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Original Article / Özgün Araştırma

# The Effect of Anakinra on Paclitaxel-Induced Peripheral Neuropathic Pain in Rats

## Sıçanlarda Paklitaksel İlişkili Periferik Nöropatik Ağrı Üzerine Anakinra'nın Etkisi

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Abstract / Öz

**Objective:** Paclitaxel is used in the treatment of cancer, and it may cause interleukin-1 beta (IL-1β)-related peripheral neuropathic pain. While our primary aim was to investigate the analgesic efficacy of an IL-1β antagonist, a secondary outcome was to assess whether a correlation exists between analgesic effects and antioxidant activity.

**Methods:** A total of 24 albino Wistar male rats were divided into the following groups: paclitaxel-control, paclitaxel+50 mg $kg^{-1}$ anakinra, paclitaxel+100 mg $kg^{-1}$ anakinra and healthy group (HG). After the normal paw pain threshold in all animal groups was measured using a Basile algesimeter, a single dose of 2 mg $kg^{-1}$ paclitaxel was intraperitoneally administered on the 1st, 3rd, 5th and 7th days. Anakinra was intraperitoneally administered following the final paclitaxel administration. The paw pain thresholds in the groups were measured before and seven days after paclitaxel administration and at the 1st and 3rd hours after anakinra administration. After the third hour of measurement, the rats were killed with high doses of ketamine, and the paw tissues were removed. Malondialdehyde, myeloperoxidase and total glutathione levels were measured in claw tissues, and IL-1β gene expression was determined. The biochemical results were compared with the results of the HG; in the meanwhile the claw pain threshold results were compared with the results obtained after the last paclitaxel and the results obtained from the 1st and 3rd hours after the anakinra application.

**Results:** The claw paw pain threshold of the rats decreased one and three hours after anakinra administration. Further, 100 mg $kg^{-1}$ anakinra had greater analgesic activity than 50 mg $kg^{-1}$ anakinra. A correlation was found between the antioxidant and analgesic activities of 100 mg $kg^{-1}$ anakinra.

**Conclusion:** Anakinra may be useful to reduce paclitaxel-induced neuropathic pain; further, 100 mg $kg^{-1}$ anakinra may have greater analgesic and antioxidant activities.

**Keywords:** Paclitaxel, anakinra, peripheral neuropathy, pain, oxidative stress

**Amaç:** Paklitaksel kanser tedavisinde kullanılan ve IL-1 Beta ilişkili periferal nöropati meydana getirebilen bir ilaçtır. Primer amacımız Paklitaksel ilişkili periferal nöropatik ağrıda Anakinra'nın analjezik etkinliğini araştırmak iken bir diğer hedefimiz ise anakinranın analjezik etkinliği ile antioksidan aktivitesi arasında korelasyon olup olmadığını araştırmaktır.

**Yöntemler:** Toplamda 24 Albino Wistar deney hayvanları, paklitaksel uygulanan kontrol (PAC), paklitaksel+50 mg kg-1 anakinra (PAC-50), paklitaksel+100 mg $kg^{-1}$ anakinra (PAC-100) ve herhangi bir işlem uygulanmayan sağlıklı gruplara (HG) ayrıldı. Tüm hayvan gruplarının normal pençe ağrı eşikleri Basile Aljezimetre kullanılarak ölçüldükten sonra, sıçanların PAC, PAC-50 ve PAC-100 gruplarına paklitaksel 2 mg $kg^{-1}$ tek doz 1, 3, 5, ve 7. günlerde intraperitoneal olarak toplam dört kez uygulandı. Son Paklitaksel dozundan sonra sıçanlara intraperitoneal yoldan Anakinra verildi.Tüm sıçanların pençe ağrı eşikleri son paklitaksel dozundan sonra ayrıca Anakinra verildikten 1 ve 3 saat sonra ölçüldü. Üçüncü saatteki ölçümden hemen sonra sıçanlar yüksek doz ketamin anestezisi ile öldürülerek ayak pençe dokuları çıkartıldı. Pençe dokularında malondialdehit, myeloperoksidaz ve total glutatyon düzeyleri ölçüldü ve IL-1β gen ekspresyonu tayini yapıldı. Elde edilen biyokimyasal sonuçlar HG grubu ile; pençe ağrı eşikleri ise son paklitaksel dozu sonrası ile Anakinra 1. ve 3. saatler karşılaştırılarak değerlendirildi.

**Bulgular:** Elli ve 100 mg $kg^{-1}$ anakinra uygulanan hayvanların ayak pençesinde ağrı eşiği birinci ve üçüncü saatlerde anlamlı düşüş gösterdi. Ayrıca anakinra uygulanan PAC-100 grubunun analjezik aktivitesi PAC-50 grubundan daha fazla saptandı. 100 mg $kg^{-1}$ Anakinra'nın antioksidan ve analjezik aktiviteleri arasında korelasyon bulundu.

**Sonuç:** Anakinra paklitaksele bağlı nöropatik ağrının azaltılmasında yararlı olabilir. Ayrıca 100 mg $kg^{-1}$ anakinra daha etkin analjezik ve antioksidan aktivite sağlayabilir.

**Anahtar kelimeler:** Paklitaksel, anakinra, periferal nöropati, ağrı, oksidatif stres



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## Introduction

Paclitaxel is a taxane-derived drug that is used in the treatment of lung, ovarian and breast cancers (1). Peripheral neuropathic pain is the most serious long-term adverse effect of paclitaxel, in addition to short-term disorders, such as diarrheoa, nausea and muscle and joint pain. Peripheral neuropathy patients frequently present with numbness and tingling, experienced either spontaneously or mechanically, as well as cold stimulation-induced pain. It has been reported that neuropathic pain commences seven days after the administration of paclitaxel and that it may continue for up to one year after the cessation of chemotherapy drugs (2, 3). Treating peripheral neuropathic pain increases the quality of life of patients while decreasing morbidity. However, it has not yet been determined whether pain treatment methods that are currently accepted as the standard are adequately effective. The current analgesic treatment strategies are known to result in dose limitations, patient morbidity and minimalize advers effect (4, 5).

It is highly desirable to identify less toxic and more effective drugs to treat neuropathic pain caused by paclitaxel; therefore, neuropathic treatment strategies continue to constitute a major area of research. To this end, narcotic analgesic drugs are used in the treatment of peripheral neuropathic pain (6). However, narcotic analgesics cause addiction and tolerance and have serious side effects, such as respiratory depression and constipation, which restrict their use.

This suggests that drugs that are responsible for impeding the pathogenesis of paclitaxel-related peripheral neuropathic pain would be useful in its treatment. It has been found in numerous studies that reactive oxygen species (ROS) cause nerve injury-related pain and inflammatory pain (7). Paclitaxel has also been reported to activate proinflammatory cytokine expression (8-11). Thus, the administration of paclitaxel results in the production of interleukin-1 beta (IL-1β) and other proinflammatory cytokines and their release from microglial cells. It has also been claimed that IL-1β, in particular, contributes to the development of peripheral neuropathic pain (12); IL-1β has also been shown to play a role in the regulation of pain perception in various inflammatory situations (13).

Anakinra, an arthritis drug, is commonly used to treat rheumatoid arthritis and auto-inflammatory disease. Its efficacy against paclitaxel-induced peripheral neuropathic pain was evaluated in the current study. Anakinra is a competitive inhibitor of IL-1 receptors and an anti-inflammatory protein that regulates the biological activity of IL-1β by preventing signal transduction. It also inhibits the hyperalgesic response of cytokines and demonstrates antioxidant activity (14). Baamonde et al. (15) reported that anakinra prevented mechanical hyperalgesia induced by osteosarcoma.

Accordingly, we hypothesised that based on the aforementioned evidence, anakinra is useful in treating paclitaxel-induced peripheral neuropathic pain, although to the best of our knowledge, there have been no reports in the literature on the effects of anakinra on paclitaxel-induced peripheral neuropathic pain.

Mitochondrial dysfunction and oxidative stress have been reported to be physiopathologically involved in paclitaxel-related peripheral neuropathy (16). Mitochondria are a major source of ROS, while oxidative stress has been demonstrated to play an important role in the pathogenesis of paclitaxel-induced peripheral neuropathic pain. Anakinra has been shown to prevent oxidant injury and decrease inflammation (17).

Accordingly, in this study, we aimed to determine the influence of anakinra on the mechanisms of paclitaxel-induced peripheral neuropathic pain and its ability to suppress oxidant parameters. Therefore, the primary objective of this study was to investigate the analgesic efficacy of this IL-1β antagonist on paclitaxel-induced peripheral neuropathy by evaluating the paw pain threshold in rats and by calculating IL-1β gene expression. A secondary outcome was to assess whether a correlation exists between the analgesic effects and antioxidant activity of anakinra by examining malondialdehyde (MDA), myeloperoxidase (MPO) and total glutathione (tGSH) levels.

## Methods

### Animal testing

Animal testing was performed in accordance with the National Research Council of the National Academies Guide for the Care and Use of Laboratory Animals and was approved by the local Animal Ethics Committee of Atatürk University, Erzurum, Turkey (Ethics Committee Number 2015/9, dated 27 November 2015). All rats were provided by Atatürk University's Medical Experimental Application and Research Center. Twenty-four male albino Wistar rats, weighing between 210 g and 220 g, were included in the experiment. The animals were maintained and fed in groups under appropriate conditions at a room temperature of 22°C in the pharmacology department laboratory.

### Chemical substances

The paclitaxel (Taxol), anakinra (Kineret) and ketamine (Ketalar) used in the experiment were supplied by Actavis (Little Island, Co. Cork, Ireland), Sobi (Waltham, Sweden) and Pfizer (Istanbul, Turkey), respectively.

### Experimental groups

The test animals were divided into the following groups: paclitaxel-administered control (PAC), paclitaxel plus 50 mg $kg^{-1}$ anakinra (PAC-50), paclitaxel plus 100 mg $kg^{-1}$ anakinra (PAC-100) and a healthy group (HG) to which no procedure was applied. Mechanical hyperalgesia induced by osteosarcoma was blocked with 100 mg $kg^{-1}$ and 300 mg $kg^{-1}$ of intravenously administered anakinra (15). Nayki et al. (17) studied the effects of 50 and 100 mg $kg^{-1}$ doses of anakinra on ischaemic injury.

**Experimental procedure**

After the normal paw pain threshold of the animal groups was measured using a Basile® algesimeter (18), a daily single dose of 2 mg $kg^{-1}$ paclitaxel was intraperitoneally administered to the rats in the PAC, PAC-50 and PAC-100 groups for a total of four times on days 1, 3, 5 and 7, with a two-day interval between each administration (17, 19). At the end of day 7, 50 mg $kg^{-1}$ and 100 mg $kg^{-1}$ doses of anakinra were intraperitoneally injected to the rats in the PAC-50 and PAC-100 groups, respectively. Distilled water in the same volume was given as a solvent to the PAC and HG groups. The anti-inflammatory effect of anakinra has been reported to be immediate in a study in which the analgesic effect of the drug was evaluated one and three hours after administration (18). The paw pain thresholds of the rats in the PAC, PAC-50 and PAC-100 groups were measured on the seventh day after the administration of the last paclitaxel dose and one and three hours after the administration of anakinra. The paw pain threshold values before and seven days after the administration of paclitaxel, as well as those measured one and three hours after the administration of anakinra, were compared in all study groups, with the exception of the HG. It was anticipated that the paw pain threshold in the PAC, PAC-50 and PAC-100 groups before the administration of paclitaxel would be equal to that in the HG.

Analgesic activity was calculated one and three hours after the administration of distilled water and anakinra using the following formula:

Analgesic activity (%)=100–[(100×paw pain threshold measured on day 7 after the administration of paclitaxel (g)/paw pain threshold measured after the administration of anakinra (g)] (20).

Immediately following the measurements taken three hours after the administration of anakinra, the rats were sacrificed with a high dose of ketamine anesthaesia, and the tissue in their paws was removed to measure the MDA, MPO and tGSH levels. IL-1β gene expression was determined. The biochemical measurements of the study groups were compared with those in the HG.

**Biochemical procedures**

**Preparation of samples**

Potassium phosphate buffer (pH 6) containing 0.5% hexadecyl trimethyl ammonium bromide (HDTMAB) was used for the determination of MPO in tissue samples; 1.15% potassium chloride solution was used for the determination of MDA. For other measurements, phosphate buffer (pH 7.5) was completed to mL and homogenised in an icy environment. Then, the homogenate was centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was used as the sample for analysis.

**MDA analysis**

The MDA analysis method was based on the spectrophotometric measurement of the absorbance of the pink complex formed by thiobarbituric acid (TBA) and MDA at a high temperature (95°C) at a wavelength of 532 nm. The homogenates were centrifuged at 5000 g for 20 min, and the supernatants were used for the determination of MDA levels. In total, 250 µL homogenate, 100 µL 8% sodium dodecyl sulfate (SDS), 750 µL 20% acetic acid, 750 µL 0.08% TBA and 150 µL distilled water were pipetted into capped test tubes and vortexed. After the mixture was incubated at 100°C for 60 min, 2.5 mL n-butanol was added to the mixture, and it was spectrophotometrically measured. The intensity of the red color was read at 532 nm using 3 mL cuvettes, and the MDA level in the sample was determined with a standard diagram created using an MDA stock solution that was previously prepared by considering dilution coefficients.

**Determination of MPO activity**

Potassium phosphate buffer (pH 6) containing 0.5% HDTMAB was prepared for the determination of MPO in homogenates of paw tissue. The mixture was centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was used as the sample for analysis. For the determination of MPO activity, an oxidation reaction was performed with MPO-mediated $H_2O_2$ using 4-amino antipyrine/phenol solution as the substrate.

**tGSH analysis**

The level of GSH in the homogenate was measured according to the method used by Sedlak and Lindsay with some modifications (13). The sample was weighed and homogenised in 2 mL 50 mmol $L^{-1}$ Tris-HCl buffer containing 20 mmol $L^{-1}$ EDTA and 0.2 mmol $L^{-1}$ sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL 25% trichloroacetic acid. The precipitate was removed upon centrifugation at 4200 rpm for 40 min at 4°C, and the supernatant was used to determine the GSH level. A total of 1500 µL measurement buffer [200 mmol $L^{-1}$ Tris-HCl buffer containing 0.2 mmol $L^{-1}$ EDTA at pH 7.5, 500 µL supernatant, 100 µL DTNB (10 mmol $L^{-1}$) and 7900 µL methanol] was added to a tube, vortexed and incubated at 37°C for 30 min. 5.5-Dithiobis (2-nitrobenzoic acid) (DTNB), which forms a yellow complex with sulfhydryl groups, was used as a chromogen. The absorbance was measured at 412 nm using a spectrophotometer (Beckman DU 500, USA). The standard curve was obtained using reduced glutathione.

**Gene expression of IL-1β**

**RNA isolation**

RNA was isolated from the homogenised tissue samples using a Roche Magna Pure Compact LC device (Mannheim, Germany) with a MagNA Pure LC RNA Isolation Kit (Roche Diagnostics). The quantity and quality of the isolated RNA were assessed using a nucleic acid measurement device (Maestro, Nano). In total, 50 µL RNA samples were stored at –80°C.

Table 1. The effect of anakinra on the paw pain threshold in paclitaxel-administered rats

| Groups | Pain threshold (g) | | | |
|---|---|---|---|---|
| | Pain threshold before PAX administration | 7th day pain threshold after PAX administration | 1st hour after drug administration | 3rd hour after drug administration |
| PAC (n=6) | 49±4.2 | 24±3.2* | 24.5±2.9[&] | 24±3[&] |
| PAC-50 (n=6) | 54.5±3.4 | 24.4±4.5[#] | 31.8±1.9[#] | 30.3±2.2[#] |

PAX: paclitaxel; PAC: paclitaxel-control; PAC-50: paclitaxel+50 mg $kg^{-1}$ anakinra; PAC-100: paclitaxel+100 mg $kg^{-1}$ anakinra; *p=0.017; [&]p=0.357, [#]p=0.018.

**cDNA synthesis**

cDNA was synthesised from the isolated RNA samples using a Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics). For each subject, 1 µL $ddH_2O$, 10 µL RNA and 2 µL random primer were combined and incubated in a thermal cycler at 65°C for 10 min. After incubation, 4 µL reaction buffer, 0.5 µL RNAase, 2 µL deoxynucleotide mix and 0.5 µL reverse transcriptase were added. The reactions were incubated at 25°C for 10 min, at 55°C for 30 min and at 85°C for 5 min and were then maintained at 4°C.

**Quantitative gene expression evaluation by real-time polymerase chain reaction (qPCR)**

For each cDNA sample, the gene expression of IL-1β and the reference gene (G6PD) was analyzed using a Roche LightCycler 480 II Real-Time PCR instrument (Mannheim, Germany). Each PCR reaction mixture contained 5 µL cDNA, 3 µL distilled water, 10 µL LightCycler 480 Probes Master (Roche Diagnostics) and 2 µL primer probe set (Real-Time Ready single assay, Roche) to a final volume of 20 µL. The cycle conditions of relative quantitative PCR (qPCR) were preincubation at 95°C for 10 min, followed by 45 amplification cycles of 95°C for 10 s, 6°C for 30 s, 72°C for 1 s and cooling at 40°C for 30 s. Analysis of qPCR and calculation of quantification cycle (Cq) values for relative quantification were performed using LightCycler 480 Software, Version 1.5 (Roche Diagnostics). Relative quantitative amounts were calculated by dividing the target gene by the expression level of the reference gene. The reference gene was used for the normalisation of target gene expression.

**Statistical analysis**

Retrograde power analysis was performed using Russ Lenth's power and sample size calculations. The required number of samples was calculated to be 24 to achieve 86% power and a 5% alpha in the event of an increase in the paw pain threshold from 24% to 30%. Normal distribution and variance analysis was evaluated using the Kolmogorov-Smirnov test and kurtosis and skewness histograms. Numerical data were presented as the mean and standard deviation. The Kruskal-Wallis test was used for between-group comparisons; the Wilcoxon signed ranks test was used to assess which groups had differences between them. Tukey's HSD or nonparametric tests were used for multiple comparisons. All data were analysed using Statistical Package for Social Sciences (IBM Corp.; Armonk, NY, USA) version 20.0. In all analyses, statistical significance was considered to be p<0.05.

Table 2. Analgesic activity in groups (%)

| Groups | Analgesic activity (%) | |
|---|---|---|
| | 1st hour after anakinra administration | 3rd hour after anakinra administration |
| PAC (n=6) | 2 | 4 |
| PAC-50 (n=6) | 36.88 | 33.7 |
| PAC-100 (n=6) | 66.3 | 54.5 |

PAC: paclitaxel-control

## Results

**Pain test**

As can be seen in Table 1, a significant difference was observed between the paw pain thresholds before paclitaxel and after seven days of paclitaxel administration (p=0.017). However, there was no significant difference between the pain thresholds at the end of seven days of paclitaxel administration and at the first or third hours after administration of distilled water (p>0.05).

A significant difference was noted regarding the paw pain threshold in the PAC-50 group before the administration of paclitaxel and that measured on the seventh day of paclitaxel administration (p=0.018). When the pain threshold on day 7 of the administration of paclitaxel was compared with that measured one and three hours after the administration of anakinra, a significant difference was observed (p=0.018). A statistically significant difference was found between the paw pain thresholds measured at one and at three hours after the administration of anakinra in the PAC-50 group (p=0.017). A significant difference was noted regarding the paw pain threshold measured before the administration of paclitaxel and that measured on day 7 of paclitaxel administration in the PAC-100 group, as well as between the pain thresholds measured on day 7 of paclitaxel administration and one and three hours hours after the administration of 100 mg $kg^{-1}$ anakinra (p=0.018).

A statistically significant difference was also recorded between the paw pain thresholds measured in the PAC-100 group (p=0.018) one and three hours after the administration of anakinra.

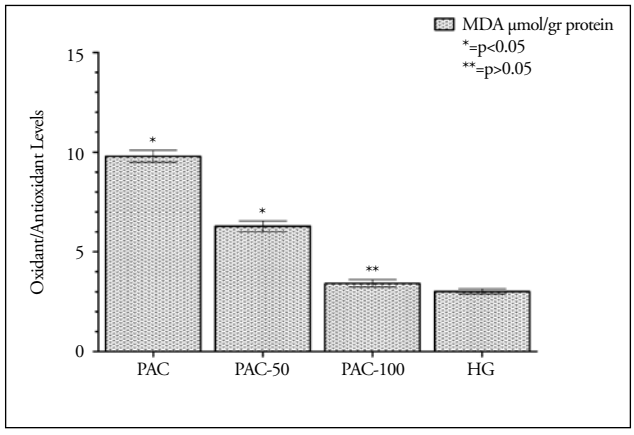


Figure 1. Malondialdehyde levels in groups

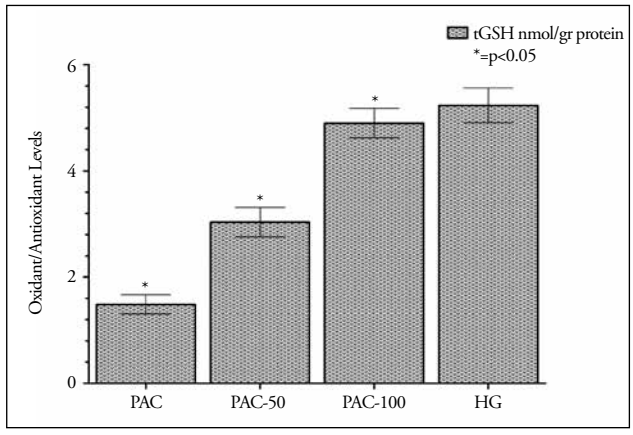


Figure 3. Total glutathione levels in groups

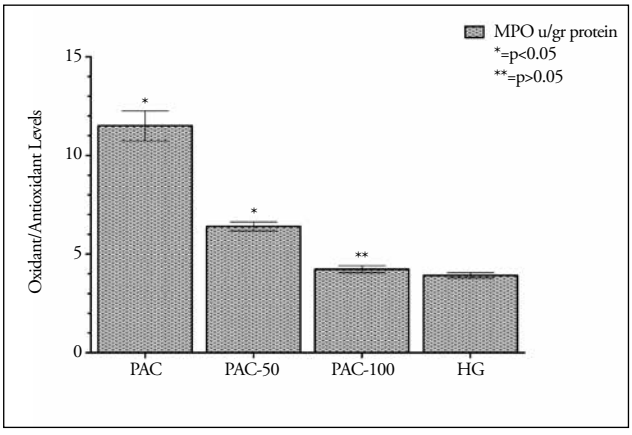


Figure 2. Myeloperoxidase levels in groups

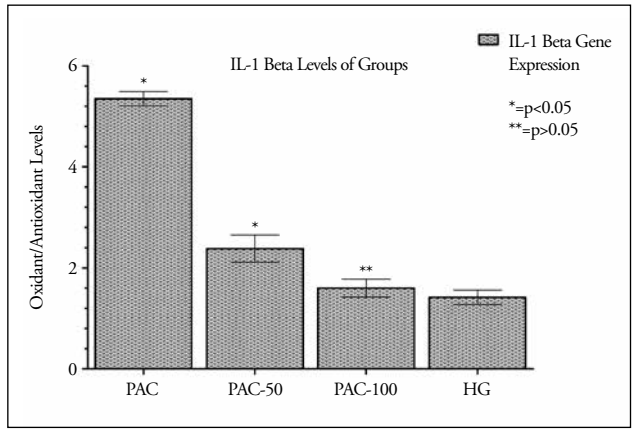


Figure 4. Interleukin-1 beta levels in groups

Following comparative analysis of the analgesic activities in the study groups, the rates of analgesic activity in the anakinra-administered PAC-50 group were 36.8% and 33.7% measured at one and three hours, respectively, compared to 66.3% and 54% at one and three hours, respectively, in the PAC-100 group. Thus, a greater degree of analgesic activity was demonstrated in the PAC-100 group than in the PAC-50 group at those times (Table 2).

**Biochemical results**

The amount of MDA was shown to be 3.02±0.28 µmol $g^{-1}$ protein in the paw tissue of the HG, increasing to 9.74±0.68 µmol $g^{-1}$ protein in the PAC (control) group ($p<0.05$) (Figure 1). Anakinra decreased the MDA amount that had been increased by paclitaxel to 6.27±0.61 µmol $g^{-1}$ protein at the 50 mg $kg^{-1}$ dose ($p<0.05$) and to 3.35±0.43 µmol $g^{-1}$ protein at the 100 mg $kg^{-1}$ dose. Statistically significant differences were observed between the PAC and PAC-50 groups and the HG ($p<0.05$), while no statistically significant difference was found between the PAC-100 group and the HG ($p>0.05$). Paclitaxel increased MPO activity to 11.71±1.79 µmol $g^{-1}$ protein in paw tissue (Figure 2). Compared to the paclitaxel group (PAC), anakinra significantly inhibited MPO activity at a dose of 50 mg $kg^{-1}$ (6.37±0.50 µ $g^{-1}$ protein) and even more significantly at a dose of 100 mg $kg^{-1}$ (4.24±0.40 µmol $g^{-1}$ protein). The MPO value in the HG was 3.9±0.31 µmol $g^{-1}$ protein. While no statistically significant difference between the HG and PAC-100 groups was observed ($p>0.05$), the difference between the PAC and PAC-50 groups and the HG group was statistically significant ($p=0.018$).

Paclitaxel decreased the amount of tGSH in rat paws compared to the amounts measured with the use of anakinra and that recorded in the HG (Figure 3). The smallest amount of tGSH was 1.45±0.41 nmol $g^{-1}$ in the paclitaxel-administered group (PAC); the amounts were 3.10±0.64 nmol $g^{-1}$, 4.82±0.64 nmol $g^{-1}$ and 5.24±0.73 nmol $g^{-1}$ in the PAC-50, PAC-100 and HG groups, respectively. Statistically significant differences were found between the HG and the other groups ($p<0.050$).

**IL-1β gene expression results**

Paclitaxel significantly increased IL-1β gene expression in rat paws compared to that measured with the use of anakinra and in the HG (Figure 4). The highest level of IL-1β gene expression (5.35±0.31) was measured in the paclitaxel control group (PAC); it was evaluated as being 2.40±0.60 in rats to whom a 50 mg $kg^{-1}$ dose of anakinra had been administered

and as 1.57±0.41 in rats to whom a 100 mg $kg^{-1}$ dose of anakinra had been administered. The lowest level of IL-1β gene expression (1.38±0.32) was recorded in the HG. While a statistically significant difference was not recorded between the HG and the PAC-100 group (p>0.050), statistically significant differences were observed between the HG group and the PAC and PAC-50 groups (p=0.018).

## Discussion

The focus of this study was the impact of paclitaxel on peripheral neuropathy, as the latter is one of the most frequent side effects of chemotherapeutics, especially chemotherapy drugs (Taxol®) (21). Chemotherapy-associated peripheral neuropathy may even lead to the cessation of treatment. Allodynia and hyperalgesia frequently result from the systemic administation of paclitaxel, vincristine and cisplatin, depending on how frequently the chemotherapeutic drug is used (21-23). Reyes-Gibby et al. (24) reported that neuropathic pain was experienced by 40% of cancer patients. A significant decrease in the paw pain threshold of animals to whom paclitaxel had been administered was observed in the present study; this finding is supported by other studies in the literature. It was demonstrated in our study that anakinra significantly inhibited paw pain caused by paclitaxel in a dose-dependent manner. In addition, when anakinra was administered at a dose of 100 mg $kg^{-1}$, it resulted in a more significant decrease in the pain threshold than that achieved with a dose of 50 mg $kg^{-1}$. The recorded value for the paw pain threshold one hour after the administration of anakinra was higher than that recorded at the baseline in the PAC-100 group. This indicated that a higher dose of anakinra (100 mg $kg^{-1}$) was more effective than a lower dose as an analgesic and increased the pain threshold considerably higher than that at the baseline.

It has been reported in several studies that paclitaxel is responsible for the development of peripheral neuropathic pain by increasing proinflammatory IL-1β expression (8-12). The IL-1β gene expression level increased in the paclitaxel group (PAC) compared to that in the HG and decreased in the groups in which anakinra was administered. This can be interpreted as evidence that anakinra is a competitive antagonist of the IL-1 receptor and has an anti-inflammatory effect. Similarly, the findings in the present study that IL-1β gene expression increased in the paclitaxel group (PAC) compared to that in the HG and that the pain threshold decreased in the paclitaxel group (PAC) are compatible with other findings that have been reported in the literature. Oxidative stress was identified in one study in relation to paclitaxel-related neuropathy (16). However, no correlation between paclitaxel-induced neuropathic pain and MDA, MPO and tGSH levels has been reported in the literature. It has been reported elsewhere that MDA levels increased proportionally in relation to a decrease in the paw pain threshold (20), while Cetin et al. (25) observed that MDA decreased in paw tissue with a corresponding increase in pain threshold. In another study, MDA was shown to increase in the presence of neuropathic pain (26). In support of the findings in the aforementioned studies, in the present study, an increase was also recorded in the level of MDA in the paw tissue of rats to whom paclitaxel was administered. It has been reported in the literature that oxidant substances that occur due to oxidative stress cause inflammatory pain (7). Odabaşoğlu et al. (27) proved that the level of MPO activity increased in inflamed tissue. Additionally, inflammation has been reported to play an important role in neuropathic pain. Muthuraman *et al.* (28) also demonstrated that MPO activity significantly increased in the presence of neuropathic pain.

In the present study, it was observed that MPO activity, an indicator of inflammation in the paw tissue of rats to whom paclitaxel had been administered, also increased significantly.

It was found in the current study that when the MDA and MPO levels were high in the paw tissue of rats, tGSH levels correspondingly decreased. This suggests that paclitaxel caused oxidative stress by altering the oxidant-antioxidant balance in the paw tissue of rats in favour of the oxidants.

In previous studies, it has been reported that anakinra suppresses MDA, the final product following lipid peroxidation, resulting in antioxidant activity (29). It has also been suggested that inhibiting MPO activity reduces muscle pain. tGSH levels are also believed to be maintained in tissues in which anakinra causes an analgesic effect (30).

Aside from the demonstrated antioxidant activity of anakinra, it is mainly known to be an anti-inflammatory drug and a natural competitive antagonist of IL-1 receptors. Therefore, its analgesic properties correlate with the inhibition of cytokines and antioxidant activity (14).

Parallel to the findings in the literature, in the present study, an increase in oxidant products, such as MDA and MPO, was observed, depending on the degree to which paclitaxel was administered, together with a decrease in the parameters of antioxidants such as tGSH. On balance, the respective increase and decrease was shown to favor oxidative stress. Similarly, anakinra was demonstrated to have antioxidant properties on the basis of the reported increase in antioxidant levels and decrease in oxidant substances at both doses of the drug in the groups in which anakinra was administered. Both the 50 mg $kg^{-1}$ and 100 mg $kg^{-1}$ anakinra doses were shown to inhibit increases in MDA, MPO and IL-1β levels with a corresponding decrease in tGSH levels in the paw tissue of rats following the administration of paclitaxel; however, the 100 mg $kg^{-1}$ dose, in particular, was shown to cause antioxidant and oxidant levels similar to those found in the HG.

Anakinra is administered subcutaneously in clinical studies; however, due to the challenges of subcutaneous administration in experimental studies, we systemically administered the drugs. This was the limitation of our study.

## Conclusion

Hyperalgesia developed in the paws of rats to which paclitaxel was administered. It was found to increase in proportion to the increases in MDA, MPO and IL-1β levels and to a decrease in tGSH levels; these increases and decrease could have been prevented in the two groups in which anakinra had been administered. However, the 100 mg/kg anakinra dose provided an oxidant-antioxidant balance in favour of the antioxidants, which was similar to that in the HG; this dose also provided more efficient analgesia.

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