significantly to 18.4 mv ± SE 2.5 (Table 1).

Effects on conduction velocity of sciatic nerve in rats

The mean conduction velocity of sciatic nerve in the control group was 56.24 m/s ± SE 5.1 (Table 1); it was reduced significantly in the paclitaxel receiving group rats to 29.04m/s ± SE 1.46 (Table 1). In the benfotiamine and paclitaxel receiving group the conduction velocity was 54.46 m/s ± SE 3.95. There wasn't a significant change (Table 1), whereas the conduction velocity in methylcobalamin and paclitaxel receiving group was also reduced to 42.7 m/s ± SE 3.26 (Table 1) but better than in the paclitaxel group.

Effects of paclitaxel, methylcobalamin and benfotiamine on Tail thermal threshold in mice

Tail immersion test for cold allodynia (10±2°c) in mice

The mean withdrawal latency of cold allodynia for all groups of mice was measured weekly and is shown in Figure 1.

On day 28 the withdrawal latency of the control group was 17.8 sec. ± SE 1.7 and in the paclitaxel group was reduced significantly to 10.3 seconds. ± SE 2.6 ($P=0.042$). While compared to the control group the paclitaxel and benfotiamine receiving group was 16.5 seconds ± SE 1.6 no significant change was observed. The paclitaxel and methylcobalamin receiving group was 17.7 seconds. ± SE 1.7 compared to the control group and no significant differences were seen.

Tail immersion test (cold hyperalgesia 4±1°c) in mice.

There was a significant reduction in the mean withdrawal latency for cold hyperalgesia in the paclitaxel receiving group which was 3 seconds ± SE 1.09 ($P = 0.0001$) compared to the

Table 1: Effect of paclitaxel, paclitaxel and methylcobalamin and paclitaxel and benfotiamine on motor nerve conduction study (n=24)

MNCS sciatic nerve with recording at gastrocnimeus muscle	Control	paclitaxel	paclitaxel & benfotiamine	paclitaxel & methylcobalamin
Latency (millisecond) **ms**	0.92 ± 0.086 a	1.74 ± 0.087 c	0.94 ± 0.074 a	1.2 ± 0.094 b
Amplitude millivolt **(mv)**	27.5 ± 2.1 A	12.02 ± 0.92 b	26.66 ± 1.22 a	18.4 ± 2.5 b
Conduction velocity (meter per second) **m/s**	56.24 ± 5.1 A	29.04 ± 1.46 c	54.46 ± 3.95 a	42.7 ± 3.26 b

• The same letters mean that there is no significant difference

• The different letters mean there is a significant difference at $P < 0.05$

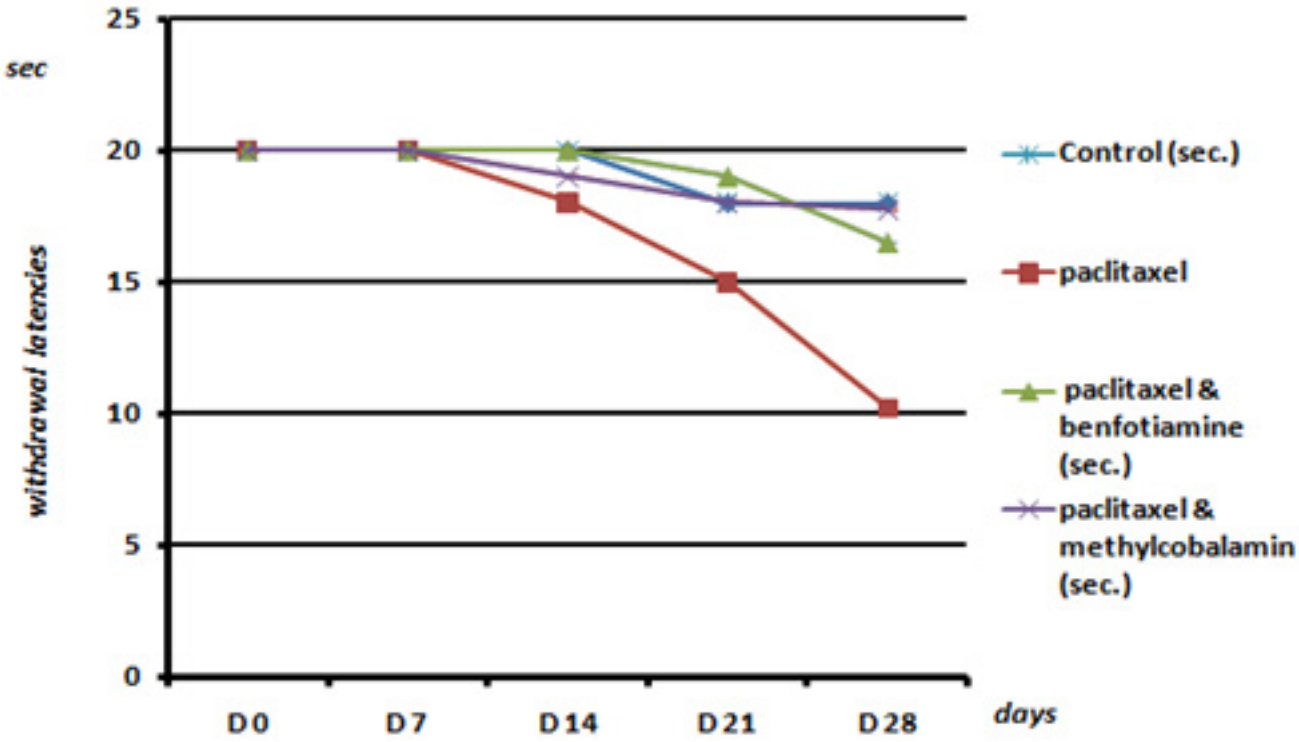

Figure 1: Tail immersion tests for cold allodynia (10±2°c), withdrawal latencies (sec.) measured on weekly bases for all the groups each group with six mice

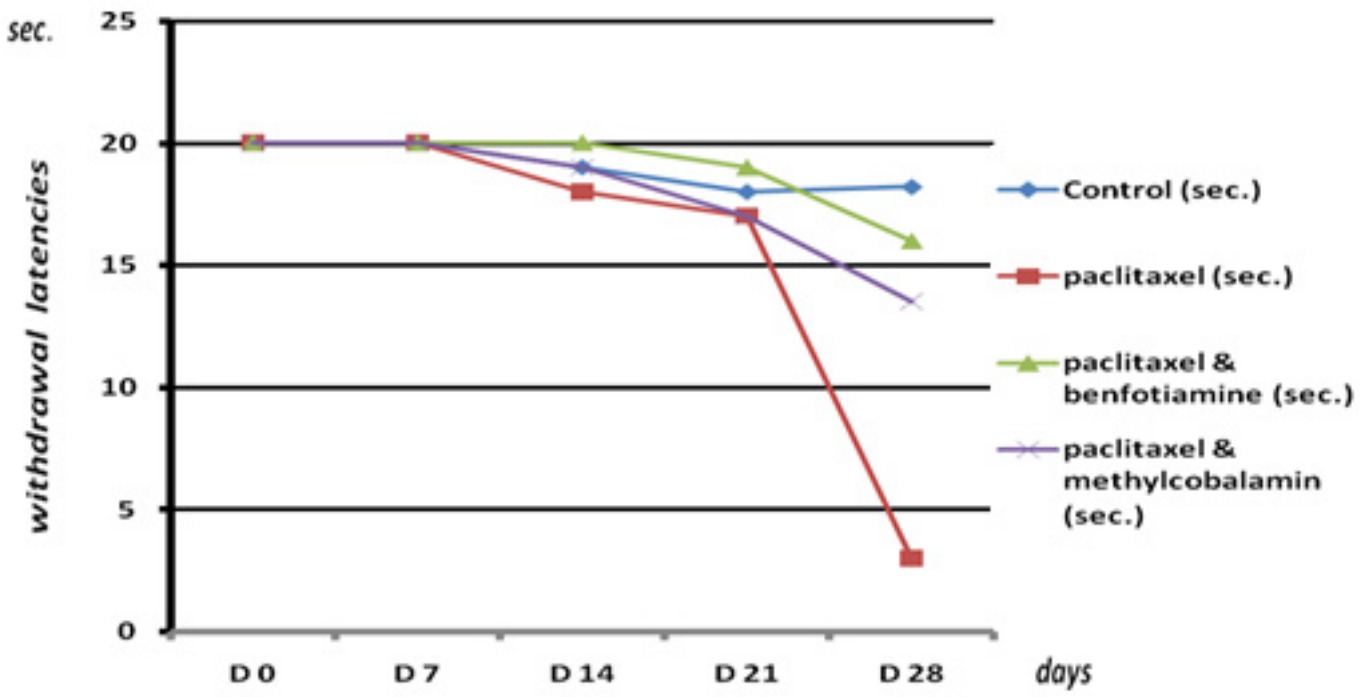

Figure 2: Tail immersion tests for cold hyperalgesia (4 ± 1°c), withdrawal latencies (sec.) measured on weekly bases for all the groups each group with six mice.

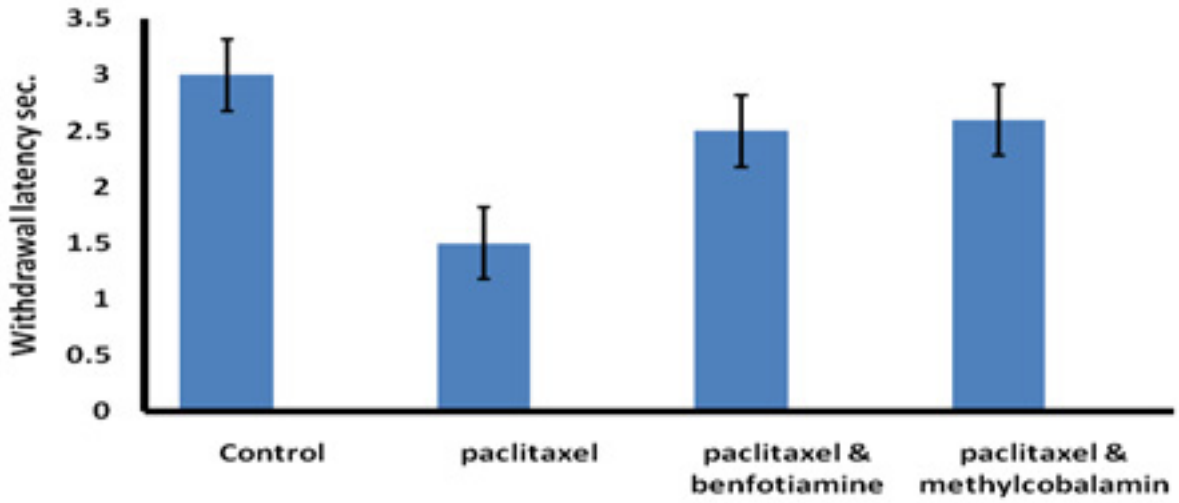

Figure 3: The mean paw withdrawal latencies in four mice groups, each group (n=6) 7 days after last dose of paclitaxel (28th day).

control group. Whereas in the paclitaxel and benfotiamine receiving group it was 16 ± SE 2.5 (P = 0.46) which is non significant compared to the control group. The mean tail withdrawal latency in the paclitaxel and methylcobalamin receiving group was (13.5± SE 2.9, P = 0.19), compared to the control group where no significant change was seen (Figure 2).

Effects of paclitaxel, methylcobalamin and benfotiamine on paw thermal threshold in mice.

The mean paw withdrawal latency in the control group was 3 sec± SE 0.44, (Figure 3). Compared to the control group the mean paw withdrawal latency in the paclitaxel group was reduced significantly to 1.5 sec ± SE 0.22 (P= 0.017) whereas compared to the control group no significant changes were observed in mean paw withdrawal latency for both paclitaxel and benfotiamine and paclitaxel and methylcobalamin groups. The mean paw withdrawal latencies were 2.6seconds ± SE 0.24 (P= 0.45) and 2.5 ± SE 0.22 (P= 0.34) respectively.

In vitro study: Histopathological section of rat's sciatic nerve stained by E &H stain.

Sciatic nerve for control group:

The histopathological results of this study showed normal architecture of rat's sciatic nerve fibers in the control group in which most of the nerve fibers were equal in size, diameter with regular thickness of myelin, continuation of myelin cell basement membrane and normal nucleus of myelin cell (Figure 4 - next page).

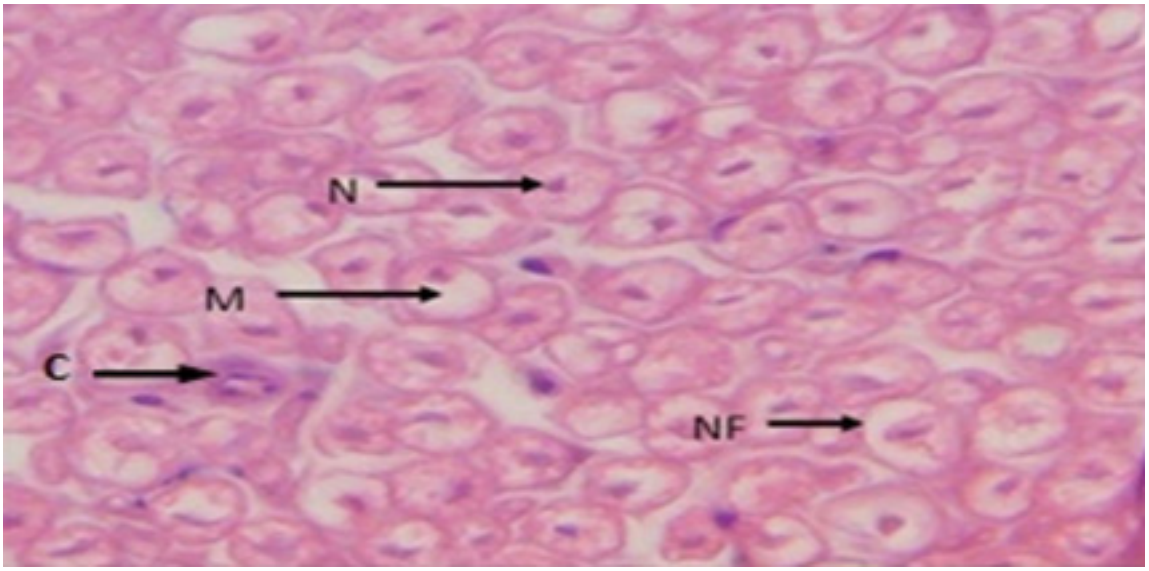

Figure 4: Cross section from the SN of a normal control specimen showing normal appearance of the individual nerve fibers (nucleus and myelin) of Schwann cells (arrows) (N= nucleus , M= myelin, NF= nerve fiber, C=capillary) E & H stain X 400.

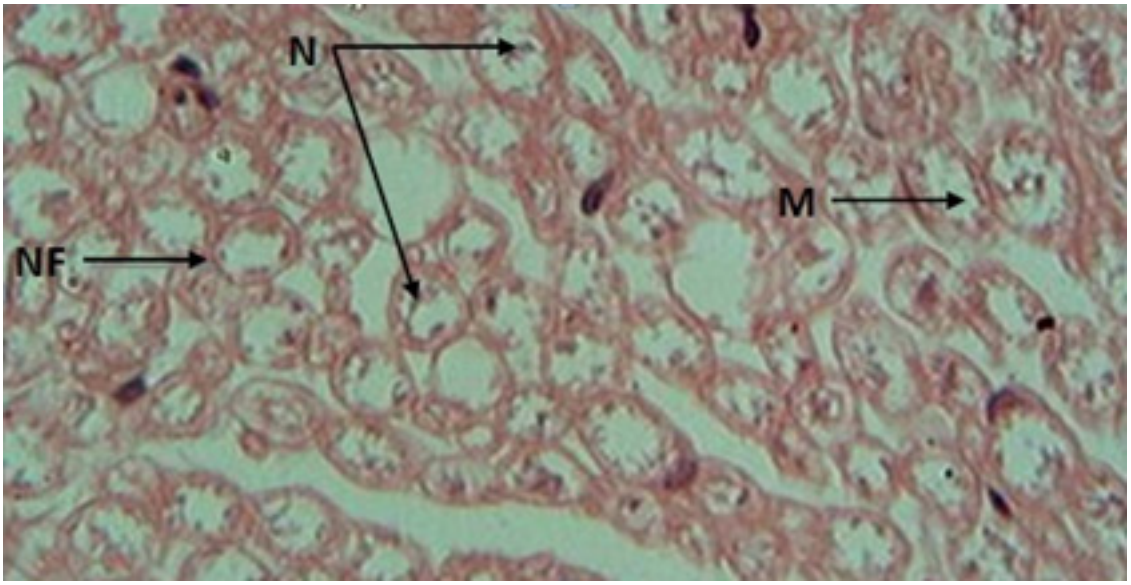

Figure 5: Cross section of SN from the paclitaxel receiving group specimen showing abnormal appearance of the individual nerve fibers (nucleus degeneration and disruption of myelin sheath) of Schwann cells and multiple different size nerve fibers (arrows) (N= nucleus , M= myelin , NF= nerve fiber,) E & H stain X 400.

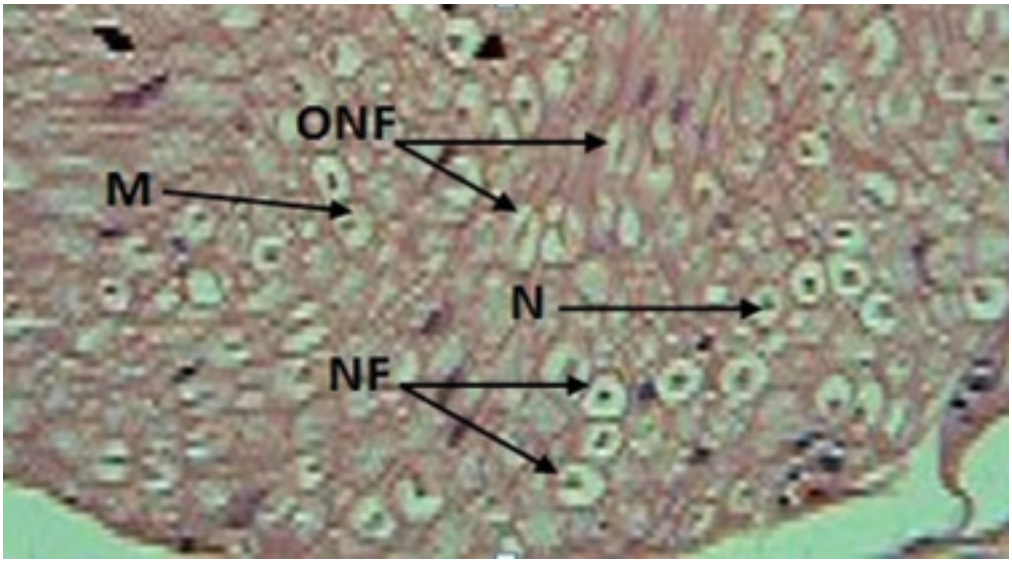

Figure 6: Cross and oblique sections of SN from paclitaxel and benfotiamine receiving group specimen showing most nerve fibers normal, with normal nucleus, normal myelin thickness and maintenance of basement membrane of Schwann cells (arrows) (N= nucleus , M= myelin, NF= nerve fiber, ONF= oblique view nerve fiber) E & H stain X 400.

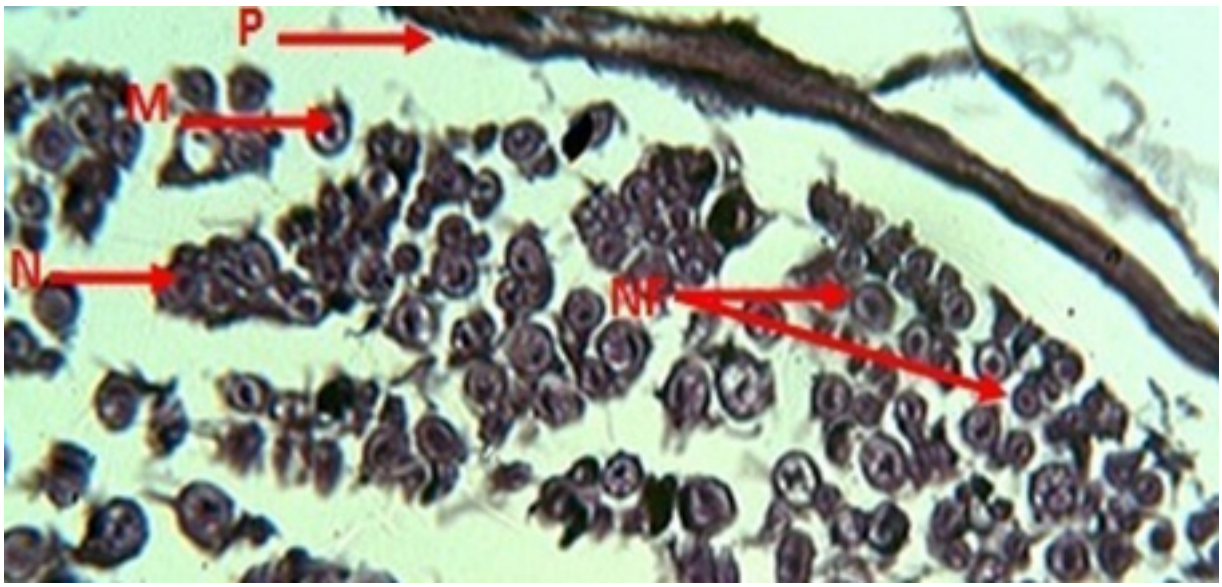

Figure 7: Cross section of SN- B (lower) - Cross and oblique section of SN, from paclitaxel and methylcobalamin receiving group specimen showing marked reduction in myelin thickness and multiple different size nerve fibers but, with maintenance of basement membrane of Schwann cells (arrows) (N= nucleus , M= myelin , NF= nerve fiber, ONF= oblique view nerve fiber, P = perineurium) E & H stain X 400.

Effect of paclitaxel on sciatic nerve

The result of this study showed that 6 mg/kg paclitaxel once a week for four weeks resulted in marked destruction of sciatic nerve fibers in rats, as shown in Figure (5).

Effect of paclitaxel and benfotiamine on sciatic nerve

In this study the results showed that with daily administration of benfotiamine 100mg/kg together with paclitaxel and continued for four weeks after last dose of paclitaxel preserves most nerve fibers from destruction (Figure 6)

Effect of paclitaxel and methylcobalamin on sciatic nerve

The result of this study showed that administration of methylcobalamin 500 µg /kg, intraperitoneally twice weekly together with paclitaxel in rats for eight weeks inhibits destruction of Schwann cells but, resulted in significant reduction of myelin thickness in most of the cells (Figure 7).

Discussion

Neurotoxic effect of paclitaxel on peripheral nerves in rats

Peripheral neuropathy that induced by chemotherapeutic substances such as paclitaxel, thalidomide, and cisplatin represents beside other adverse effects of these drugs, a major clinical problem due to the frequency of this toxic process and the lack of therapeutic measures to treat the resultant disability. Furthermore, this adverse effect often represents the dose-limiting factor in therapeutic oncologic regimen, where higher doses might be otherwise desirable (3,19, 20).

In this study, a motor nerve conduction study (MNCS) of rat's sciatic nerves in the paclitaxel group showed marked prolongation in the mean latency, moreover there was a reduction in conduction velocity and amplitude compared to the control group. This pattern might explain the possibilities of axonal degeneration and demyelination with subsequent neuropathy. Axonal neuropathy is identified by nerve conduction study (NCS) as a low compound muscle action potential; pure demyelinating neuropathy is identified by NCS as slow conduction velocity and prolonged latency (21).

This result was in agreement with Lipton et al, (1989) and Sahenk et al, (1994) who have concluded that in paclitaxel-induced neuropathy, both axonal degeneration and demyelination patterns were possible on NCS.(22,23)

Authier et al, (2000) reported that exposure to paclitaxel at a single dose of 16 or 32 mg/kg did not change NCV in vitro; they concluded that this might be due to the low sensitivity of electrophysiological methods in early detection of neuropathy. The same researchers reported that NCV decreased following paclitaxel treatment 6mg/kg once a week for 5 weeks (24).

Moreover the model of paclitaxel-induced neuropathy in mature rats, with minimal effects on general health, by using two intravenous injections 12 mg/kg, 3 days apart, showed reduction in amplitudes of sensory compound nerve action potentials in the tail. Motor amplitudes were not affected, but both motor and sensory conduction velocities decreased. These effects persisted for at least 4 months after treatment (25).

This study also showed shortening of the tail withdrawal latencies for cold allodynia and hyperalgesia; there was shortening of the paw withdrawal latency in hot plate test. This is in agreement with several studies performed on laboratory animals in which Paclitaxel 6 mg/kg was given intraperitoneally (i.p.) once a week for 4 weeks which significantly shortened the paw withdrawal latency in acetone test compared with vehicle for cold hyperalgesia and decreased the travelled distance compared with vehicle in the balance beam test (11).

Flatters and Bennett, (2004) concluded that four (i.p.) injections on alternate days of 2 mg/kg paclitaxel induced a pronounced cold allodynia and hyperalgesia (26).

Polomano et al, (2001) in an experimental paclitaxel-induced painful peripheral neuropathy concluded that paclitaxel at low doses 0.5, 1 and 2 mg/kg caused heat-hyperalgesia and cold-allodynia, but had no effect on motor performance (27).

The mechanism of chemotherapy-induced neuropathy is still uncertain. Direct toxic damage to axons and Schwann cells and disturbed cytoplasmic flow are considered to be the main pathogenic factors (3).

Spontaneous improvement of nerve function over time, as observed in some animal models, suggests involvement of components of the nerve which have regenerative capacity, unlike neurons themselves (25). However, involvement of the vasa nervorum is a more attractive hypothesis since the majority of substances causing this type of neuropathy, i.e., paclitaxel, thalidomide, and cisplatin exhibit antiangiogenic properties in addition to their direct effects on tumor cells (28, 29).

Dvorak et al, (1995) and Kirchmair et al, (2005) found that the neuropathy caused by a chemotherapeutic drug was due to destruction of the blood supply of the nerve, i.e., the vasa nervorum.(30,20)

Kirchmair et al. (2005) showed that cisplatin-induced neuropathy is associated with the induction of endothelial cell apoptosis and destruction of the vasa nervorum and is reversed or inhibited by the angiogenic cytokine vascular endothelial growth factor (VEGF) (20).

The mechanism of chemotherapy-induced neuropathy also could be due to chemotherapeutic drugs that cause high levels of oxidative stress and are thought to rely, in part, on using this stress mechanism to kill cancer cells, but Perumal and Shanthi (2005) concluded that oxidative stress might actually reduce the overall effectiveness of chemotherapy because oxidative stress slows the process of cell replicationbut, during cell replication, chemotherapy actually kills cancer cells, therefore slower cell replication can mean lower effectiveness of chemotherapy. One approach to addressing this problem is the addition of certain antioxidants at specific dosages to lessen oxidative stress, thus making the chemotherapy treatment more effective (31).

Cameron and Cotter (1997) in an experimental study have shown that reactive oxygen species (ROS) also have effects on blood vessel function, which compromise perfusion of several organs including peripheral nerves. That was responsible for the earliest defects in nerve function in experimental models and will exacerbate nerve damage by causing further ROS-dependent ischemia-reperfusion effects (32).

Protective effect benfotiamine on paclitaxel neurotoxicity

The result obtained in this study from daily administration of benfotiamine 100 mg /kg orally for eight weeks together with paclitaxel 6 mg/kg weekly for four weeks, showed significant decrease in latency of sciatic nerve and subsequently an increase in nerve conduction velocities. An increase in amplitude of compound motor action potential reached that of the control group. The protective effect of benfotiamine against paclitaxel induced neuropathy could be explained by radical scavenging property of benfotiamine, because benfotiamine exhibited an antioxidant effect by reducing the oxidative stress and genomic damage caused by mitogenic model compounds; such effect was found to be related to the direct antioxidant effect of benfotiamine (33).

Cameron and Cotter, (1999) in an antioxidant study observed that oxidative stress makes a marked contribution to the etiology of nerve dysfunction in experimental diabetes because reactive oxygen species (ROS) cause vascular endothelium dysfunction which reduces NO mediated vasodilatation and increases local vasoconstrictor production and reactivity. This reduces nerve perfusion, causing endoneurial hypoxia which results in conduction deficits (33).

However nitric oxide (NO) is an important vascular target for ROS. Superoxide neutralizes NO and the peroxynitrite formed is a source of hydroxyl radicals that can cause endothelial damage (34, 35).

Regarding peripheral nerves, Nagamatsu et al, (1995) and Low et al, (1997) suggested that ROS can directly damage neurons and Schwann cells (36,37).

Recently, a new study showed that benfotiamine reduces superoxide and hydroxyl radical levels in the heart of diabetic mice by inducing the activation of pentose phosphate pathway, which regenerates the antioxidant NADPH (38).

Cascinu et al, (2002) suggested that increased levels of the reduced form of glutathione may be one of the possible mechanisms to prevent neurotoxicity because of glutathione's possible mechanism in reducing neurotoxicity of platinum-based drugs. Reactive oxygen species generated by platinum drugs result in neuronal cell death. GSH, as an ROS scavenger, may prevent such damage (39).

This result is supported by an in vitro study in which cisplatin induced apoptosis of mouse neurons, was prevented by pre incubation with N-acetylcysteine, a precursor to GSH (40).

The result of this study is in agreement with the result of a study performed on experimental animals where NCV was normalized by benfotiamine after three months of administration (41, 42).

Moreover in this study, compared to the control group no significant changes were observed in withdrawal latency of tail immersion test for cold (allodynia and hyperalgesia) and paw withdrawal latency in hot plate test in mice treated with oral benfotiamine 100 mg/kg, daily for six weeks and paclitaxel 6 mg/kg, i.p. once a week for four weeks, This result is in agreement with Winkler et al, (1999) who concluded that benfotiamine is effective in large doses and even in smaller daily dosages in treatment of painful diabetic neuropathy(43).

Protective effects of methylcobalamin on paclitaxel neurotoxicity

In this study, the results obtained from administration of methylcobalamin 500 µg /kg, intraperitoneally twice weekly and paclitaxel 6 mg/kg once a week for four weeks, showed decrease in latency of sciatic nerve and subsequently increase in nerve conduction velocity in comparison to the paclitaxel receiving group but did not reach that of the control group.

While the amplitude of compound motor action potential (CMAPs) was very low in sciatic nerve compared to the control group, this may be due to insufficient dose of methylcobalamin in this study as Yamatsu et al, (1976) observed that daily injection of 500 µg /kg of methylcobalamin produced a significant increase in the weight of the soleus muscle which recovered to the extent of being the same weight of the contra lateral 4 weeks after the nerve-crush. These results suggest that methylcobalamin may have an inhibitory effect on Wallerian degeneration and also a facilitatory effect on the neural regeneration of the crushed sciatic nerve of rats (44). Watanabe et al, (1994) examined the effects of ultra-high dose of methylcobalamin on the rate of nerve regeneration in rats with acrylamide neuropathy, using the amplitudes of compound muscle action potentials (CMAPs) after tibial nerve stimulation as an index of the number of regenerating motor fibers. Those treated with ultra-high dose showed significantly faster CMAP recovery than saline-treated control rats, whereas the low-dose group showed no difference from the control (45).

Furthermore the result of this study, did not show significant change in withdrawal latency of tail immersion test for cold allodynia/hyperalgesia and paw withdrawal latency in hot plate test in mice treated with oral methylcobalamin 500 µg /kg, daily for six weeks and paclitaxel 6 mg/kg once a week for four weeks compared to the control (saline treated) group. This is in agreement with Mizukami et al, (2011) who suggested that correction of neural oxidative stress may be attributed to the beneficial effects of methylcobalamin (10 mg /kg every other day, intramuscularly which is a higher dose than the dose used in this study) in normalization of nerve conduction velocity of diabetic nerve (46).

Histopathology

In this study histological examination by light microscope showed features of segmental demyelination, such as a thinning and destruction of myelin sheaths, nucleus degradation of Schwann cells and multiple different size cells in the paclitaxel receiving group compared to the control group. The result of this study was in agreement with Hashimoto et al. (2004) who concluded that the local paclitaxel injection showed features of segmental demyelination, such as a marked decrease in large-diameter myelinated nerve fibers, thinning and destruction of myelin sheaths, and atrophy of axons (47). Furthermore histological changes in the paclitaxel group agreed with Kawashiri, (2009) who concluded that Paclitaxel (6 mg/kg, i.p.) induced the decrease in the density of myelinated fibers and the degeneration of myelinated fibres in rat sciatic nerve(11).

In this study, light microscope histopathology examination of sciatic nerve in a group that received benfotiamine 100 mg /kg daily and paclitaxel 6 mg/kg weekly for four weeks, showed nerve fiber architecture near to that of the control group, in which most of the cell had normal nucleus, normal myelin thickness and maintenance of basement membrane of Schwann cells. This result might be explained by improvement of a nerve conduction study in this group as Raso et al, (2005) and Mazzer et al, (2008) concluded that maintenance of the basement membrane of Schwann cells surrounding the original nerve fibers were intact despite the disrupted axon enabled Schwann cells to provide pathways to guide the regenerating axons (48,49). After contact with the periphery is established, the regenerating nerve fibers enlarge and myelinate (50).

Light microscope histopathologic examination of sciatic nerve in rats which received methylcobalamin 500 µg /kg, (i.p.) two times weekly and paclitaxel 6 mg/kg once a week for four weeks, showed a marked reduction in myelin thickness and multiple different size nerve fibers but, with maintenance of basement membrane and nucleus of Schwann cells. This result showed that methylcobalamin 500 µg /kg, two times weekly enhanced improvement of nerve but did not reach that of the control group, as Yagihashi et al, (1982) observed that methylcobalamin at high daily dose of 500 µg /kg for 16 weeks resulted in decreased demyelination and protection of nerve fiber density and size in streptozotocin-diabetic rats (51).

Conclusion

Benfotiamine 100mg/kg was very efficient in prevention of sensorimotor neuropathy induced by paclitaxel. Whereas the suggested methylcobalamin (500µg/kg) twice weekly did not sufficiently prevent peripheral motor nerve destruction induced by paclitaxel, while the administration of high dose methylcobalamin every day is efficient in removal of thermal nociception induced during paclitaxel treatment.

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