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DHP107, a novel oral paclitaxel formulation induces less peripheral neuropathic pain and pain-related molecular alteration than intravenous paclitaxel preparation in rat

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ABSTRACT: Paclitaxel is used in the treatment of cancer especially in breast, stomach and ovarian cancer. However, peripheral neuropathic pain (PNP) induction is the most common devastating side effect of paclitaxel treatment. The objective of this study was to evaluate the PNP related behavioral changes in rats following oral administration of a novel oral paclitaxel formulation DHP107 (Liporaxel) in comparison with another popular intravenous paclitaxel preparation (Taxol). The rats were equally divided in to three groups namely, NC group (normal control): was treated with saline in a matched volume, LPX group (Liporaxel were administered orally) and TAX group (Taxol was administered intravenously). Less pain like behaviors were observed in LPX group in comparison with TAX group evidenced by significant higher level of hot plate paw withdrawal threshold (PWT), von Frey filament PWT and mechanical PWT than TAX group. Reduced lipid peroxidation and elevated antioxidant activities in serum, dorsal root ganglion (DRG) and sciatic nerve (SN) in LPX group than TAX group. In addition, cell apoptosis and expression of pain and neuropathy related proteins activation in LPX group was found lowered and myelin sheath thickness was higher in DRG and SN but not significantly different from the TAX group. Therefore, oral DHP107 could be a promising chemotherapeutic agent due to inducing less PNP.

KEYWORDS: Paclitaxel, oral formulation, neuropathic pain.

INTRODUCTION

Chemotherapy-induced peripheral neuropathic pain (CIPN) following neurotoxicity is one of the unwanted devastating dose-limiting side effects of antineoplastic drugs treatment. It triggers sensory, motor and autonomic system dysfunction as well as deteriorates the long-term quality of life of cancer survivors [1]. It occurs up to 50% of patients treated with standard doses of chemotherapy but almost 100% of patients given with second dose or high doses [2, 3]. This circumstance ultimately made a panic situation for patients to continue chemotherapy. In addition, many considerable strategies such as OPTIMOX (stop and go) have been proposed for CIPN control [4, 5]. However, dose modification or halting can enhance cancer-related morbidity and mortality. So, overcoming the neuropathic pain induced by pain is crucial and challenging. For controlling CIPN, analgesic drugs such as amitriptyline or gabapentin have been using but not successful to alleviate CIPN in randomized, placebo-controlled clinical trials [6, 7]. Moreover, chronic gabapentin treatment induces significant adverse effects, including peripheral edema, sedation, dizziness, and ataxia [8]. Therefore, an alternative safe approach of chemotherapy is important

to treat or prevent CIPN. Safe and less inducing neurotoxicity drugs could be the best solution of this problem.

Paclitaxel is a taxane derived microtubule-binding antineoplastic drug that is first line treatment for solid tumors [9], most commonly used in the treatment of breast, stomach ovarian, non-small cell lung carcinomas, and Kaposi sarcoma [10-13]. Paclitaxel is commonly used intravenous formula, such as, Taxol® and Abraxane® for the treatment of cancer. Unfortunately, Taxol® or Abraxane® induced peripheral neuropathy is a common side effect of anti-cancer treatment with an incidence of 30 to 50% following a single dose, elevating to more than 50% after a second dose [3, 14]. Thus, the devastating effects of CIPN limit the choosing of cytostatic drugs, delays subsequent treatment cycles, and leads to discourage of therapy. DHP107 (Liporaxel®), is a novel lipid-based single-agent oral paclitaxel formulation developed by Daehwa Pharmaceutical Co. Ltd.(Seoul, Republic of Korea) that is systemically absorbed without the need for P-glycoprotein inhibitors or cremophor EL [15, 16]. The absorption, mechanism and anti-cancer activities of Liporaxel® had been extensively studied previously [15-20]. However, the objective of this study was to evaluate the CIPN related behavioral changes in rats following oral administration of a novel oral paclitaxel formulation (Liporaxel®) in comparison with another popular intravenous paclitaxel preparation Taxol®.

MATERIALS AND METHODS

Animals and experimental design

White Sprague-Dawley male rats (Orient Bio, Gapyeong, Gyeonggi-do, Korea) were used for this study. The rats were housed in controlled environment with temperature of (23±2)°C, humidity of (50 ± 5)% with a 12–12 h light-dark cycle with lighting time with 150~300 Lux illumination and ventilation frequency of 10 to 20 times/hr. Food and water were available ad libitum before and after started experiment. After a week of adaptive feeding, average body weight was 264 ±1.18 g.

The 24 rats were equally divided in to three groups namely, NC group (normal control): was treated with saline in a matched volume, LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol®, Bristol-Myers Squibb Company, New York, United States) administered intravenously once a week, 10 mg/kg in at 10 ml/kg). The dosage and routes were selected in accordance with the clinical application and formulation of this drug in the field. All experimental protocols employed herein were approved by the committee on the care of laboratory animal resources, KNOTUS Co., Ltd, Republic of Korea, (Certificate number: IACUC 17-KE-355).

Pain related behavioral tests

Paw pressure test

The nociceptive threshold in the rat was measured by an analgesiometer (Ugo Basile, Varese, Italy). Briefly, application of continuously increasing pressure to a small area of the dorsal surface of the paw was performed using a blunt conical probe by a mechanical device. The paw withdrawal threshold of mechanical pressure was expressed as gram (g) and applied in lightly restrained rats until vocalization or a withdrawal reflex occurred. Experiments were performed blindly for three times. Paw pressure test was performed before and after once a week after drug administration.

Von Frey test

In order to observe the painful behavior von Frey filament (diameter of about 0.5 mm) was used. The animal was placed in a plastic box with a wire net on the floor and stabilized for about 20 minutes to adapt to the environment. Mechanical stimulation is applied to the foot pad by increasing the strength gradually by avoiding sharpen or violent application. The flinching time for the filament stimulation was limited to the positive reaction and at that time the filament strength was measured. For accurate measurement, the von Frey filament was repeatedly pressed three times at intervals of about 10 seconds under pressure until it was bent slightly. Before the test substance administration, after once/week.

Hot plate test

Sensitivity to thermal stimulation was performed before and once a week after the drug administration by hot-plate test. The animals were placed on a transparent acrylic chamber with a hot plate metal floor, then record the time in seconds when the pain-related behaviors (ie,

lifting and licking of the hind paw) response was first observed. The hot plate temperature was maintained at 49-50°C and the cut-off time was 60 seconds. The examination was carried out for three times and the mean was calculated at 10 seconds intervals.

Measurement of oxidative stress profiles

At 8 weeks after measurement of all pain related behaviors the rats were sacrificed after anesthesia in an induction chamber by isoflurane 3.5% for 2–4min as previously described [21]. Blood serum, sciatic nerve and dorsal root ganglion of lumbar 5 and 6 vertebra were collected for the measurement of biochemical profiles analysis. Concentrations of malondialdehyde (MDA) in serum and cardiac tissue were measured with lipid Peroxidation (MDA) Colorimetric/Fluorometric Assay Kit (Biovision, Mountain View, CA, USA). Serum and tissue levels of superoxide dismutase (SOD) were quantified using Superoxide Dismutase (SOD) Activity Colorimetric Assay Kit; Glutathione Peroxidase (GPx) Activity Colorimetric Assay Kit and levels of glutathione (GSH) were measured using a Glutathione Colorimetric Assay Kit; (Biovision, Mountain View, CA, USA) according to the protocol instruction.

Histopathological examination

To observe the demyelination, glutaraldehyde fixed gluteal ganglion and sciatic nerve tissue (4 µm-thick) were fixed with osmium, and finally processed into resin-embedded plastic blocks. Blocks were sectioned to semithin thickness and stained with toluidine blue O. After the ROI (region of interest) was determined in a 400 × magnification image obtained with cellsense software, the area of stained Toluidine blue (% respectively).

Immunohistochemistry staining was performed using 4 µm-thick tissue sections to quantify the number of activated GFAP (Glial fibrillary acidic protein)-positive astrocytes and ionized calcium-binding adapter molecule (Iba-1)-positive microglia, to quantify protein oxidative modification by nitrotyrosine and MPZ (Myelin protein zero) to measure level of demyelination. The tissue sections were first washed twice with PBS and incubated with blocking reagent (4 % Bovine Serum Albumin in PBS containing 0.3 % Triton-X 100) for 30 min. The sections were then incubated overnight with the primary antibodies anti-GFAP (1:1000), anti-Iba-1 (1:1000), anti-nitrotyrosine (1:800) and anti-MPZ (1:800) at 4°C. The sections were washed and incubated with secondary anti-rabbit (GFAP and anti-Iba-1) or anti-mouse (nitrotyrosine) antibody for 30 min at room temperature. Sections were then washed and counter stain Mayers Hematoxylin, mounted on slides. Immunohistochemically staining was performed using an image analyzer (Zen 2.3 blue edition, Carl Zeiss, Germany), and histopathologic changes were observed using an optical microscope (Olympus BX53, Japan). Percentage of stained Area (%) was compared between groups.

TUNEL assay

TUNEL assay of tissue sections used ApopTag® Peroxidase In Situ Apoptosis Detection Kit (S7100, Millipore, USA). Incubated the tissue slices with proteinase K for 10 min followed by 3 % H2O2 in methanol for 15 min to inactivation of endogenous peroxidase. TdT was added at room temperature and incubated overnight. Dark brown color showed DNA breaks after incubation with DAB (3, 3'-diaminobenzidine tetrachloride) and hydrogen peroxide, followed by counterstaining with methyl green. Percentages of positive TUNEL staining cells within cardiac areas were estimated.

Statistical Analysis

Data were expressed as means± standard error of the mean (SEM). Differences between groups were evaluated by Bonferronipost hoc test following one-way ANOVA or two-way ANOVA. We analyzed the differences by using Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $p < 0.05$.

RESULT

Effects of drugs administration on the body weight

Before the drug administration, the body weight (BW) of NC, LPX and TAX groups were 265.33±6.05, 264.20±5.40 and 264.11±6.55 g, respectively. However,

after the drugs administration, the weekly body weights of LPX and TAX groups were lowered until the end of the experiment but BW of TAX group was significantly differed from 2 weeks ($p < 0.001$) while LPX group from 5 weeks ($p < 0.05$) than NC group. In addition the BW of TAX group was significantly lowered since 3 weeks ($p < 0.01$) until the end of the experiment ($p < 0.01$) than LPX group. At the end of the experiment (8 weeks) the BW of three groups were 520.89±33.87, 476.49±33.44 and 411.45±28.56 g, respectively (Fig 1 A).

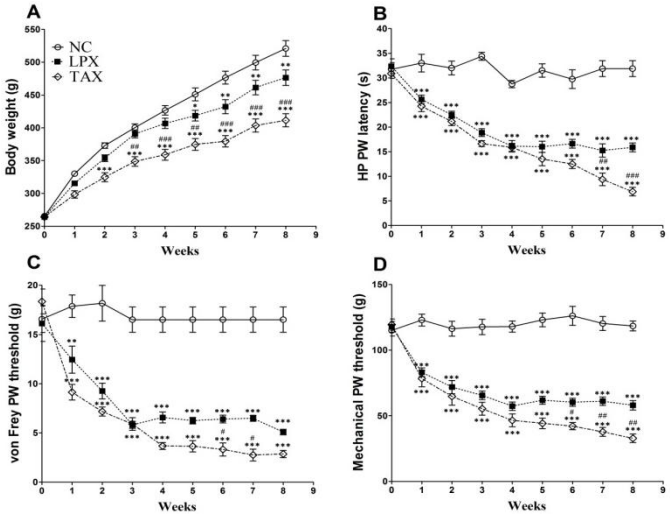

Figure 1. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on body weight and mechanical thresholds in rats. NC group (normal control): was treated with saline in a matched volume; LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol® was administered intravenously once a week, 10 mg /kg in at 10 ml/kg). HP, hot plate; PW, paw withdrawal. The data are reported as the mean ±SEM (n = 10).*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, Bonferronipost hoc test following two-way ANOVA versus the NC group. #, $p < 0.05$; ##, $p < 0.01$ and ###, $p < 0.001$, Bonferronipost hoc test following two-way ANOVA versus the LPX group.

Effects of drugs administration on pain related behaviors

The pain related behavioral changes were measured by thermal paw withdrawal latency, von Frey filament test and analgesiometer before and after drug administration weekly. Before drug administration there were no significant differences among all groups in all tests (Fig 1 B, C, D). However, after the drugs administration thermal paw withdrawal latency in both LPX and TAX groups significantly lowered when compared to NC group until the end of the experiment. Interestingly, the thermal latency of TAX group was significantly lowered than LPX from 7 weeks ($p < 0.01$). At 8 weeks the time of latency of TAX group was significantly lowered (6.9±2.4 s, $p < 0.001$) than LPX group (15.9±2.5s) where as in NC group it was 30.8±4.7s (Fig 1 B). On the other hand paw withdrawal thresholds (PWT) measured by analgesiometer were also significantly lowered after drug administration in both LPX and TAX group significantly lowered in comparison with NC group from 1 weeks to the end of the experiment. Moreover, at 6 to 8 weeks the PWT of TAX group (32.9±9.1) was significantly reduced when compared LPX group. At 8 weeks the PWT of TAX group was significantly lowered (32.9±9.1g, $p < 0.01$) than LPX group (58.0±10.1g) where as in NC group it was 118.5±10.6g (Fig 1 C).

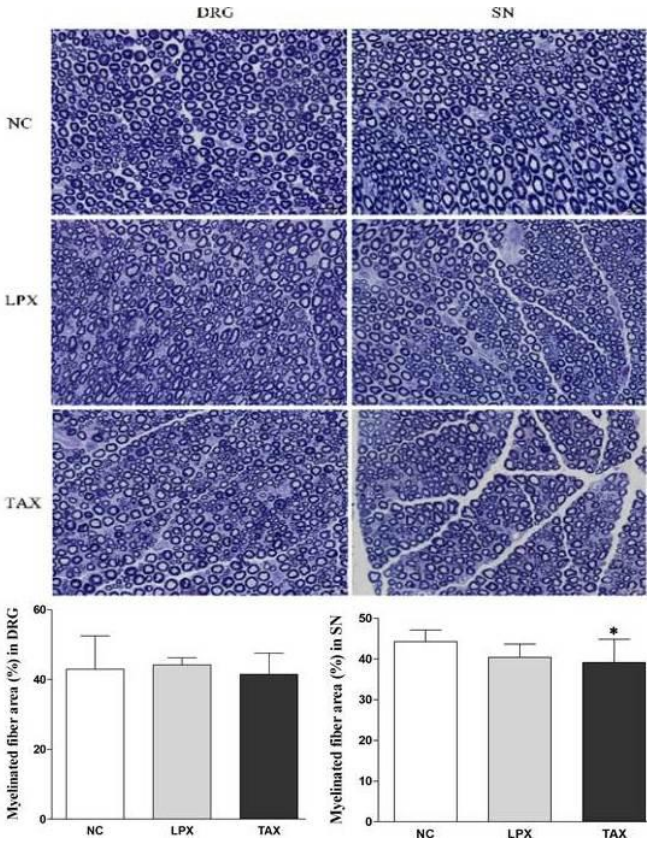

Figure 2. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on morphometric changes of dorsal root ganglion and sciatic nerve. NC group (normal control): was treated with saline in a matched volume; LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol® was administered intravenously once a week, 10 mg /kg in at 10 ml/kg). DRG, dorsal root ganglion; SN, sciatic nerve. The data are reported as the mean ±SEM (n = 10).*, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the NC group. #, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the LPX group but no significance change was observed.

Pain like behavior was also further confirmed by von Frey filament test and like before, similar tendency was also confirmed. After drug administration in both LPX and TAX group significantly lowered in comparison

with NC group from 1 week to the end of the experiment. At 8 weeks the PWT of LPX group (5.1±0.8g) was higher than TAX group (2.9±1.0g) where as in NC group it was 17.8±4.0g (Fig 1 D).

Effects of drugs administration oxidative stress and antioxidant activities

Drug administration both in LPX and TAX group caused a significant increase in lipid per oxidation/MDA concentration in serum, DRG (L5 and L6) and sciatic nerve compared with the NC group. However, in TAX group the lipid per oxidation level were significantly higher in comparison with LPX group. In addition, the amounts of GSH, Gpx activity and SOD activity were significantly reduced in serum, DRG (L5 and L6) and sciatic nerve following drug administration in both LPX and TAX group when compared with NC group. Interestingly, these parameters were also significantly higher in the LPX group than TAX group (Table 1).

Table 1. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on antioxidant activities in rat

	Sample name	NC	LPX	TAX
MDA	Serum (nmol/ml)	15.68±1.22	19.75±0.81***	23.49±0.63***###
	Sciatic nerve (nmol/mg prot)	0.18±0.01	0.14±0.01**	0.16±0.01***###
	DRG L5 (nmol/mg prot)	0.12±0.02	0.15±0.02	0.18±0.05**
	DRG L6 (nmol/mg prot)	0.11±0.01	0.12±0.01**	0.14±0.01**###
SOD activity	Serum (inhibition %)	82.09±10.49	64.37±6.25**	55.78±8.21***
	Sciatic nerve (inhibition, %)	86.98±7.60	68.679±8.31***	46.89±9.91***###
	DRG L5 (inhibition, %)	81.89±4.96	62.74±10.41**	51.42±15.22***
	DRG L6 (inhibition, %)	78.77±13.79	54.06±12.33**	43.02±12.87***
Gpx activity	Serum (mU/mL)	3.64±1.35	2.37±0.74*	1.66±0.75**
	Sciatic nerve (mU /mg prot)	3.61±0.90	2.42±0.46**	1.97±0.54***
	DRG L5 (mU /mg prot)	6.72±1.12	2.89±1.50***	1.60±0.81***
	DRG L6 (mU /mg prot)	5.87±1.17	3.04±1.57***	1.52±1.06***#
GSH	Serum (% to NC)	100.00±14.66	75.94±10.16***	59.19±14.54***##
	Sciatic nerve (% to NC)	100.03±3.90	76.86±4.43***	70.01±3.00***##
	DRG L5 (% to NC)	100.00±7.83	85.84±7.86**	70.18±5.99***###
	DRG L6 (% to NC)	100.00±13.91	70.99±7.47***	58.332±4.34***#

NC, normal control; LPX, Liporaxel® treated group; TAX, Taxol® treated group. MDA, malondialdehyde (Lipid peroxidation); SOD, superoxide dismutase; Gpx, glutathione peroxidase; GSH, total glutathione; DRG, dorsal root ganglion. The data are reported as the mean ±SEM (n = 10). *, p< 0.05; **, p< 0.01; ***, p< 0.001, Bonferronipost hoc test following one-way ANOVA versus the NC group. #, p< 0.05; ##, p< 0.01 and ###, p< 0.001, Bonferronipost hoc test following one-way ANOVA versus the LPX group.

Effects of drugs administration on histopathology alterations

The analysis of the changes in the area of the nerve fibers using electron microscopy, the area of the nerve fibers in the sciatic nerve of the TAX group was significantly lower than the NC group (p <0.05). In the LPX group, there was no significant difference in the area of the dorsal root ganglion and the sciatic nerve fibers in the sciatic nerve compared to the normal control group (Fig 2). In addition, as a result of analysis using immunohistochemical staining in this test, there was no significant difference in all test groups. The levels of GFAP, IBA1 and Nitro in LPX group were lower than the TAX group in posterior ganglion (DRG) tissues (Fig 3) and left sciatic nerve (Fig 4). In addition, TUNEL assay for confirming tissue apoptosis showed that the number of apoptotic-positive cells in sciatic nerve and DRG was also lower in LPX group than the TAX group (Fig 5).

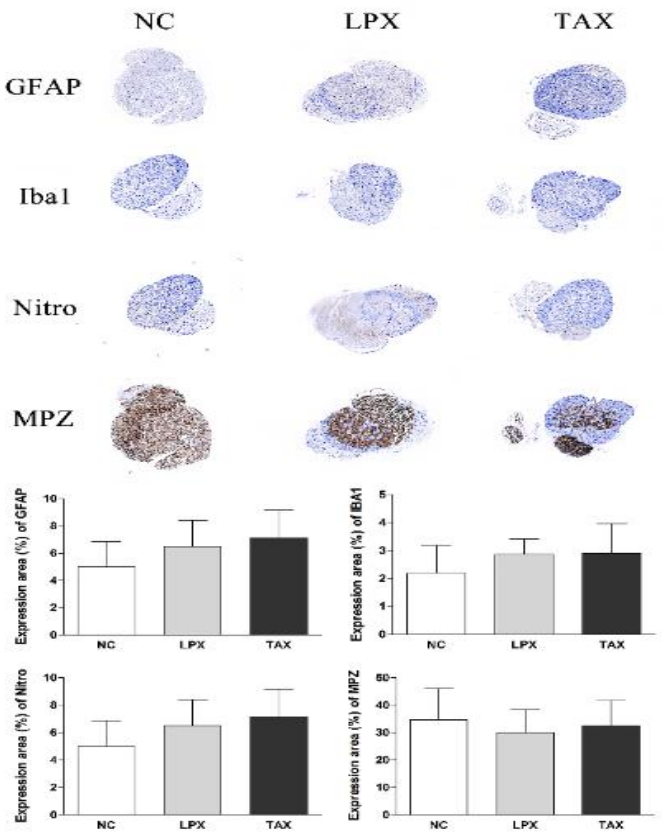

Figure 3. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on the expression of GFAP, Iba1, Nitro and MPZ in the dorsal root ganglion and sciatic nerve in rat. NC group (normal control): was treated with saline in a matched volume; LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol® was administered intravenously once a week, 10 mg /kg in at 10 ml/kg). GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding

adapter molecule-1; Nitro, nitrotyrosine and MPZ, Myelin protein zero. The data are reported as the mean ±SEM (n = 10).*, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the NC group. #, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the LPX group but no significance change was observed.

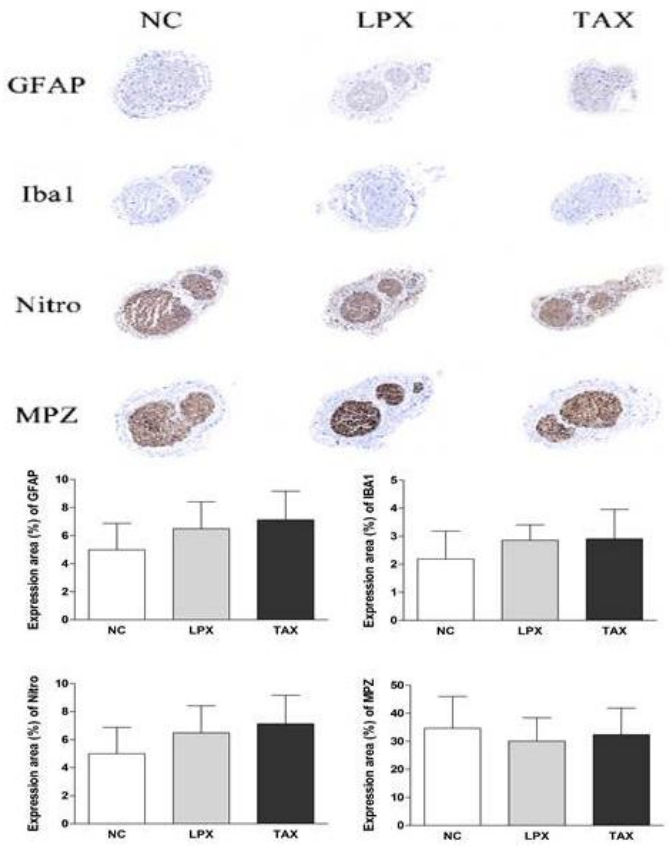

Figure 4. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on the expression of GFAP, Iba1, Nitro and MPZ in the sciatic nerve in rat. NC group (normal control): was treated with saline in a matched volume; LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol® was administered intravenously once a week, 10 mg /kg in at 10 ml/kg). GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium-binding adapter molecule-1; Nitro, nitrotyrosine and MPZ, Myelin protein zero. The data are reported as the mean ±SEM (n = 10).*, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the NC group. #, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the LPX group but no significance change was observed.

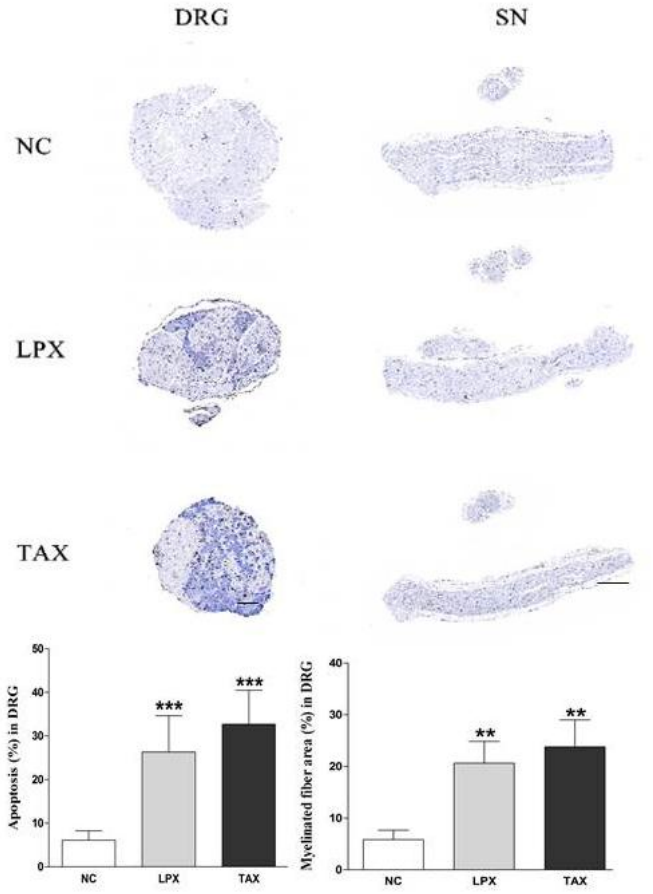

Figure 5. Effect of oral paclitaxel (Liporaxel®) and intravenous paclitaxel (Taxol®) on the apoptosis in the dorsal root ganglion and sciatic nerve in rat. NC group (normal control): was treated with saline in a matched volume; LPX group (Liporaxel® soln were administered orally at 25 mg/kg body weight at 2.5 ml/kg, twice a week) and TAX group (Taxol® was administered intravenously once a week, 10 mg /kg in at 10 ml/kg). DRG, dorsal root ganglion; SN, sciatic nerve. (Scale bar in case of DRG = 200 µm and incase of SN 1000 µm). The data are reported as the mean ±SEM (n = 10).*, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the NC group. #, $p < 0.05$ Bonferronipost hoc test following one-way ANOVA versus the LPX group but no significance change was observed.

DISCUSSION

Paclitaxel treatment induced neuropathic pain in normal rats in this experiment reflected by the significant increased pain like behaviors such as lowered PW latency in hot plate test, von Frey filament PWT threshold and mechanical PW threshold in comparison with NC group. Interestingly, when comparison in between LPX and TAX group, it was clearly showed that all of these parameters were significantly higher in the LPX group indicating the less neuropathic pain inducing effects. To elucidate the underlying molecular mechanism, lipid per oxidation, antioxidant activities in serum, DRG and sciatic nerve were measured. Neuron morphometry (myelinated area), expression of pain and

neuropathy related activated proteins and cellular apoptosis were investigated in DRG and sciatic nerve in all groups.

The oxidative stress is one of the key pathogenic mechanisms involved in nerve damage and pain following chemotherapy. It is the central mediator of neuro inflammation, apoptosis, mitochondrial dysfunction metabolic disturbances and bioenergetics failure in neurons [22]. These mechanisms are directly related with cellular oxidative stress, which damages antioxidant defense system and neuronal damage and finally result in cell death [22, 23]. Therefore, many studies measured the antioxidant activities and several oxidative stress markers to assess the state of oxidative stress after chemotherapy in animals, and concluded that chemotherapy can induce excessive oxidative stress and cause peripheral neurotoxicity [16, 22-24]. Similar types of alteration after paclitaxel administration were also reported [9, 25]. Based on this reports, stress-associated mechanism of CIPN was tested in this study. In this study it was also observed that lipid peroxidation increased significantly and GSH, Gpx and SOD activities were reduced significantly following paclitaxel administration in both LPX and TAX group in comparison with NC group in serum, DRG and sciatic nerve tissue. However, these alterations in LPX group were significantly better than TAX group which might be responsible for inducing less neuropathic pain. In addition, the expression of nitrotyrosine was also investigated in this study to evaluate the level of protein oxidative modification as nitrotyrosine is considered to be a marker of oxidative stress [23, 26, 27]. The DRG and sciatic nerve sections of treated groups showed no significance difference but increased compared with NC group but it was lowered in LPX group in comparison with TAX group. This result also further confirmed that oral Liporaxel® treatment induced less oxidative damage on sciatic nerve and DRG.

Following oxidative stress, astrocytic cell are activated which contributes to mechanical allodynia in a rat chemotherapy induced neuropathic pain model [28]. Similarly, glial cells also activated in chemotherapy induced neuropathic pain [29, 30]. Basically, astrocytes and microglia are important special glial cells populations in the nervous cells are involved in maintaining homeostasis regulation of pH and ionic balance, uptake of neurotransmitter and degradation, and neuro inflammation manipulation in physiological and pathophysiological conditions in the nervous systems [30, 31]. GFAP is a specific marker of astrocytic activation and Iba-1 is specific marker microglial activation in the nervous system [31, 32]. Therefore, we have evaluated the expression of GFAP and Iba1 proteins in the sciatic nerve and DRG. It was found that the expression of GFAP and Iba1 were increased in both LPX and TAX groups than NC but both the markers were slightly lower in LPX group accompanied a lower pain threshold than TAX group.

Furthermore, paclitaxel induced peripheral neuropathy and pain is represented by decreased density of myelinated fibers, axonal degeneration, and demyelination of nerve fibers [33]. In this study, such paclitaxel induced significant demyelination of nerve fibers were observed in sciatic nerve in the TAX group than NC group but in the LPX group demyelination was also observed but not markedly. These results also accurately reflected with the severity of neuropathic pain behavioral responses in the LPX and TAX groups of this study. In addition we have measured the MPZ (Myelin protein zero) expression in sciatic nerve and DRG. MPZ is a major component of the myelin sheath of the peripheral nervous system and decreased in chronic neuropathic pain state [34]. Accordingly, linking with the neuropathic pain behavior and demyelination MPZ expression was also decreased in the treatment groups (LPX or TAX group) as compared to NC group, however, such difference is not significant.

Apoptosis has taken main target of chemotherapy to induce programmed cell death in cancerous tissues. Unfortunately, conventional chemotherapeutic agents not only elicit apoptosis in cancerous cells but also in other normal tissue of body [35, 36]. If any drugs inhibit the diversity of apoptosis in normal cell, it would be more effective and less toxic chemotherapeutic agent. In this study, we indeed have investigated apoptotic cells in rats and found that less apoptosis in DRG and sciatic nerve tissue in LPX group as compared to the TAX group. This result also consistent with the pain like behavior in the LPX and TAX groups.

In the view of above results and discussion, it is concluded that Liporaxel® administration induced significant lowered pain behavior in rats compared to the Taxol®-treated group along with less oxidative stress, apoptosis, pain and neuropathy related molecular protein

alterations than Taxol® treated group. Therefore, Liporaxel® could be a promising chemotherapeutic agent due to inducing less PNP as compared to the intravenous paclitaxel formula, e.g., Taxol®.

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AUTHOR CONTRIBUTIONS

Md. Mahbubur Rahman, Minhee Son, In-Hyun Lee, Junhee Jang and Sokho Kim designed the experiment and draft the manuscript. Md. Mahbubur Rahman, Minhee Son, Hyun Kim, In-Hyun Lee, Ha-young Jeon Myung-Jin Kim, Hansol Kwon and Sung-Jin Park carried out the experiments and analyzed the data. The manuscript was carefully revised by all authors. In-Hyun Lee, Junhee Jang and Sokho Kim supervised the research work and finalized the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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