Research report

Description of a short-term Taxol®-induced nociceptive neuropathy in rats

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Abstract

This work describes a new animal model of neuropathic pain produced by the single intraperitoneal administration of Taxol® (32 mg/kg) to male Sprague–Dawley rats. During the course of the experiment, the clinical status of the rats remained satisfactory and motor function was not altered. A number of classical behavioural tests of nociception as well as histological and electrophysiological investigations were performed. Taxol administration produced an important and rapidly developing mechanical hyperalgesia, a thermal hypoalgesia but no mechanical or thermal allodynia. Degenerative changes were observed in the sciatic nerve, the nerve fibres in the paw subcutaneous tissue and in the lumbar spinal cord. When Taxol or vehicle (a mix of Cremophor and ethanol) were repeatedly injected once a week for 5 weeks, similar nociceptive disorders were observed in addition to a decrease in peripheral nerve conduction velocity. The selective dysfunction of high-diameter myelinated fibres observed after one single administration of Taxol® (32 mg/kg) may be attributable to paclitaxel-induced neuropathy, however other mechanisms causing neurochemical dysfunction must also be involved.

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1. Introduction

Studying the mechanisms eliciting neuropathic pain necessitates reliable animal models reproducing as accurately as possible the symptoms observed in patients. However, the animal models of nerve injury actually available are few and are produced by either trauma [4,21] or metabolic changes [14]. Considering that anti-neoplastic agents frequently produce painful peripheral neuropathies, it may be possible to use these agents to create new animal models of neuropathic pain. Among these anti-cancer agents, Taxol® i.e. paclitaxel, widely used in cancer chemotherapy because of its ability to increase the stability of tubulin polymers [15,28], is one of the compounds most frequently implicated in the onset of painful peripheral neuropathy.

The change in the sensory nervous system produced by Taxol® explains the high incidence (50–90%) of patients developing painful peripheral neuropathies after a single dose of 250 mg/m²; Taxol\textsuperscript{®}, or after repeated administration of lower doses [30]. These well-documented neurotoxic effects are, in frequency, the primary adverse effects occurring in patients treated with Taxol®. Clinically, these peripheral neuropathies can cause painful complaints,
n numbness and paraesthesia of the extremities [23,30,36], an increase in the vibratory threshold and the loss of deep tendon reflexes [30]. Nerve conduction velocities and amplitudes of the sensory nerve action potentials are always reduced [11,23,30]. Moreover, there is no really effective therapy for the neuropathy-induced pain.

Previous studies of Taxol®-induced neuropathy in animals have described electrophysiological and histological changes [1,7–9,12] but none have fully described all types of nociception in animals after single dose or chronic Taxol® administration. The intraperitoneal route of administration, previously proposed by Apfel et al. [1], Hamers et al. [18] and Cavaletti et al. [9] is particularly suited to increasing the intratumoral disposition of the active principle of Taxol®, leading to high drug levels in liver, colon, pancreas and ovaries. Previously we have observed that five intraperitoneal injections, one per week, do not modify the elimination kinetics of Taxol® [13] and are of too short a duration of administration to induce ascites [9]. Consequently, this schedule of administration was used initially.

To fully describe the animal model of Taxol®-induced painful neuropathy in terms of pain, we have used the common battery of behavioural pain tests using noxious or non-noxious mechanical or thermal stimuli and the animal strain commonly used for pain studies, i.e., the male Sprague-Dawley rat. The schedule of administration was adapted in order to preserve a satisfactory clinical status in the animals. In addition, electrophysiological and neuropathological studies were performed using both light and electron microscopy in order to describe possible changes in nervous structures, to evaluate the functionality of the peripheral nervous fibres, and to determine the site of the neurotoxic action of Taxol®.

2. Materials and methods

2.1. Animals

Two hundred and fifty two male Sprague-Dawley rats (Charles River, St-Aubin-Lès-Elbeuf, France) weighing 180 to 200 g at the beginning of the induction were used (nine rats/test/dose) and housed in plastic cages on a 12-h light/12-h dark cycle with access to water and food ad libitum. Room temperature was maintained at 22°C and relative humidity was usually between 50 and 60%. Animals were allowed a 1-week acclimatisation period before use in experiments.

2.2. Drugs

2.2.1. Taxol-treated groups

Paclitaxel (Taxol®, generously provided by Bristol-Myers-Squibb, Paris, France), in vehicle (see below), (5.9 mg/ml), was diluted in normal saline (NaCl 0.9%) just before administration to a final concentration of between 0.9 to 1.3 mg/ml, depending on the animal weight and ensuring that volumes of less than 5 ml would be injected into the peritoneal cavity.

2.2.2. Control groups

The vehicle, made up of Cremophor EL (Sigma, L’Isle d’Abeau, France) and absolute ethanol (Merck, Darmstadt, Germany) in equal parts, was diluted at the time of Taxol® administration. The intraperitoneal route of administration, previously proposed by Apfel et al. [1], was used initially. The guidelines of the IASP Committee for Research and Ethical Issues were followed for all experiments [40].

2.3. Experimental procedures

Body weights were measured before each injection, every 3 days for 3 weeks after the fifth administration, and for 15 days following the single injection. Motor activity was monitored every 4 days using an actimeter Actisystem (Apelex, Passy, France). In addition, all rats were examined every day to detect clinical signs such as piloerection or hindlimb weakness. Body temperature was assessed at regular intervals.

2.5. Behavioural assessment

All behavioural tests were conducted before each injection of Taxol®. The guidelines of the IASP Committee for Research and Ethical Issues were followed for all experiments [40].

2.5.1. Grip strength test [25]

After strain gauge was zeroed, the rat was placed with both forepaws inside the front grip grid. When the rat gripped the grid, it was steadily pulled backwards by the tail until its grip was broken. Reading on strain gauge was recorded, the strain gauge was zeroed, and the rat retested until readings on three successive trials were obtained.

2.5.2. Paw pressure test

The paw pressure test has been previously described by Randall and Selitto [29]. Nociceptive thresholds, expressed in grams, were measured by applying increasing pressure to the right hind paw using an Ugo Basile analgesimeter (Apelex, Passy, France). The parameter used to quantify
the nociceptive threshold was the vocalization of the animal. Rats were habituated to the testing procedures and handling by the investigator during the week prior to the experiment. Experiments were performed until two similar consecutive pressure values were obtained. The cut-off pressure was 450 g.

2.5.3. Von Frey hair test [10]

To assess changes in mechanical nociceptive thresholds, rats were placed on a plastic mesh floor covered by transparent Plexiglas cages. On the week before the first administration of Taxol® or saline, rats were placed in the test environment each day for 15 min. For testing, rats were allowed to acclimatise for 10 min. Successively greater diameter von Frey nylon monofilaments (Stoelting, Wood Dale, IL, USA) (pressures 1.202, 1.479, 2.041, 3.630, 5.495, 8.511, 11.749, 15.136 and 28.840 g) were applied to the medial plantar surface of both hind paws. For each filament, a series of five stimuli were applied within a 2–3 s period per paw. Paw withdrawal threshold was defined as the minimum pressure required to elicit a withdrawal reflex of the paw.

2.5.4. Plantar test [19]

The rats were placed on a glass plate under a Plexiglas cage for 15 min before each experiment. On the week before the first administration of Taxol® or saline, rats were placed in the test environment each day for 15 min. A radiant heat stimulus was applied to the plantar surface of each hind paw by aiming a beam of light produced by a 50-W projection lamp (Apelex, Passy, France). When the rat lifted the paw, the light beam was turned off automatically allowing measurement of the time between the start of the light stimulus and the foot lift. This time was defined as the withdrawal latency. Heat stimuli were stopped at 20.8 s to avoid skin injury. Three trials were conducted on each hind paw, with 5 min between each trial.

2.5.5. Tail immersion test [27]

The tail of the rat was immersed in water maintained at 42°C or 48°C, until the tail was withdrawn. The duration of immersion was recorded and a cut-off time of 15 s was used. Rats were habituated to the testing procedures and to handling by the investigator during the week prior to the experiment.

2.6. Electrophysiological study

Five rats per batch at 7 and 21 days after the last injection of Taxol®. In the repeated Taxol®-injection group and one batch of five rats in the single Taxol®-injection group 7 days after injection were studied. The rats were anaesthetised by intraperitoneal injection of ethyl carbamate (2.5 mg/kg). Samples of sciatic nerves were quickly taken, preserved in a Ringer–Locke solution and then placed between two electrodes separated by 2.5 cm. After estimating the depolarization threshold of the nerve, the nerve was stimulated 50 times with a supramaximal stimulus. Nerve conduction velocity was directly calculated using a computer and a locally-made software. All measurements were performed under constant conditions in a Faraday cage.

2.7. Microscopic examination

2.7.1. Fixation of tissues

At the time of sacrifice, based on satisfactory results of behavioural tests, selected rats were deeply anaesthetized by an intraperitoneal injection of a 6% aqueous pentobarbital solution (0.5 ml/100 g body weight). After opening the thoracic cavity, the vascular system was intracardially perfused using a phosphate buffer solution (pH = 7.2–7.4, 200 ml), followed by a 4% paraformaldehyde+1% glutaraldehyde (200 ml) solution.

Samples of the lumbar spinal cord, sciatic nerve and skin with the subcutaneous tissue of the paws were stored in the fixative solution as above, until trimmed for histology and examination of the ultrastructure.

2.7.2. Histology

Samples of lumbar spinal cord, sciatic nerve and skin with subcutaneous tissue of the paws were paraffin embedded, cut at 4 μm thick, and stained by the Haematoxylin and Eosin method before examination using light microscopy.

2.7.3. Electron microscopy

Blocks of lumbar spinal cord, sciatic nerve and skin with subcutaneous tissue of the paws of approximatively 2×2×1 mm were postfixed in a 1% osmium tetroxide solution, and embedded in Epon after graded dehydration in alcohol. Semithin and ultrathin sections were obtained using a Reichert Ultracut E ultramicrotome. Semithin sections were stained with Toluidine Blue before light microscopy. Ultrathin sections were contrasted by the uranyl acetate–lead citrate method before examination with a Hitachi HU 12A electron microscope at 75 KV.

2.8. Statistical analysis

Treatments were randomised within each cage of six (five injection study) or four (single injection study) rats. Behavioural data were evaluated using analysis of variance (Fisher test) followed by a Student’s t-test to detect either differences within treatment groups between baseline and post-injections values, or differences between each treatment group on each day tested.
3. Results

3.1. Assessment of general toxicity

In the five Taxol®-injection group, two rats died, one after the first and one after the fourth Taxol® injection, after a substantial decrease in body weight and piloerection were observed. All other animals survived until the end of the study and received the five injections as scheduled in the protocol.

A significantly lower body weight gain was observed from the fourth injection to the end of the study (−15% compared to both control groups; \( P<0.01 \)) in the multiple Taxol®-treated group. Therefore, these rats showed a lower decrease in motor activity, however this was not significant compared to the control groups. No difference in cutaneous temperature was noted in the treated rats and both control groups.

There was no mortality in the single Taxol® injection groups. A significant decrease in body weight (maximal decrease: −17%, \( P<0.001 \) and −10%, \( P<0.01 \) after 3 days, for 32 and 16 mg/kg, respectively) was observed, with a total recovery within 2 weeks after the injection.

No rat in the control groups died throughout the course of the experiment and no alterations in motor activity, cutaneous temperature or clinical signs were observed. Body weight gain was similar for both saline and vehicle groups.

3.2. Behavioural examinations

3.2.1. Grip strength test

Whatever the treatment, the dose and the schedule of administration, we observed a normal increase in the motor force without any significative difference compared to saline control group.

3.2.2. Randall and Selitto test

In all experiments, mean vocalisation thresholds were not significantly different between control and drug-treated groups before the first injection.

In the multiple Taxol®-injection group, a significant decrease in vocalisation threshold (80% of rats) compared to the baseline and control groups, was observed from the first to the fifth injection (\( P<0.01 \)) (Fig. 1). The maximum decrease in vocalisation threshold (maximal decrease: −33%, \( P<0.01 \)) was observed after the fifth injection. From 2 weeks after the fifth injection, vocalisation thresholds increased progressively but up to 21 days after the last injection remained significantly lower to baseline values (\( P<0.05 \)).

In the multiple vehicle-injection group, a decrease in vocalisation threshold compared to baseline values (57% of rats) was noted from the fifth injection to the end of the study (maximal decrease: −23%, \( P<0.05 \), 14 days after the last injection). Moreover, the vocalisation thresholds were lower than in the Taxol® group for the last injection (214.0±10.4 g and 230.6±20.5 g for vehicle and Taxol®, respectively). No modification of the vocalisation thresholds was observed at any time in saline-treated group.

In the single Taxol®-injection group, vocalisation thresholds decreased both in the 16 and 32 mg/kg groups, from the 4th to the 9th day (16 mg/kg) and to the 14th day (32 mg/kg) after the injection, with a maximal decrease on the 7th day after injection, i.e., −25% (\( P<0.01 \); in 80% of rats) and −30% (\( P<0.01 \); in 90% of rats), respectively. No decrease in vocalization threshold was observed in the control groups during the course of the experiment (Fig. 2).

3.2.3. Von Frey hairs test

A significant reduction (\( P<0.01 \)) in the withdrawal threshold to von Frey filaments (−87% and −81% compared to baseline values) was observed in two rats (14%) repeatedly treated with Taxol® from the fourth injection to the end of the experiment. No significant difference in mean withdrawal threshold was observed in treated rats compared to control groups.

In the Taxol® single injection group, at either 16 or 32 mg/kg, thresholds were not significantly modified. In both
control groups, withdrawal thresholds were not decreased at any time.

3.2.4. Plantar test
In repeated Taxol®-injected rats, compared to control groups, significant increases in paw withdrawal times from the second injection \((P<0.01)\) to 7 days after the last injection \((P<0.05)\), were observed. The maximal increase was +31% compared to baseline \((P<0.01)\). This thermal hypoalgesia affected 57% of treated rats (Fig. 3).

After one single Taxol® injection at 32 mg/kg, compared to control groups, a significant increase in withdrawal latency threshold was noted 4 and 7 days after the injection, with a maximum of +17% on day 7 \((P<0.05)\). No modification was noted after administration of 16 mg/kg (Fig. 4).

Whatever the administration schedule, there was no alteration in the withdrawal latencies of the control groups.

3.2.5. Tail immersion test
When thermal allodynia was assessed, tail withdrawal times did not increase at any time in either the control or the Taxol®-treated rats, whatever the schedule of administration.
difference was observed between the Taxol® and the control groups.

3.4. Morphological examination

Results of the morphological examination (light and electron microscopy) are summarised in Table 1.

3.4.1. Light microscopy

Degenerative changes were observed on both paraffin and semithin sections, in the sciatic nerve, the nerve fibres in the paw subcutaneous tissue, and in the lumbar spinal cord. In the five injection Taxol® or vehicle groups, at the 7 or 21 day necropsies after the last intraperitoneal injection, lesions were found in all organs examined. In the single Taxol® injection group, a few changes were found in the subcutaneous nerves (16 and 32 mg/kg) and in the sciatic nerve (32 mg/kg). In the single vehicle injection group, and in all saline control groups, no lesion could be detected. In the five injection Taxol® or vehicle groups no major differences could be found between the Taxol® or the vehicle-treated groups, and between the 7 versus 21 days post-injection groups.

3.4.1.1. Sciatic nerve (Figs. 6 and 7). In the longitudinal paraffin sections rare Wallerian degeneration and axonal swellings (giant axons) were observed. In Epon semithin sections, degenerative changes were obvious, with a zonal pattern in the most severe cases. In the affected area, in myelinated fibres identified as large diameter fibres, the axonal clear centre of the fibre was replaced by dark myelinic debris, as confirmed by electron microscopy.

3.4.1.2. Spinal cord. Degenerative changes, similar to the lesions described above in the sciatic nerve, were observed, scattered in the white matter of the lumbar spinal cord.

| Table 1 |
| Summary of histological examinations after one or five Taxol® injections in rats® |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nerve of paw subcutaneous tissue</th>
<th>Sciatic nerve</th>
<th>Lumbar spinal cord</th>
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<tr>
<td>Taxol® 5×16 mg/kg Sac 7 days PI</td>
<td>++</td>
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<td>Vehicle 5 injections Sac 7 days PI</td>
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<tr>
<td>Taxol® 1×16 mg/kg Sac 7 days PI</td>
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<td>Vehicle 1 injection Sac 7 days PI</td>
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® 0, no detectable change; + to ++++, degenerative changes as described in text, graded from the less to the most severe. Sac, rats sacrificed 7 or 21 days after the last intraperitoneal injection. Ax; Myel; Schwann, axons, myelin sheaths and/or non-myelinated Schwann cells were affected in the group.
cord. Neurons in the gray matter remained unaffected in all groups.

3.4.1.3. Subcutaneous tissue (Fig. 8). In the fine subcutaneous nerves from the paws, degenerative changes affected almost all nerve fibres, including those of both large and small diameter. Lesions were similar to those noted for the sciatic nerve, and phagocytosis of degenerative myelinic debris was clearly detected at high magnification.

3.5. Ultrastructure

At the ultrastructural level, axonal and/or Schwann cells changes were found in all Taxol® or vehicle groups, except in the single injection vehicle group. No changes were noted in saline control groups.

In the Taxol® and vehicle five injection groups, as under the light microscope, there were no major ultrastructural differences between the Taxol® and the vehicle-treated groups. The most conspicuous changes were found in the paw subcutaneous nerve fibres.

3.5.1. Subcutaneous nerve fibres (Figs. 9 and 10)

Axons were degenerated. They were darkened, with organelle accumulation, mostly neurotubules and/or vesicles, and often seemed compressed by Schwann cells changes as described below. Vesicles were empty or filled with lamellar debris. Most myelinated and a few non-myelinated axons were affected.

Schwann cells and myelin sheaths: vacuolation of enlarged axonal and adaxonal parts of the Schwann cells was frequent, with accumulation of various debris, giving the axon an angular shape. In the most severe cases, myelin sheaths were also affected by partial delamination of collapsed myelin sheaths (ovoids) or complete destruc-
Fig. 10. Degenerative subcutaneous nervous lesions — ultrathin section of fine subcutaneous nerve fibres in the paw of a Taxol®-treated rat (five injections at 16 mg/kg) showing several degenerated myelinated axons (arrows), beside few normal nerve fibres (star). Degenerative changes are also found in non-myelinated schwann cells (arrowheads). Uranyl acetate and lead citrate ×5000.

tion and subsequent phagocytosis. Non-myelinated Schwann cells were also affected and showed various degrees of degeneration, mostly vacuolisation.

3.5.2. Sciatic nerve (Fig. 11) and spinal cord

In the affected areas of transverse sections of the sciatic nerve, the nervous fibres showed the various degrees of alteration described above for subcutaneous fibres, with the same decreased incidence in the semithin sections from the nerve to the spinal cord. It is also of note that non-myelinated fibres remained unaffected, in contrast to the subcutaneous tissue. Neurons of the spinal gray matter had a normal ultrastructural aspect, confirming the observations made with light microscopy.

In the Taxol® single injection group (Fig. 12), some degenerated axons were found with accumulation of organelles and vacuoles in the sciatic nerve (32 mg/kg) and subcutaneous tissue (16 and 32 mg/kg). Schwann cells and myelin sheaths remained normal and no change was detected in the spinal white matter. No ultrastructural change was found in the corresponding vehicle group (Fig. 13).

4. Discussion

This study demonstrates that either one single or five repeated intraperitoneal Taxol® injections induce a nociceptive peripheral neuropathy in rats, associated at the highest cumulative doses with electrophysiological and histological alterations. Despite the long-standing knowledge of the side-effects of Taxol® and the widespread use

Fig. 11. Increased density of neurotubules — ultrathin section of the sciatic nerve from a Taxol®-treated rat (five injections at 16 mg/kg) showing increased density of neurotubules. Uranyl acetate and lead citrate ×37,500.

Fig. 12. Axonal degenerative changes — ultrathin section of fine subcutaneous nerve fibres in the paw of a Taxol®-treated rat (a single injection at 32 mg/kg) showing axonal degenerative changes (stars) while myelin sheaths remain within normal limits (arrows). Uranyl acetate and lead citrate ×8700.

Fig. 13. Vehicle-induced nervous lesions — ultrathin section of fine subcutaneous nerve fibres in the paw of a vehicle-treated rat (a single injection of Cremophor) showing axons (stars) and myelin sheaths (arrows) within normal limits. Uranyl acetate and lead citrate ×5000.
of this effective drug in cancer therapy, no complete description of the associated nociceptive signs could be found in the literature. Moreover, it must be emphasised that such a description is necessary firstly, to understand the neurotoxic mechanisms involved in the genesis and transmission of the pain syndromes observed after Taxol® chemotherapy and secondly, to develop new and more efficient pain treatments in these neuropathies which will be based on a better knowledge of their pathophysiological mechanisms. A few studies [8,9,12,18], mainly describing electrophysiological and/or morphological modifications induced by Taxol® are available, but in these publications, all behavioural results were obtained with animals whose strain or sex were not routinely used in studies assessing nociception in animals. A strain, like the Sprague–Dawley rat, with a weak stress seems to be more adapted to realise behavioural tests [39]. Thus these results published previously cannot be used to describe the nociceptive symptoms generated by peripheral Taxol® neuropathy in the rat.

It should be noted that the Taxol® single and multiple doses used in our study are similar to those administered either intraperitoneally by Cavaletti et al. [9] and Hamers et al. [18], (single dose: 8 and 16 mg/kg/week, 6, 9 and 12 mg/kg/week, respectively; cumulative dose: 40 and 80 mg/kg, 54 and 66 mg/kg, respectively) or intravenously by Cavaletti et al. [8] and Cliffer et al. [12] (single dose: 5 mg/kg/day and 18 mg/kg/week, respectively; cumulative dose: 25 mg/kg, 72 mg/kg, respectively). The schedule of repeated administration, one injection per week for 5 weeks, was previously used by Cavaletti et al. [9] in female Wistar rats. This dose is a good compromise between the appearance of nociceptive disorders and the preservation of good clinical status. It was important to avoid severe health alterations in the rats since they may severely compromise the predictability of behavioural pain tests. No significant change in body weight was observed until the fourth Taxol® injection and the loss of weight never exceeded 15%. As expected, no significant decrease in motor activity and motor force was observed whatever the schedule of Taxol® administration.

With regard to the behavioural assessments, the modifications observed after five Taxol® injections were mechanical hyperalgesia in 86% of rats and thermal hypoalgesia in 80% of rats. However, only mechanical hyperalgesia appeared 1 week after the first administration and persisted until the end of the treatment. We also observed mechanical hyperalgesia after five weekly injections of the vehicle which is a mixture of Cremophor EL and ethanol. This observation led us to assess the kinetics of the pain disorders produced after a single 16 or 32 mg/kg dose Taxol® injection. Each dose induced a state of hyperalgesia of similar intensity to that after the five injections which lasted for five and 10 days respectively. These results encouraged us to perform pain tests after a single injection of Taxol® thereby avoiding the vehicle interference and preserving a good clinical status.

Concurrent to this mechanical hyperalgesia, the application of a non-noxious, mechanical or thermal, stimulus did not modify nociceptive thresholds, irrespective of the Taxol® dose and the number of injections. These results show that allodynia is absent and are important because mechanical and thermal non-noxious stimuli have not previously been assessed in the published literature.

Thermal hypoalgesia was observed after two Taxol® injections at 16 mg/kg or after one injection at 32 mg/kg. A higher Taxol® dose was needed to produce thermal hypoalgesia than mechanical hyperalgesia suggesting that thermal hypoalgesia requires a more substantial nervous system injury than mechanical hyperalgesia. As regards to the literature, only thermal hypoalgesia status has been previously described in experiments carried out using the tail flick test in CD1 mice [1] and Wistar rats [7,8]. However Cliffer et al. [12] did not observed a decrease in a heat nociception test with a cumulative dose of 72 mg/kg Taxol®. Nociceptive signs observed after one Taxol® injection in rats were well correlated with the disorders of sensation observed in patients treated by one course of Taxol®, such as numbness, tingling and hypoesthesia [30]. Moreover negative symptoms such as loss of sensitivity, and positive symptoms, such as pain and dysesthesia, are frequently associated with human sensory neuropathies [26,38].

Electrophysiological ex vivo study of the sciatic nerves of rats treated with repeated Taxol®, vehicle or saline injections showed a significant decrease in the nerve conduction velocity after five injections of Taxol® compared to the control groups, but no modification after one single injection. These results may be due to the low sensitivity of electrophysiological studies to detect early signs of neuropathy. The results of the present study agree with those published previously in the literature [7–9,12]. An increase in the nerve conduction velocity was observed in control rats, probably due to the degree of maturation of the nervous system in these relatively young animals [9].

With regard to morphological examination, all nervous structures, including axons, Schwann cells and myelin sheaths, were affected after the fifth 16 mg/kg Taxol® injection or in the vehicle groups at the 7 or 21 day necropsies after the last intraperitoneal injection. These findings are consistent with previous publications in which Wallerian degeneration, spheroids/giant axon formation and microtubule accumulation [8,9,12,31,34], as well as changes in Schwann cells (microtubules increased in number, debris accumulation and vacuolation by cisternae dilatation), were regularly described after various protocols of Taxol® administration. The recovery observed after the seventh week in the paw pressure test, 21 days after the last injection, might be explained by some regeneration process which was rarely observed in our samples. Regeneration is not a commonly described process in this area of publication, and only Royitta has mentioned remyelination 4 weeks after Taxol® administration (with
DMSO as the vehicle), latter than last sampling time of the present experiment [31].

In subcutaneous tissue, after five intraperitoneal injections, all nervous fibres were affected, irrespective of their diameter and the presence of myelin sheath, but in the sciatic nerve only large myelinated fibres were affected. A reason for this discrepancy could be that the fine terminal fibres are ramifications of the large upper sciatic fibres, and that subcutaneous fine non-myelinated fibres may be myelinated at an upper level. This highlights the unsolved question of why in the sciatic nerve entirely non-myelinated fibres are not degenerated: an explanation could be the potentiation of Taxol® toxicity by the lipophilic Cremophor, which is known to block multidrug resistance and to induce neurotoxic side effects [5,6,16,30,37]. Such an action could be enhanced in the myelin sheath which is rich in lipid. Fiber alterations have already been revealed in subcutaneous tissues of patients suffering from sensory neuropathy [20,22].

In the single Taxol® injection group only axonal changes were found, and no lesions were found in the single injection vehicle group. This suggests that the primary lesion is axonal and due to Taxol® alone and not the vehicle. Myelin sheaths could be affected later as a consequence of axonal degeneration, but predominantly by the direct delayed toxicity of the vehicle. This secondary myelinic toxicity occurred after repeated injections in the case of the five injection groups, and is consistent with the findings of human sural nerve biopsies after Taxol® therapy [32] in which the loss of large myelinated and of unmyelinated fibres was noted. Furthermore, the absence of neuronal body changes in the spinal cord, and the decreased severity of lesions from the subcutaneous nervous fibres to the spinal cord indicate that the primary lesion is axonal and retrograde, most probably progressing from the terminal fibres to the cellular body. A study of the spinal ganglia remains to be done to further explore the retrograde toxicity of Taxol®.

An important issue is to link the alterations in nociceptive behaviour observed, i.e., mechanical hyperalgesia and thermal hypoalgesia, with axonal changes. Mechanical hyperalgesia could be related to the injury of large fibres which normally exert an inhibitory feedback on the small fibres [24,35] which convey mechanical hyperalgesia. This inhibition may be increased and the noxious impulses conveyed by the small Aδ fibres may be amplified. However, the absence of allodynia associated with an alteration of large fibres seems paradoxical as these fibres would take an active part in the occurrence of this type of sensitivity disorder by the appearance of ectopic discharges, the formation of ephapses with small gauge fibres and abnormal connections to convergent neurons conveying specific noxious impulses [2,3,17,33,38]. Additional studies regarding spinal pain mediator levels will add further information about the mechanisms involved in the pathophysiology of pain, thereby allowing the behavioural responses observed to be explained.

In conclusion, one single intraperitoneal administration of a 32 mg/kg Taxol® dose can be used as a good validated animal model predictive of the painful conditions observed in human peripheral Taxol® neuropathy without revealing the neurotoxicity of the vehicle (Cremophor EL). This animal model of peripheral neuropathy is of interest as the neuropathy is easily induced in animals, it is of quick onset and the nociceptive symptoms are quantitatively substantial while a good clinical status is observed, and the recovery of the neuropathy is possible as observed in man.

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