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Phenyl *N*-tert-butylnitrone, a Free Radical Scavenger, Reduces Mechanical Allodynia in Chemotherapy-induced Neuropathic Pain in Rats

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ABSTRACT

Background: Paclitaxel is a widely used chemotherapeutic drug for breast and ovarian cancer. Unfortunately, it induces neuropathic pain, which is a dose-limiting side effect. Free radicals have been implicated in many neurodegenerative diseases. The current study tests the hypothesis that a free radical scavenger plays an important role in reducing chemotherapy-induced neuropathic pain.

Methods: Neuropathic pain was induced by intraperitoneal injection of paclitaxel (2 mg/kg) on four alternate days (days 0, 2, 4, and 6) in male Spraue-Dawley rats. Phenyl *N*-tert-butylnitrone (PBN), a free radical scavenger, was administered intraperitoneally as a single dose or multiple doses before or after injury. Mechanical allodynia was measured by using von Frey filaments.

Results: The administration of paclitaxel induced mechanical allodynia, which began to manifest on days 7–10, peaked within 2 weeks, and plateaued for at least 2 months after the first paclitaxel injection. A single injection or multiple intraperitoneal injections of PBN ameliorated paclitaxel-induced pain behaviors in a dose-dependent manner. Further, multiple administrations of PBN starting on day 7 through day 15 after the first injection of paclitaxel completely prevented the development of mechanical allodynia. However, an intraperitoneal administration of PBN for 8 days starting with the first paclitaxel injection did not prevent the development of pain behavior.

Conclusions: This study clearly shows that PBN alleviated mechanical allodynia induced by paclitaxel in rats. Furthermore, our data show that PBN given on days 7 through 15 after the first paclitaxel injection prevented the development of chemotherapy-induced neuropathic pain. This clearly has a clinical implication.

What We Already Know about This Topic

- Paclitaxel chemotherapy is often limited by painful neuropathy
- Free radicals may be involved in other types of neuropathic pain, but their role in chemotherapy-induced neuropathy has not been examined

What This Article Tells Us That Is New

- In rats, the free radical scavenger phenyl-*N*-tert-butylnitrone reduced hypersensitivity from paclitaxel but did not prevent it when coadministered
- Free radical scavengers may be useful in the treatment of paclitaxel-induced neuropathy

CHEMOTHERAPY-INDUCED peripheral neuropathy is a major side effect of many chemotherapeutic agents, including taxanes, platinum-based agents, and vinca alkaloids. Paclitaxel (Sigma, St. Louis, MO), one of the taxanes, is a widely used chemotherapeutic drug for the treatment of breast cancer, cervical cancer, ovarian cancer, non-small cell lung carcinomas, and Kaposi sarcoma.[1,2] It binds to β-tubulin of microtubules, thereby stabilizing microtubules and enhancing polymerization, thereby exerting its anticancer mechanism of action.[3,4] However, it produces peripheral neurotoxicity, which is characterized by pain, numbness, tingling, and burning sensation.[5] This peripheral neuropathic pain is a dose-limiting side effect. This is reported with paclitaxel monotherapy at the dose of greater or equal to 250 mg/m^2 body surface area in as many as 22–100% of patients[6,7] or as a combination therapy with cisplatin or vincristine. There are no effective treatment options for chemotherapy-induced neuropathic pain partly due to its unknown pathomechanisms.[8]

Free radicals, including superoxide radical, hydrogen peroxide, nitric oxide, and nitroperoxide, have been implicated in many neurodegenerated diseases such as Alzheimer disease, amyotropic lateral sclerosis, and brain dysfunction due to injury or aging.[8–11] They easily react with nucleic acid, protein, and lipid and usually have short-half life. In normal conditions, they play a role in a number of biologic processes, including cell signaling process, and are removed by antioxidant systems such as catalase, superoxide dismutase, gluta-

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thione, and glutathione peroxidase. Thus, their levels are precisely controlled by antioxidant systems. However, in pathologic conditions, their levels increase due to either increased production or impaired antioxidant system.

Phenyl *N*-tert-butylnitrone (PBN) is a spin-trap reagent and a potent free radical scavenger. To date, PBN has been shown to have an analgesic effect in various animal models of pain including the spinal nerve ligation model, capsaicin-induced secondary hyperalgesia, formalin-induced pain, and zymosan-induced visceral pain.[12–15] Furthermore, other free radical scavengers, such as vitamin E and Tempol, produced significant analgesic effects in both neuropathic pain[16–18] and inflammatory pain models.[19–21] However, the effect of PBN on chemotherapy-induced neuropathic pain has not been reported.

Thus, the aims of this study were to determine: (1) whether PBN ameliorates paclitaxel-induced neuropathic pain in rats and (2) whether PBN prevents paclitaxel-induced neuropathic pain in rats.

Materials and Methods

Experimental Animals

Male adult Sprague-Dawley rats weighing 200–350 g (Harlan Sprague-Dawley Co., Houston, TX) were used in this study. The animals were housed in groups of two or three in plastic cages with soft bedding and free access to food and water under a 12/12-h light–dark cycle (dark cycle: 7:00 PM–7:00 AM). All animals were acclimated in their cages for 1 week before any experiments were performed. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Miami (Miami, Florida) and were carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Paclitaxel-induced Neuropathic Pain Model

Paclitaxel (Sigma) was dissolved in dimethyl sulfoxide at a concentration of 50 mg/ml and stored in a freezer (−60°C). The solution was mixed with an equal volume of Tween 80 and then diluted in sterile saline to a concentration of 2 mg/ml just before injection. Paclitaxel (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6; cumulative doses of 8 mg/kg) as previously described[22] to induce painful peripheral neuropathy. Control animals were injected with the same volume of vehicle (4% dimethyl sulfoxide and 4% Tween 80 in saline). The time course of the mechanical allodynia was measured before and at various time points after paclitaxel injection.

Phenyl N-tert-butylnitrone Administration

PBN (molecular weight, 177.24) was obtained from Sigma Chemical Company. It was dissolved in saline to two different concentrations, namely, (1) to a concentration of 10 mg/ml for the dose of 50 mg/kg and (2) to a concentration of 20 mg/ml for the dose of 100 mg/kg. Thus, all animals received a volume of 5 ml/kg.

Multiple Injection of PBN in Normal Rats. To examine the sedative and hyperalgesic effect of PBN, PBN (100 mg/kg) was injected intraperitoneally twice daily for 3 consecutive days starting on day 0 in normal rats.

Single or Multiple Injection of PBN in Established Paclitaxel-induced Neuropathic Pain Model in Rats.

Single Injection. The analgesic effect of PBN was evaluated when PBN was given as a single intraperitoneal injection in an established paclitaxel-induced neuropathic pain model in rats. Mechanical allodynia was measured after various doses of PBN by using the randomized Latin square design to minimize the number of rats.[12,18,23] Hence, treatment of paclitaxel was done on four alternate days (days 0, 2, 4, and 6) in six rats. Beginning on the 34th postpaclitaxel injection day, mechanical allodynia was measured, and rats were randomly divided into three groups (saline, PBN 50, and PBN 100). Rats in each group received an intraperitoneal injection of one of the two doses of PBN (50 or 100 mg/kg) or saline as a control. Then, behavioral tests were repeated at 0.5, 1, 2, 4, and 6 h postinjection. After wash-out periods of 3 or 4 days, all rats were then crossed over and got and the next injection, and behavior testing was repeated at the 39th and 42nd postpaclitaxel injection days. Thus, each rat received all three treatments (two doses of PBN and saline) in a random order by the end of the testing period.

Multiple Injections. The analgesic effects of PBN were determined when multiple intraperitoneal injections of PBN were given after paclitaxel-induced pain had developed. At the 35th postpaclitaxel injection day, 11 rats were divided into two groups, namely, PBN group and control group. Rats in the PBN group received PBN (100 mg/kg) twice daily at 12-h intervals for 3 consecutive days, and the control group received saline (5 ml/kg) twice daily for 3 consecutive days. Behavioral tests to determine mechanical allodynia were performed a day before the injections and the rest of the experimental period.

Multiple Injections of PBN to Prevent Paclitaxel-induced Neuropathic Pain in Rats. PBN (100 mg/kg, intraperitoneal) was given twice a day in two different paradigms: (1) starting with the first injection of paclitaxel for 8 days (days 0–7) and (2) starting 7 days after the first injection of paclitaxel for 9 days (days 7–15). Mechanical allodynia was measured repeatedly before and on experimental days 9, 11, 14, 16, 18, and 21 for experiment I or experimental days 2, 4, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 21, 23, 25, 28, 30, 32, 35, 37, and 39 for experiment II after each treatment of PBN.

Behavioral Tests for Mechanical Allodynia

Behavioral tests were conducted blindly so that the experimenter who conducted the tests did not know the nature of the experimental treatment. The behavioral tests measured were foot withdrawal thresholds (as indicator for mechanical allodynia) in response to mechanical stimuli applied to the

left and right hind paws.[24] For each test, the animals were placed in a plastic chamber (8.5 × 8.5 × 28 cm) and habituated for at least 15 min. The chamber was placed on top of a mesh screen, so that mechanical stimuli could be administered to the plantar surface of the left and right hind paws. Thresholds were determined by the up–down method[25] by using a set of von Frey monofilaments (von Frey filament values: 3.65, 3.87, 4.10, 4.31, 4.52, 4.74, 4.92, and 5.16; equivalent to: 0.45, 0.74, 1.26, 2.04, 3.31, 5.50, 8.32, and 14.45 g values). A von Frey filament was applied perpendicularly to the most sensitive areas of the plantar surface at the center area of paw or the base of the third or fourth toes with sufficient force to bend the filament slightly for 3–4 s. An abrupt withdrawal of the foot during stimulation or immediately after stimulus removal was considered as a positive response. The first stimulus was always the 4.31 filament. When there was a positive response, the next lower filament was used, and when no response was observed, the next higher filament was applied. This testing pattern continued until responses to the sixth von Frey stimuli from the first change of response (either higher or lower than the first stimulus depending on whether the first response was negative or positive) were measured. The responses were then converted into a 50% threshold value using the formula: 50% threshold $= 10^{(X + kd)}/10^4$, where X is the value of the final von Frey hair used in log units, k is the tabular value for the pattern of positive or negative responses, and d is the mean differences between stimuli in log units (0.22). When positive or negative responses were still observed at the 3.65 or 5.16 filament, values of 0.3 or 18.6 g were assigned, respectively, by assuming a value of ±0.5 for k in these cases.

Tests for Sedation

The following assessments were made to see whether PBN or paclitaxel induces sedation, which interferes with posture and righting reflexes, as opposed to analgesia which does not.[12,26]

Five-point scale for posture:

0, normal posture, rearing, and grooming;
1, moderate atonia and ataxia. Weight support, but no rearing;
2, weight support, but severe ataxia;
3, muscle tone but no weight support and only small purposive movements; and
4, flaccid atonia, fully immobilized with no attempts at movement.

Five-point scale for righting reflexes:

0, rat struggles when placed on its side, followed by rapid forceful righting;
1, moderate resistance when the rat is placed on its side, with rapid but not forceful righting;
2, no resistance to the rat being placed on its side, with effortful but ultimately successful righting;
3, unsuccessful righting; and
4, no movements.

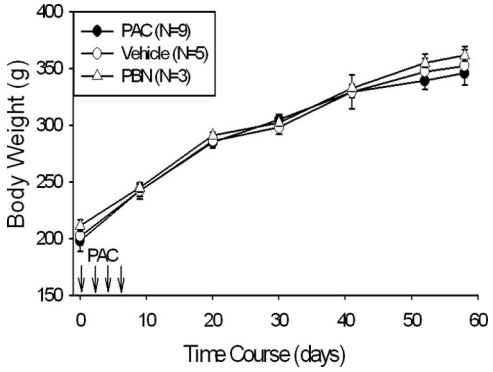

Fig. 1. The time courses of body weight in paclitaxel (PAC)-induced neuropathic pain in rats. PAC (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6, *downward arrows*) in rats. The vehicle group received equal volume (1 ml/kg) of 4% dimethyl sulfoxide and 4% Tween 80 in saline. Phenyl *N*-tert-butylnitrone (PBN) (100 mg/kg) was injected intraperitoneally twice a day for 3 days beginning day 0 in normal rats. There was no significant difference in body weight between the groups. Data are expressed as means ± SEMs.

Rats were scored immediately after each behavioral test in all experiments.

Data Analysis

Data are presented as means ± SEMs and analyzed using the SigmaStat program (Systat Software, Inc., Chicago, IL). Statistical analyses were done using two-way repeated-measures analysis of variances with two-repeated factors followed by Tukey *post hoc* test for the experiment of Latin square design or two-way repeated-measures analysis of variances with one-repeated time factor followed by Tukey *post hoc* tests. In all cases, $P < 0.05$ was considered statistically significant.

Results

Sedation

Because sedation can increase mechanical threshold, all rats treated with paclitaxel or PBN were assessed for sedation throughout the experimental periods. All rats in this study scored 0 indicating that they were not sedated for both posture and righting reflexes, suggesting that increase in mechanical allodynia observed in PBN-treated animals was indeed the result of its analgesic effect.

Paclitaxel-induced Neuropathic Pain Model in Rats

Rats treated with paclitaxel or PBN showed normal gain of body weight compared with control groups (fig. 1). The baseline mechanical thresholds of all rats before paclitaxel injection was 18.6 g, which was the value as the maximal cutoff point (fig. 2). Paclitaxel decreased the mechanical threshold of both hind paws. Further, the mechanical threshold of both hind paws did not differ significantly from each other. Thus, we used the right hind paw as the site to measure mechanical threshold. After paclitaxel treatment (2 mg/kg) on days 0, 2, 4, and 6, mechanical thresholds began to decrease on days 7–10 and reached its lowest levels (0.8 ±

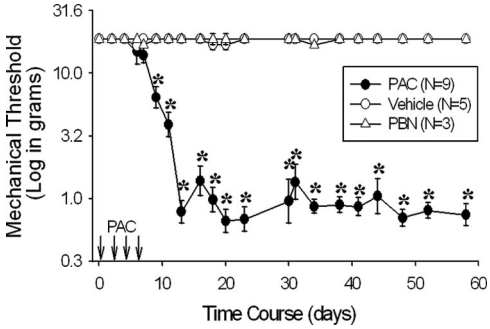

Fig. 2. The time courses of mechanical threshold in paclitaxel (PAC)-induced neuropathic pain in rats. PAC (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6; cumulated doses of 8 mg/kg, *downward arrows*) in rats. The vehicle group received equal volume (1 ml/kg) of 4% dimethyl sulfoxide and 4% Tween 80 in saline. Phenyl *N*-tert-butylnitrone (PBN) (100 mg/kg) was injected intraperitoneally twice a day for 3 days beginning on day 0 in normal rats. Note that PAC significantly decreased mechanical thresholds compared with the vehicle group. PBN or vehicle group did not increase mechanical threshold when given to normal animals. Data are expressed as means ± SEMs. *Asterisks* indicate values significantly different ($P < 0.05$) from corresponding vehicle values by using a two-way repeated measures analysis of variance with a repeated time factor, followed by Tukey *post hoc* test.

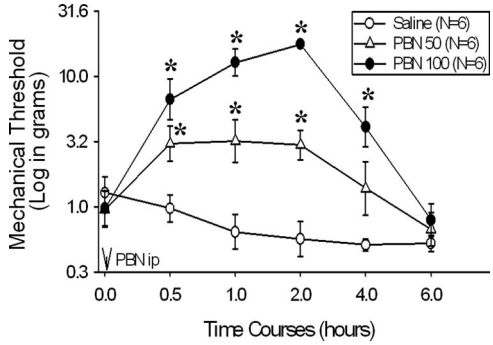

Fig. 3. Analgesic effect of systemic injection of single phenyl *N*-tert-butylnitrone (PBN) on established paclitaxel-induced neuropathic pain in rats. After measurements of mechanical thresholds for 2 days (days 0 and 1), paclitaxel (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6) in six rats. On the 34th postpaclitaxel injection day, the rats were divided into three groups, and behavioral tests were performed for 6 h followed by 3 or 4 days of wash-out period. The process was repeated on the 39th and 42nd post-paclitaxel injection day, so that each rat received all three treatments followed by behavioral testing after each treatment. Thus, each point represents the results from six rats. Note the normal or almost normal threshold level after the 100 mg/kg of PBN injections. Data are expressed as means ± SEMs. *Asterisks* indicate significant differences from the saline control group by a two-way repeated measures analysis of variance (two repeated factors), followed by the Tukey *post hoc* test. ip = intraperitoneal.

0.2 g) on days 12–16, and then plateaued for 45 days. Vehicle treatment (4% dimethyl sulfoxide and 4% Tween 80 in saline) did not change mechanical thresholds for 2 months. Furthermore, intraperitoneal injection of PBN (100 mg/kg, twice a day for 3 days starting day 0) in normal rats did not induce changes in mechanical thresholds (fig. 2).

Analgesic Effect of Single Injections of PBN on Established Paclitaxel-induced Neuropathic Pain

Changes in mechanical thresholds in paclitaxel-induced neuropathic rats after intraperitoneal injections of PBN (50 and 100 mg/kg) were shown in figure 3. The 100 mg/kg of PBN significantly increased mechanical threshold up to normal (>10 g of mechanical threshold) at 2 h after injection and returned to baseline at 6 h (fig. 3).

Analgesic Effect of Multiple Injections of PBN on Established Paclitaxel-induced Neuropathic Pain

To examine the prolonged analgesic effects of PBN, it was intraperitoneally injected twice daily at 12-h intervals for 3 days (a total of six doses) at the dose of 100 mg/kg during days 34–36 (fig. 4). The control group received an intraperitoneal injection of saline. Mechanical allodynia was measured once a day in the morning before injections of PBN. Repeated injections of PBN (100 mg/kg) significantly increased mechanical threshold at day 35 and maintained it for 4 days (fig. 4). These data indicate that repeated injections of PBN (100 mg/kg) produce analgesic effects in paclitaxel-induced pain behaviors without sedation.

Preventive Effect of Multiple Injections of PBN on the Development of Paclitaxel-induced Neuropathic Pain in Rats

To examine preventive analgesic effects, PBN (100 mg/kg) was given intraperitoneally twice daily starting either 1 h before the first paclitaxel injection (day 0) for 8 days (paradigm I) or on days 7 through 15 (for 9 days; paradigm II). The early treatment of PBN (paradigm I) did not affect the development of pain behaviors (fig. 5). However, the treatment of PBN starting on day 7 through day 15 completely prevented the development of mechanical allodynia for at least 30 days (fig. 6). Thus, PBN had potent preventive ef-

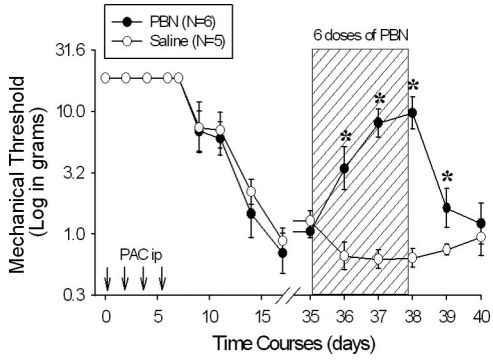

Fig. 4. Analgesic effect of systemic multiple phenyl *N*-tert-butylnitrone (PBN) administrations on established paclitaxel (PAC)-induced neuropathic pain in rats. Paclitaxel (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6, *down arrows*) in 11 rats, and subsequently, the thresholds were significantly reduced. PBN (100 mg/kg) was injected intraperitoneally twice daily at 12 h interval for 3 days beginning on day 35. The control group received an intraperitoneal (ip) injection of saline. Mechanical thresholds were measured once a day before injection of PBN. Repeated injection of PBN significantly increased mechanical threshold the next day (day 36) and maintained it for 4 days. Data are expressed as means ± SEMs. *Asterisks* indicate significant differences from the saline control group by a two-way repeated measures analysis of variance, followed by the Tukey *post hoc* test.

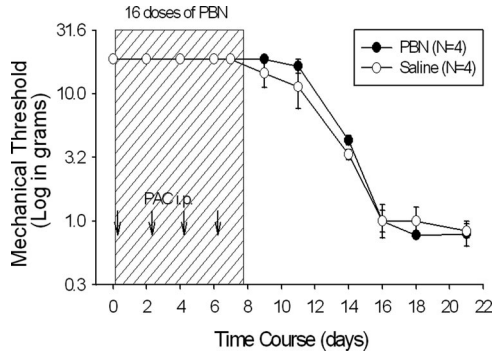

Fig. 5. Preventive effects of early treatment of phenyl *N*-tert-butylnitrone (PBN) on the development of paclitaxel (PAC)-induced neuropathic pain in rats. PAC (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6, *downward arrows*) in rats, and mechanical thresholds were measured. PBN (100 mg/kg) was given intraperitoneally twice a day starting 1 h before the first paclitaxel injection for 8 days (day 0 through day 7, ▨). Control animals received the same volume of saline (5 ml/kg). Note that PBN did not change the development of pain behaviors in rats. Data are expressed as means ± SEMs. ip = intraperitoneal.

fects in paclitaxel-induced neuropathic pain in rats when given in the early phase of developing mechanical allodynia.

Discussion

It is well known that oxidative stress plays an important role in the pathogenesis of degenerative diseases,[27] and neurodegenerative diseases are widely regarded as at least a partial consequence of reactive oxygen species (ROS) damage associated with increased levels of proinflammatory cytokines.[9,28,29] Moreover, ROS have been implicated in both neuropathic and inflammatory pain conditions because their production has been shown to be increased in injured peripheral nerve and inflamed tissue.[30–33] According to Kim *et al.*,[12] a barrage of primary afferent injury discharges at the time of nerve injury followed by a steady flow of ectopic discharges into the spinal cord in the neuropathic condition may increase mitochondrial respiration and intracellular calcium and, consequently, lead to increased ROS production. The authors also speculate that it is also possible that excessive ROS builds up in glial cells, which then leaks to produce neuronal damage and dysfunction. Further, it has also been speculated that ROS triggers second messenger system as second messengers are involved in sensitization of dorsal horn neurons[34,35] and also possibly activates spinal glial cells, which in turn play an important role in chronic pain.[36] Further, ROS accumulation was also observed primarily in the mitochondria of dorsal horn neurons after capsaicin treatment in mice,[19] suggesting that increased mitochondrial ROS may be an important mechanism of capsaicin-induced hyperalgesia and central sensitization.

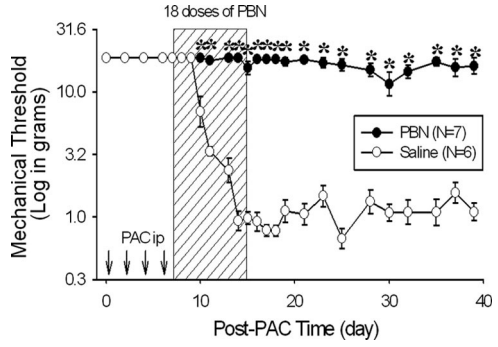

Fig. 6. Preventive effects of phenyl *N*-tert-butylnitrone (PBN) starting day 7 for 9 days on the development of paclitaxel (PAC)-induced neuropathic pain in rats. PAC (2 mg/kg) was injected intraperitoneally on four alternate days (days 0, 2, 4, and 6, *downward arrows*) in rats, and mechanical thresholds were measured. PBN (100 mg/kg) was given intraperitoneally twice a day starting on day 7 for 9 days (day 7 through day 15, ▨). Control animals received the same volume of saline (5 ml/kg). Note that PBN completely prevented the development of pain behaviors for more than 30 days. Data are expressed as means ± SEMs. *Asterisks* indicate values significantly different ($P < 0.05$) from corresponding control values by using a two-way repeated measures analysis of variance, followed by the Tukey *post hoc* test. ip = intraperitoneal.

Free radicals are derivatives of molecular oxygen and nitrogen and consist of superoxide, hydroxyl radical, hydrogen peroxide, and peroxynitrite.[37] These molecules are ubiquitously present in the body and participate in many normal cellular processes including ion transport, transcription, neurotransmission, neuromodulation, and immune responses.[37] Sources of free radicals are both mitochondrial oxidative metabolisms to produce adenosine triphosphate and several enzymes such as xanthine oxidae, phospholipase A2, cytochrome P450, monoamine oxidase, and tyrosine hydroxylase. They are normally removed by antioxidant systems including superoxide dismutase, catalase, glutathione, glutathione peroxidase, ascorbate, and α-tocopherol. Thus, their levels are precisely controlled by antioxidant systems. However, in pathologic conditions, levels of free radicals may increase due to the increased production or decreased antioxidants level.[38,39]

Conversely, antioxidants produce analgesia in both neuropathic[12] and inflammation pain.[13,21] However, the underlying mechanism by which ROS reduction alleviates pain remains unclear. Nonetheless, several investigators tried to address this using various neuropathic pain models. In the chronic constriction injury model of neuropathic pain in rats, systemic injection of antioxidants reduced heat hyperalgesia, suggesting that free radicals induced heat hyperalesia.[16,30] Others reported that systemic ROS scavengers ameliorate the behavioral signs of mechanical allodynia in the spinal nerve ligation model of neuropathic pain.[12] Further, in the capsaicin-induced pain model, an intradermal injection of capsaicin produced primary and secondary hyperalgesia as a result of peripheral sensitization and central sensitization, respectively.[40] Most importantly, ROS scavengers were observed to reduce pain in capsaicin-induced secondary hyperalgesia, suggesting ROS involvement in the spinal cord.[13]

The molecular mechanisms underlying paclitaxel-induced neuronal damage is a complex phenomenon that needs to be defined. However, Selimovic *et al.* addressed this

by studying the mechanism of apoptosis in human melanoma cell lines A375 treated with taxol. The authors found that the apoptotic neuronal damage was the result of activation of apoptosis signal-regulated kinase, c-jun NH2-terminal kinase, p38 mitogen-activated protein kinase, and extracellular-regulated kinase together with the down-regulation of uncoupling protein 2.[41] They also reported that taxol-induced ROS enhanced DNA-binding activity of the transcription factors activator protein-1, activating transcription factor-2 and Ets LiKe gene1, release of cytochrome *c*, and cleavage of caspases-9 and -3 and poly (ADP-ribose) polymerase. Further, the authors showed that pretreatment of melanoma cells with the c-jun NH-terminal kinase inhibitor[2] or the p38 inhibitor blocked taxol-induced uncoupling protein 2 down-regulation, ROS generation, and apoptosis. However, extracellular-regulated kinase inhibitor had no such effect. Their conclusion was that taxol-induced mitochondrial stress occurs through the activation of c-jun NH2-terminal kinase and p38 pathways and suggested a novel role for uncoupling protein 2 in the modulation of taxol-induced apoptosis of melanoma cells.

Conversely, PBN has been shown to have a neuroprotective property. Tsuji *et al.*[42] examined the effects of PBN on the p38 mitogen-activated protein kinase pathway and heat shock proteins and reported that the intraperitoneal administration of PBN before ischemia–reperfusion enhanced the activation of extracellular-regulated kinase, suppressed the activation of stress activated protein kinase/c-jun NH2-terminal kinase and p38, and increased the expression of heat shock protein 27 and heat shock protein70 *in vivo*. Their results indicate that PBN, which has a radical-scavenging property, protects against delayed neuronal death by regulating the p38 mitogen-activated protein kinase signaling pathway and induction of a tolerant state by upregulating heat shock protein in the brain. However, whether this phenomenon applies using our model of injury is yet to be seen.

When PBN is injected intraperitoneally in rats, it rapidly penetrates all organs including brain, spinal cord, and liver within 20 min, and is subsequently excreted in urine and has a half-life of approximately 134 min.[43] Thus, the sites of action of PBN are organs including both central nervous systems (brain and spinal cord) and peripheral nervous systems (sensory nerves, dorsal root ganglia, and nerve endings).

Even though it is not the subject of this study to investigate the site of action of PBN, we think it is important to address this by presenting the findings of other authors using a different model of injury, namely, spinal nerve ligation model. Given the fact that ectopic discharges originating from the injured peripheral nerve or dorsal root ganglia cells drive central sensitization that underlies neuropathic pain behavior[44–46] and the fact that systemic PBN (100 mg/kg), which ameliorated the neuropathic pain behaviors, did not reduce the ectopic discharges suggests that the site of action of PBN might not be these peripheral locations.[12] Specifically, we reported that PBN, ROS scavenger, did not change the rate of ectopic discharges originating from the dorsal root ganglia or peripheral nerve, and intrathecal injection produced a strong analgesic effect, suggesting that the major site of action was the spinal cord. Furthermore, we reported that a systemic administration of the spin-trap reagents PBN and 5,5-dimethyl-pyrroline-*N*-oxide had the greatest analgesic effect, intrathecal administration had almost as much of an effect, and intraventricular administration had a significant but much smaller effect, and the interpretation of our results was that the reagents worked primarily at spinal levels and somewhat at supraspinal levels.[12] Again, whether this is true for chemotherapy-induced neuropathic pain is to be determined.

Our study shows that PBN, a free radical scavenger, prevented and ameliorated pain behavior as measured by mechanical allodynia in the paclitaxel-induced neuropathic pain in rats. Further, PBN did not induce sedations at the doses we used, so that the behavioral changes are interpreted as analgesia. Our results also show that the time point of administering PBN is critical in preventing pain behaviors, namely, (1) systemic administration of PBN starting on day 0 through day 7 (before the induction of mechanical allodynia) did not show the preventive effect; (2) however, systemic administration of PBN starting on day 7 through day 15 (during the development of mechanical allodynia) completely prevented the development of pain behaviors; and (3) systemic single or repeated administration of PBN after day 15 (after the induction of pain behaviors) only temporarily ameliorated pain behaviors (mechanical allodynia).

It is well known that paclitaxel-induced neuropathic pain is a dose-limiting side effect, and its mechanism is not fully understood. In this model, nonsteroidal antiinflammatory drugs have little or no effect, and opioids have an analgesic effect only when given at high doses.[47] Conversely, anticonvulsants, antidepressants, and sodium channel blockers have effect only after repeated administrations.[47] Recently, Wolf *et al.*[8] reviewed the analgesic effects of intravenous calcium and magnesium treatment, vitamin E, glutamine, glutathione, *N*-acetylcysteine, oxcarbazepine, and xaliproden in clinical and preclinical studies and concluded that currently no drugs have been proven to prevent chemotherapy-induced neuropathy. In this study, PBN had a potent analgesic effect when given before mechanical allodynia was established. It also had a significant mechanical allodynia-reducing effect when given after mechanical allodynia was established. This indicates that free radical scavengers are potential candidates for the treatment of chemotherapy-induced neuropathic pain.

It is quite interesting to note that repeated injections of PBN on days 7 through 15 completely prevented the development of paclitaxel-induced neuropathic pain in rats. However, when PBN was given on days 0 through 8, this effect was not observed. This suggested that the period between days 7 and 15 is critical in the development of paclitaxel-induced neuropathic pain.

In this study, PBN had potent analgesic effects in both established and developing neuropathic pain. PBN has been reported as an analgesic agent in pain models including the

spinal nerve ligation, capsaicin-induced secondary hyperalgesia, formalin-induced pain, and zymosan-induced visceral pain.[12–15] In those models, it has been reported that the mechanisms of action of PBN is not only to be a free radical scavenger or antioxidant but also to suppress the genes induced by proinflammatory cytokines or other mediators.[48] Also, other free radical scavengers, such as vitamin E and Tempol, produced significant analgesic effects in the aforementioned pain models,[16–21] and thus, it was suggested that PBN had analgesic effects mainly *via* its antioxidant activities.

In conclusion, systemic administration of PBN, a free radical scavenger, ameliorated the marked mechanical allodynia that developed in paclitaxel-induced neuropathic pain in rats. This analgesia can be prolonged by repeated injections with no side effects such as sedation. Furthermore, we were able to show that chemotherapy-induced neuropathic pain could be prevented when PBN was administered on days 7 through 15. We, thus, conclude that free radical scavengers might be useful in alleviating or preventing chemotherapy-induced neuropathic pain in a clinical setting.

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ANESTHESIOLOGY REFLECTIONS

Young's Maquette by Rhind of Crawford Long

As a young surgeon-anesthetist, Hugh H. Young (1870–1945) of Johns Hopkins popularized Georgian physician Crawford W. Long (1815–1878) as the world's first serial etherizer. Later, as a renowned urologist, Young officiated in 1926 when the State of Georgia unveiled a marble of Long in the National Statuary Hall Collection of the U.S. Capitol. The gold-painted plaster maquette above is one of at least three models of Long fashioned by sculptor J. Massey Rhind (1860–1936) before chiseling the final statue from Georgian marble. Significantly, the maquette above passed through Hugh Young's hands on its way to the Wood Library-Museum of Anesthesiology. (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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