Effects of Ganglioside on Paclitaxel (Taxol) Induced Neuropathy in Rats

VILAI CHENTANEZ, MD, PhD*,
SOMPOL SANGUANRUNGSIRIGUL, BSc, MSc, MD**,
NAWASORN PANYASAWAD, BSc, MSc*

Abstract

The effects of ganglioside on paclitaxel induced neuropathy were studied in 15 female Wista rats. The animals were equally divided into 3 groups based on the type of administrated drug. The first (C-group) received an intraperitoneal weekly injection of 1 ml of NSS for five weeks. The second (P-group) received 9 mg/kg of a paclitaxel intraperitoneal weekly injection for five weeks. The third (PG-group) received both ganglioside and paclitaxel. Sensory evaluation and electrophysiologic studies of the tail nerve were performed before the administration of the first dose and at the end of the experiment. Morphological evaluation of the sciatic nerve was also studied. The results revealed the mean reaction time of the tail flick test, latency, amplitude and nerve conduction velocity of the P-group in the first and seventh week were of significant difference. However, there was no significant difference detected in those of the C-group and the PG-group. There was significant difference in all parameters between the PG and P-groups but not between the PG and C-groups. Cross sections of the sciatic nerve in the P-group showed mild endoneurium edema and some degenerated axons. Small myelinated nerve fibers in the PG-group were prominent. The results of this study indicated that ganglioside treatment could produce some beneficial effects in an animal model of paclitaxel induced neuropathy.

Key word : Paclitaxel Induced Neuropathy, Taxol, Neuropathy, Ganglioside

CHENTANEZ V,
SANGUANRUNGSIRIGUL S, PANYASAWAD N
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* Department of Anatomy,
** Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Paclitaxel (Taxol), a natural extract from the bark of *Taxus brevifolia,* is a promising antineoplastic agent[1,2]. It is also known to be neurotoxic in humans[3]. Neurophysiological and morphological evaluations of taxol-induced neuropathy have been studied in several experiments[4-12]. The possibility of reducing the toxic effects of paclitaxel on the peripheral nerves would, therefore, be of great clinical interest. Nerve growth factor and adrenocorticotropic hormone analogue have shown their potentiality to reduce the toxic neuropathy[12,13]. Recently, it has been found that oral administration of glutamine 24 h after the completion of paclitaxel can reduce the severity of peripheral neuropathy[14]. Gangliosides belong to a family of sialic acid-containing glycosphingolipids that modulate cell-cell and cell-matrix interactions. Several experiments revealed the preventive effects of ganglioside treatment in diabetic neuropathy [15-19]. Ganglioside treatment can alter the abnormality of axonal transport of cytoskeletal proteins such as actin and tubulin in diabetic rats[19]. Paclitaxel binds to microtubules, specifically the β-subunit of tubulin [20] at the N-terminal domain[21]. This reduces the critical concentration of tubulin which is required for polymerization, shifting the dynamic equilibrium toward microtubule assembly and increasing the rate and yield of polymerization[22]. Paclitaxel-induced microtubules are shorter and considerably more flexible [23] than those produced normally. Nerve cells contain large quantities of microtubules which take part in the outgrowth of neuronal processes and axonal transport and they are, therefore, susceptible to the toxic effects of paclitaxel. In this regard, the authors developed an animal modeled investigation to find out whether ganglioside can prevent taxol induced neuropathy *in vivo.*

MATERIAL AND METHOD

Animal and drug administration

Fifteen young adult female Wistar rats, weighing 200-250 g each were used in the present study. They were equally divided into 3 groups. The C-group was the control group, each of which received

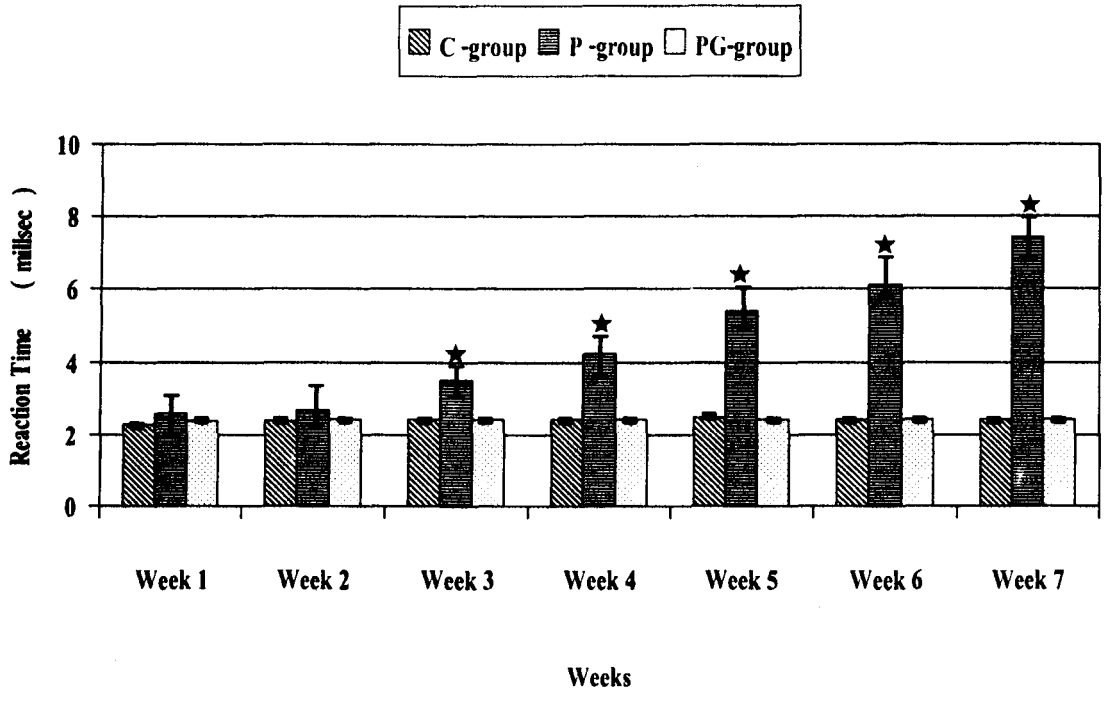

Fig. 1. Histogram of the mean reaction time for the tail flick test in each group.

★ Statistical significance at $p < 0.05$.

1 ml of normal saline intraperitoneally once a week for five weeks; the P-group, each of which received 9 mg/kg of paclitaxel intraperitoneally once a week for five weeks; the PG-group, each of which received 50 mg/kg of ganglioside intramuscularly daily for three days before the first dose of paclitaxel, then six times per week throughout the experiment. The animals in this group received placlitaxel at the same dose and were scheduled as in the P-group. From a previous study this dose of paclitaxel induced neuropathy from week 3 onwards[13]. The total period of the experiment was seven weeks.

Sensory evaluation

The rats in each group were assessed for their thermal pain threshold once a week prior to the administration of the drug or normal saline by the Harward Tail flick analgesia meter. The assessment was also done one week before and after the experiment. The tail of the rat was placed on the photocell, then the lamp that eliminated infrared which would cause heat was switched on. The time span from the elimination of the infrared until the rat flicked its tail from the photocell was measured as the reaction time which was timed five times in each turn. The mean reaction time was then calculated.

Neurophysiological evaluation

Neurophysiological evaluation was also done in the same way, the nerve conduction velocity in the tail nerve of each animal was measured by the Electromyograph Mem 3202 (Neuropack). The tail nerve was stimulated 50 times with supramaximal stimuli with ring electrodes at a distance of 5 cm and 10 cm from the fixed distal recording ring electrodes and averaged traces were used to calculate the conduction velocity directly on the oscilloscope screen.

Morphological examinations

After all measurements were performed, the rat was anesthetized by injection of 50 mg/kg sodium pentobarbital intraperitoneally. The sciatic nerve was then dissected and removed. It was fixed in 2 per cent

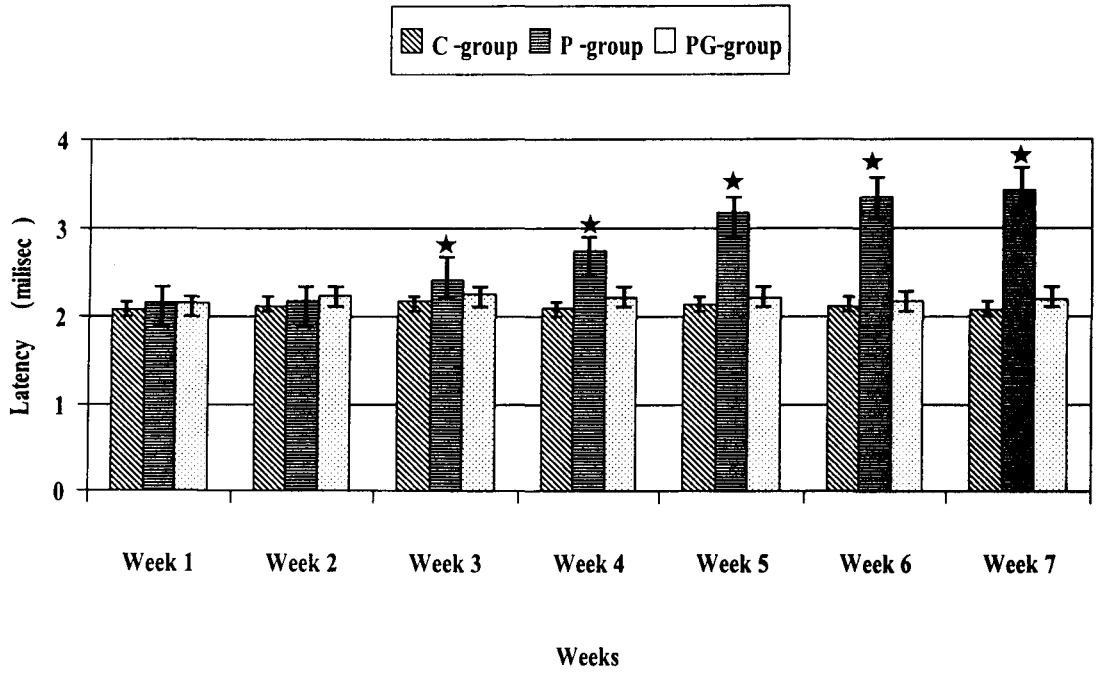

Fig. 2. Histogram of the mean latency in each group.

★ Statistical significance at $p < 0.05$.

glutaraldehyde for 2 h, rinsed in cacodylate buffer, postfixed in OsO_4 and embedded in epoxy resin. Semethin sections (1 μm) stained with methylene blue were used for light microscope morphological examination.

Statistical evaluation

The nonparametric test (Kruskal Wallis One way Analysis of Variance by Rank) was employed to compare the data obtained from the three groups.

RESULTS

Sensory evaluation

The mean reaction time of the tail flick test of each group is shown in the histogram in Fig. 1. The mean reaction time of the C-group in the first and seventh week were 2.29 ± 0.11 and 2.45 ± 0.11 msec. In the PG-group they were 2.39 ± 0.10 and 2.42 ± 0.09 msec, in the P-group, they were 2.59 ± 0.14 and 7.42 ± 0.63 msec. The reaction time was prolonged and it had statistically significant difference at $p < 0.05$ from the third week.

Neurophysiological evaluation

The mean latency, amplitude and nerve conduction velocity are shown in histograms, Fig. 2, 3 and 4, respectively. The mean latency in the control group was about 2.08 msec, but it was prolonged in the P-group (3.42 ± 0.15 msec in the seventh week). In the PG-group, the mean latency in the first and seventh weeks were 2.16 ± 0.10 and 2.2 ± 0.06 msec without any difference of statistical significance. The amplitude in the P-group was decreased, beginning from one week after the second dose of paclitaxel. 95 per cent confidence limits revealed a significant difference between the control and the P-group. The nerve conduction velocity was also significantly decreased in the P-group, but no change in the PG-group was detected.

Morphological evaluation

Transverse sections in paclitaxel treated rats showed only a slight decrease of myelinated nerve fibers and some degenerated axons were observed.

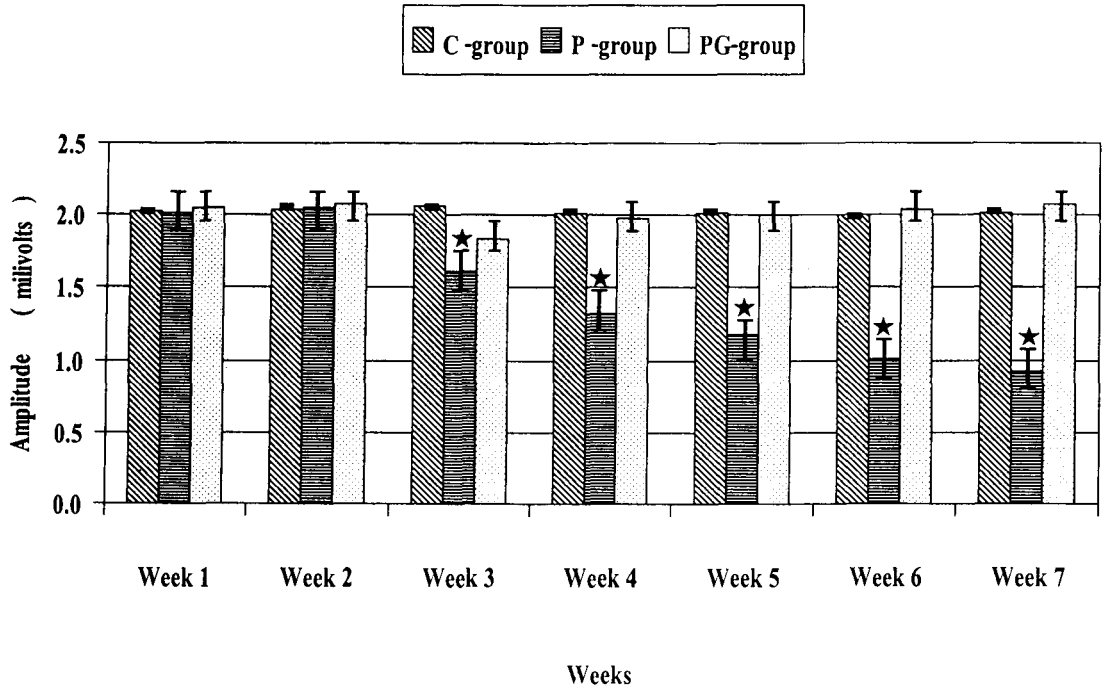

Fig. 3. Histogram of the amplitude in each group.

★ Statistical significance at $p < 0.05$.

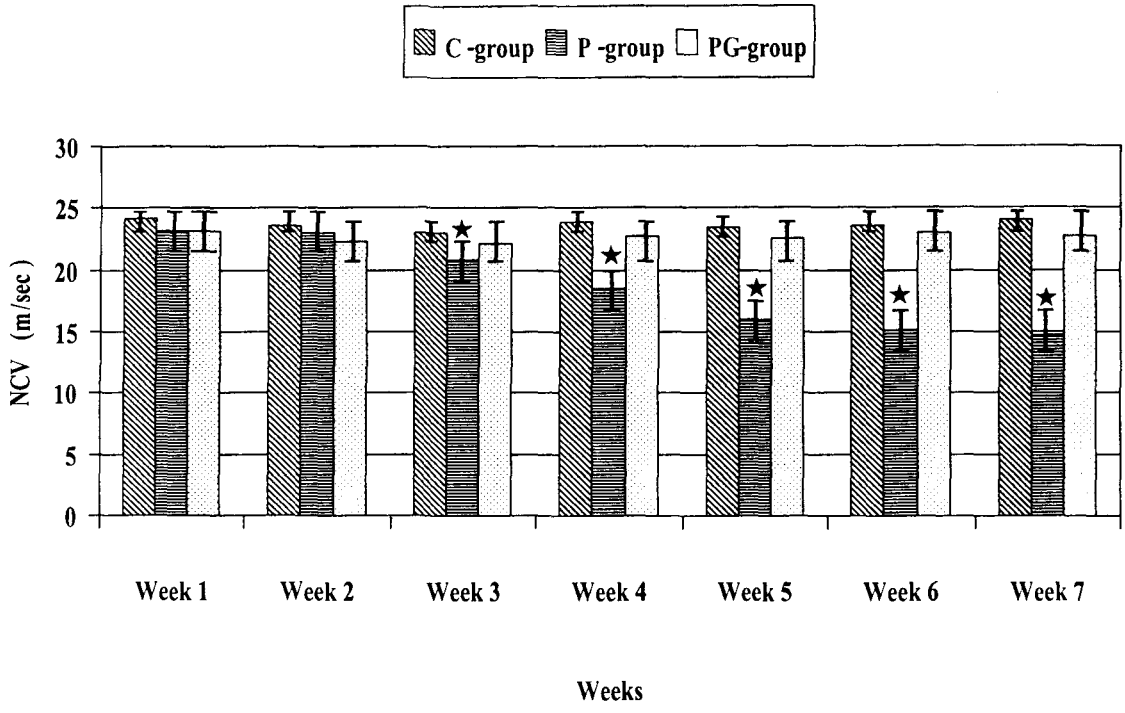

Fig. 4. Histogram of the nerve conduction velocity (NVC) in each group.

★ Statistical significance at $p < 0.05$.

Mild endoneurium edema was seen in some cases. Small myelinated nerve fibers in the PG-group were prominent.

DISCUSSION

The study demonstrates that paclitaxel administration intraperitoneally in repeated doses induces peripheral neuropathy. Gangliosides have potentiality prevents toxic neuropathy, which may due to the dose-limiting toxicity of life-saving drugs. The authors reported that rats with taxol (P-group) had reduced thermal pain sensation, demonstrated by a significantly prolonged reaction time in tail flicking compared with the control group. Rats which were treated with ganglioside as well as taxol did not have any significant difference from the control. The authors were able to demonstrate some prolongation of latency and reduction of amplitude with paclitaxel administration. Ganglioside administration prevented reduction of the compound sensory amplitude and prolongation of the latency.

However, it is not clear how ganglioside prevents taxol neuropathy. Since gangliosides can alter the abnormality of axonal transport of cytoskeletal proteins such as actin and tubulin[19], it is possible that gangliosides promote axonal transport which was interrupted by abnormal microtubules produced by paclitaxel. The preservation of the nerve conduction velocity in the PG-group may be due to the action of ganglioside on myelination of Schwann's cell[24]. Although the clear neurobiological role of gangliosides has not been defined, recent studies have proposed the gangliosides serve as complementary ligands for myelin-associated glycoprotein (MAG)[25,26]. MAG, a minor constituent of both peripheral and central myelinating glia, is localized predominantly to the periaxonal glial plasmalemma[27]. Because of its periaxonal location, it is postulated that MAG may mediate axon-glial interactions[28]. Based on the results of several experiments[29-31], it has been proposed that MAG plays an important role in maintaining the integrity of myelin and axon which is one of the glial signals that regulates axonal caliber and

in part, myelination. Mice lacking complex gangliosides, develop Wallerian degeneration and myelination defects[32].

Morphological examination in the sciatic nerve of the P-group and PG-group showed only slight changes. A slightly decreased number of large myelinated fibers was observed, and some degenerated axons were seen. The results were not different from previous studies[33]. In the PG-group, small myelinated nerve fibers were prominent.

SUMMARY

Administration of taxol to rats resulted in profound sensory neuropathy characterized by prolonged reaction time of the tail flick test, diminished amplitude of compound action potential, prolonged latency period and reduced nerve conduction velocity in the tail nerve. Coadministration of gangliosides prevented all of these signs of neurotoxicity. These findings suggest that the administration of gangliosides may prevent certain taxol induced neuropathy.

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ผลของแกงกลิโอไซด์ต่อพยาธิสภาพของเส้นประสาทที่เกิดจากยาพาคลิแทคเซล (แทคซอล)

วิไล ชินธเนศ, พบ, ปรด,*
*สมพล สงวนรังศิริกุล, วทบ, วทม, พบ**, นวสรณ์ ปัญญาสวัสดิ์, วทบ, วทม**

ได้ทำการศึกษาถึงผลของแกงกลิโอไซด์ต่อพยาธิสภาพของเส้นประสาทที่เกิดจากการได้รับยาพาคลิแทคเซล ในหนูเพศเมียพันธุ์วิสตา จำนวน 15 ตัว โดยแบ่งสัตว์ทดลองเป็น 3 กลุ่มเท่า ๆ กัน ตามยาที่ได้รับ กลุ่มแรก (กลุ่ม C) ได้รับการฉีดน้ำเกลือปริมาตร 1 มล เข้าช่องท้อง สัปดาห์ละ 1 ครั้ง เป็นเวลา 5 สัปดาห์ กลุ่มที่สอง (กลุ่ม P) ได้รับการฉีดยาพาคลิ-แทคเซล ขนาด 9 มก/กก เข้าช่องท้อง สัปดาห์ละ 1 ครั้ง เป็นเวลา 5 สัปดาห์ กลุ่มที่สาม (กลุ่ม PG) ได้รับการฉีดยาทั้งพาคลิแทคเซล และแกงกลิโอไซด์ ผู้วิจัยได้ทำการประเมินการรับความรู้สึก และศึกษาทางสรีรวิทยา ไฟฟ้าของเส้นประสาทที่หางหนู สัปดาห์ละ 1 ครั้ง โดยเริ่มต้นตั้งแต่ก่อนการให้ยา 1 สัปดาห์ จนถึงภายหลังการให้ยาอีก 1 สัปดาห์ หลังจากนั้นได้ทำการศึกษาลักษณะทางจุลกายวิภาคของเส้นประสาท Sciatic ของสัตว์ทดลองทุกตัว ผลการศึกษาพบว่าค่าเฉลี่ยของ reaction time ของ tail flick test latency amplitude และ nerve conduction velocity ในสัปดาห์ที่หนึ่ง และสัปดาห์ที่เจ็ด มีความแตกต่างอย่างมีนัยสำคัญทางสถิติในกลุ่ม P แต่ไม่พบความแตกต่างในกลุ่ม C และ กลุ่ม PC และเมื่อเปรียบเทียบกันในระหว่างกลุ่ม PG และกลุ่ม P พบว่ามีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติในทุกพารามิเตอร์ที่วัด แต่ไม่พบความแตกต่างในระหว่างกลุ่ม PG และกลุ่ม C เส้นประสาท Sciatic ที่ถูกตัดตามขวาง พบว่า ในกลุ่ม P ชั้น endoneurium บวมเล็กน้อยและมีบาง axon ที่เสื่อม ในกลุ่ม PG มีปริมาณของเส้นประสาทชนิดมีเปลือกหุ้มขนาดเล็ก ๆ จำนวนมาก ผลของการศึกษานี้ชี้ให้เห็นว่าการให้ยาแกงกลิโอไซด์ มีผลในทางที่เป็นประโยชน์ต่อการเกิดพยาธิสภาพของเส้นประสาทที่เกิดจากยาพาคลิแทคเซลในสัตว์ทดลอง

คำสำคัญ : พาคลิแทคเซล, แทคซอล, พยาธิสภาพของเส้นประสาท, แกงกลิโอไซด์

วิไล ชินธเนศ, สมพล สงวนรังศิริกุล, นวสรณ์ ปัญญาสวัสดิ์
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* ภาควิชากายวิภาคศาสตร์,
** ภาควิชาสรีรวิทยา, คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, กรุงเทพ ฯ 10330