Effects of Oxaliplatin and Cisplatin on Peripheral Nerve Excitability and Conduction

Oksaliplatin ve Sisplatinin Periferik Sinir Uyarılabilirliği ve İletimi Üzerine Etkileri

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ABSTRACT

Purpose: This study examines and compares the level of neurotoxicity of oxaliplatin and cisplatin in terms of excitability and conduction parameters over rats' caudal and sciatic nerves.
Methods: Twenty-seven Wistar rats were divided into three groups labeled as oxaliplatin (OXA), cisplatin (CIS) and control (CON). OXA and CIS groups were administered oxaliplatin (8 mg/kg/week, i.p) and cisplatin (4 mg/kg/week, i.p) respectively, for a 4.5 week follow-up period. Shortly after, threshold tracking recordings from tail caudal nerve and compound action potential recordings from isolated sciatic nerve were performed, in order to obtain the corresponding excitability and conduction parameters.
Results: Cisplatin is found more neurotoxic than oxaliplatin with regards to nerve excitability and conduction. Cisplatin is more effective on fibers having small radius (slowly conducting). The partial blockade of Na^+ channels (mostly persistent) and an increment in the activity of inward rectifier K^+ conductance due to cisplatin are noteworthy.
Conclusions: In terms of neurotoxicity, oxaliplatin may be more preferable compared to cisplatin clinically.

Key Words: Threshold tracking, excitability, conduction, oxaliplatin, cisplatin.

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ÖZET

Amaç: Bu çalışmada, oksaliplatin ve sisplatinin nörotoksisite düzeyinin sıçanların kaudal ve siyatik sinirleri üzerindeki uyarılabilirlik ve iletim parametreleri açısından karşılaştırılarak incelenmesi amaçlanmıştır.
Yöntem: Yirmi yedi Wistar türü sıçan, oksaliplatin (OXA), sisplatin (CIS) ve kontrol (CON) olarak üç gruba ayrıldı. OXA ve CIS gruplarına 4,5 haftalık takip süresi boyunca sırasıyla oksaliplatin (8 mg / kg / hafta, i.p) ve sisplatin (4 mg / kg / hafta, i.p) uygulandı. Kısa bir süre sonra, uyarılabilirlik ve iletim parametrelerini elde etmek için kuyruk kaudal sinirinden eşik izleme kayıtları ve izole edilmiş siyatik sinirden bileşik aksiyon potansiyeli kayıtları alındı.
Bulgular: Sisplatin, sinir uyarılabilirliği ve iletim açısından oksaliplatinden daha nörotoksik bulunmuştur. Sisplatinin, akson çapı küçük (yavaş ileten) lifler üzerinde daha etkili olduğu görüldü. Sisplatine bağlı olarak, Na^+ kanallarının kısmi blokajı (çoğunlukla kalıcı) ve içeri doğrultucu K^+ iletkenliğinde dikkate değer artış görüldü.
Sonuç: Nörotoksisite açısından oksaliplatin, sisplatin ile karşılaştırıldığında klinik olarak daha tercih edilebilirdir.

Anahtar Sözcükler: Eşik izleme, uyarılabilirlik, iletim, oksaliplatin, sisplatin

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INTRODUCTION

As a platinum-based chemotherapeutic agent, cisplatin and oxaliplatin are common effective cancer drugs. Despite their favourable anti-tumor properties, peripheral neurotoxicity is their one of the important side effect (1,2). In-vivo studies demonstrate that oxaliplatin disrupt Na^+ conductance by acting over activation of voltage-trigger, or in activation kinetics, however the exact neuro-pathological mechanism has not been fully understood yet (3,4,5).

Conventional nerve conduction studies regarding measurements from compound action potential (CAP) recording remain still an important tool in many respects. It is useful means for the evaluation of nerves both for the purpose of research and clinical diagnosis. Nerve excitability measurement with the threshold tracking (TT) method developed by Bostock et al. (6,7) has also gained importance in recent years. Thanks to the TT method more parameters concerning nerve excitability can be acquired reliably. The excitability of an axon can be defined as the amount of current required to yield a specific level of response. Therefore, depolarization of the axon increases its excitability and thereby decreases the strength of threshold current needed to just excite the nerve. Hence, a weaker current is needed to produce the same magnitude response, and conversely, hyperpolarization of the axon increases the amount of current needed to produce a comparable response. With this method, one can provide information related to the activity of a variety of ion channels, energy-dependent pumps, and ion exchange processes activated during impulse in the peripheral axon (23).

The excitability of a myelinated axon is determined by ion channels, pumps, and exchangers. Transient Na^+ channels (Na_t), highly dense at the node of Ranvier, represent the majority of Na^+ current and responsible for action potential generation. Persistent Na^+ channels (Na_p) may reflect differential gating of Na_t channels population, demonstrate incomplete inactivation, and its neurophysiological role is the modulation of excitability. Slow K^+ channels (K_s), highly dense at the node of Ranvier, limits ectopic firing and reduces excitability following impulse transmission. Fast K^+ channels (K_f), highly dense in juxtaparanode, damps excitability after action potential generation to prevent re-excitation. Na^+/K^+ pump, unclear localization, maintains low intracellular Na+ concentration and membrane potential (9).

The present study, using classical CAP recording and newly developed threshold tracking technique, aimed to investigate how cisplatin and oxaliplatin affect peripheral nerve conduction and nerve excitability parameters, these are complementary to each other, through both nodal and axonal alteration. In addition, it was aimed to investigate the comparative side effect levels of oxaliplatin and cisplatin on peripheral nerves in terms of excitability and conduction.

METHODS

The experiments were carried out on 27 adult (12-14 weeks old) male Wistar albino rats purchased from N.E. University Experimental Medicine and Application Center, which were selected randomly. After birth, the animals were housed up to five per cage, at ambient temperature and humidity with 12/12 h light/dark cycle, receiving food and water ad libitum. They were divided into three groups labeled as oxaliplatin (OXA), cisplatin (CIS) and control (CON). The OXA and CIS groups were intraperitoneally injected two times a week with oxaliplatin (8 mg/kg/week), and cisplatin (4 mg/kg/week), respectively, for 4.5 weeks (10). The experiments were conducted after the third day following the injection period. The experimental procedures on animals were held correspondingly the instructions of N.E.U. Meram Medical Faculty Experimental Ethics Committee (Approval No: 2011-112). During the non-invasive excitability testing and dissection of sciatic nerve for in vitro CAP recording experiments, animals were anaesthetized using a combination of 80 mg/kg Ketamine and 10 mg/kg Xylazine. Body temperature was maintained at 37°C using a heating pad (MAY RTC9404-A Animal Rectal Temperature Controller, Commat Ltd., Turkey) with a feedback of rectal probe during the experiments.

In vivo Excitability Studies

Hair in the stimulus and recording regions were removed to reach the skin to decrease electrode resistance. Ag/AgCl surface and ring type electrodes were used for both stimulus and recording, with an average distance of 35 mm in-between, and the ground electrode was placed in the middle of stimulus and recording electrodes. Stimulus electrodes were placed on the hip area (proximal of tail caudal nerve), and distal responses were recorded from tail. Conducting gel was used to minimize resistance between electrodes and tissue. Threshold electrotonus (TE) (for 40% and 20% target response) curves, threshold charge-stimulation duration relationship, current-threshold (I-V) relationship and recovery cycle (RC) curve were obtained using TRONDNF/Rodent Regular Trond protocol of QTRAC software (written by H. Bostock, copyright Institute of Neurology, London, UK) (6). The details of TT method can be found more extensively in Bostock et al. (6), and Nodera and Kaji (7).

The TE protocol of TT provides us excitability changes during and after 100 ms depolarizing or hyperpolarizing conditioning pulse. When a subthreshold conditioning current is applied to the nerve, it can polarize the internode and subsequently affect nerve excitability. The change in excitability is then assessed by a test pulse, whose strength is adjusted to obtain 20% or 40% of maximal response (6, 7). It is known from conventional nerve conduction studies that the excitability of fibers is proportional with the conduction velocity. Therefore, slower fibers contribute more in 40% of maximal CAP which can be attributed to fibers with relatively slow conduction velocity while 20% of maximal response corresponds to relatively fast fibers (10).

The term strength-duration time constant (SDTC), parametrically analogous to chronaxie, is a measure of the rate at which the threshold current for a target potential declines proportionally until it reaches rheobase (in accordance with Weiss Law). Rheobase is the threshold current strength that has infinitely long duration. These two parameters are properties of nodal membrane and are measures of excitability (6, 11), which also are voltage dependent, and this dependence is due to conductance of membrane. Near threshold, membrane conductance is mainly determined by persistent Na^+ currents; therefore, increasing the amount of Na^+ currents will produce a larger SDTC and a lower rheobase (12, 13, 14).

Rectification is the electrical property that causes current to flow more readily in one direction but only slightly or not at all in the opposite direction due to the potential difference (V) across it. This property occurs when conductance is dependent on voltage. In I-V relationship, by holding the duration of the conditioning current constant, its strength is varied from +50% to -100% of the threshold for an unconditioned test potential. Then, by delivering the test pulse of 1 ms duration at the end of polarizing current, one can obtain the I-V relationship (7, 15). This curve reflects rectification due to activation of K^+ channels, or in other words, inward rectifier (16).

Absolute refractory period is the period during which axon is not capable of excitation due to a second stimulus. Immediately following the initial depolarization, the axon will be inexcitable, even if a very strong stimulus is applied. This period is due to voltage gated Na^+ channels being inactivated (17). During relative refractory period, Na^+ channels gradually recover from the inactivation. Axonal excitability is low in this period, so to excite the axon a stronger stimulus is required. Refractory period can be determined by RC protocol within TT (7, 15).

With the TT method, it is possible to determine easily SDTC and rheobase from threshold charge vs. stimulus duration curve (Figure 2A, 2B) (6, 7).

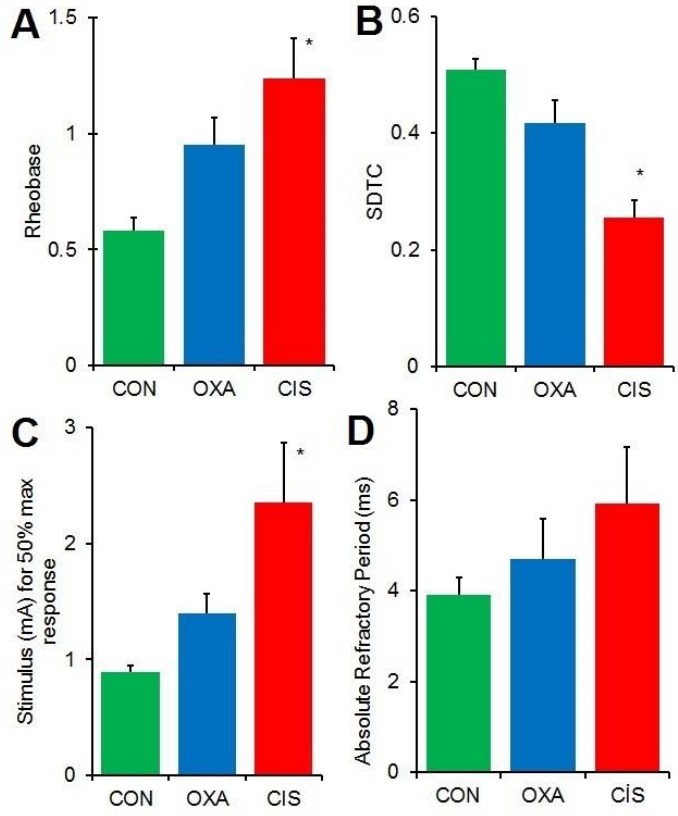

Figure 1: Threshold charge (mA.ms)-stimulus duration (ms) changes (A), the recovery cycle curves (B), threshold electrotonus curves plotted with the values obtained when the target response was set to 40% and 20% of maximum CMAP amplitude (C), current strength (% threshold)-threshold reduction (%) relationship (D) for CON, OXA, CIS groups. Values are given as mean±SEM and * represents the significant levels ($p<0.05$) as compared with CON.

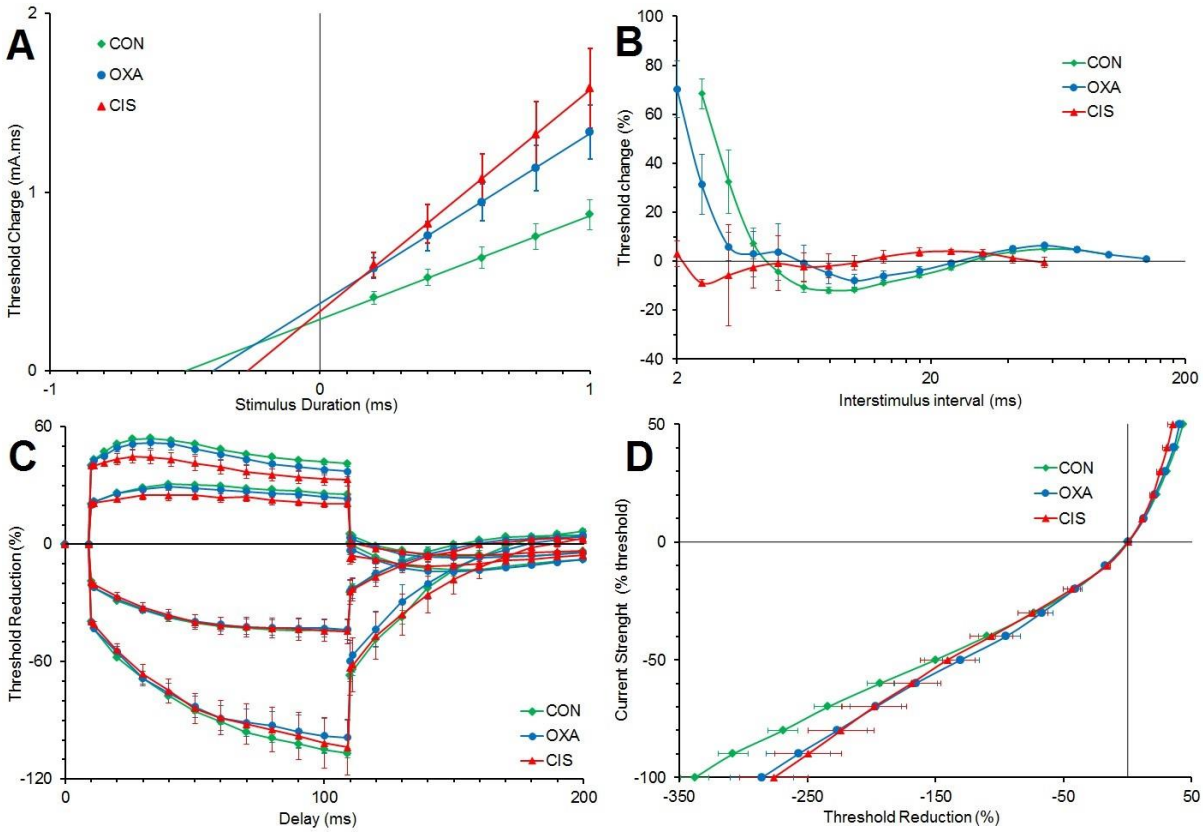

Figure 2: Rheobase (A), strength duration time constant (SDTC, ms) (B), stimulus strength (mA) to reach 50% maximum compound muscle action potential (CMAP) amplitude means (C), absolute refractory period means (D) for each group for CON, OXA, CIS groups. Values are given as mean±SEM and * represents the significant levels ($p<0.05$) as compared with CON.

In vitro Conduction Studies

After excitability testing procedures, sciatic nerves of rats were dissected and then sacrificed by cervical dislocation during anesthesia. Through providing as close to physiological conditions, the suction method is considered the most adequate method for recording compound action potentials (CAPs) from isolated nerves, obtaining detailed conduction properties (10, 18). The nerves were placed into tissue bath perfused with Krebs solution (119 mM NaCl, 4.8 mM KCl, 1.8 mM CaCl2, 1.2mM MgSO4, 1.2 mM KH2PO4, 20 mM NaHCO3, and 10 mM glucose; pH=7.4; temperature fixed at 37˚C) continuously being fed with a gas mixture (5% O2, 95% CO2). Sciatic nerves were stimulated at proximal 20 mm distant to suction recording electrodes with pulses of 0.1 ms duration provided via the stimulator (S88, Grass Instruments Co., USA). Recordings were performed using an AC pre-amplifier (CP511, Grass Instruments Co., USA) from distal end. Signals were sampled at 50 kHz and permanently stored as 20 ms sweeps using BioSigW software.

The early temporal region of a CAP signal is known to reflect the activity of fastest fibers, due to having the largest axon diameters (19). Hence in order to obtain information about these fibers, conduction velocities were calculated which incorporate the delay from stimulus artifact (latency) to the onset of CAP. The instant of maximum time derivative of CAP ($t_{\dot{v}_{max}}$) gives information about the activity of relatively faster fibers (20). A compound action potential is composed of each fiber's individual action potential within the nerve (21, 22), hence the area under the CAP is a measure of contribution of active fibers. When derived from strength-duration curve of isolated nerve recording, rheobase and chronaxie values are the major indicators of excitability of the fastest fibers (23, 24).

Analyses and Statistics

Preliminary analyses of excitability data were held via QtracP software (6). The related graphics were then generated using Microsoft Excel. Threshold charge-stimulus duration graph was fitted to a line, whose slope was then used to calculate the rheobase. Similarly, SDTC was calculated as the point at which the fitted line crosses x-axis. The CAP data from isolated nerve were also analyzed. Latency (L, ms) measured as the duration between stimulus artifact and CAP onset, conduction velocity (CV, m/s), CAP Area (mV.ms) and instant of maximum time derivative ($t_{\dot{v}_{max}}$: ms) of CAP were calculated from CAP signal using Microsoft Excel 2010. The excitability parameters of isolated nerve, rheobase (mV) and chronaxie (ms) values were also computed from stimulus strength-duration curve.

Statistical comparisons among CON, OXA and CIS groups were performed using One-Way ANOVA, after checking whether the data were normally distributed using Kolmogorov-Smirnov (K-S) test. Comparison between individual pairs was performed when necessary, using Tukey's post-hoc test. For distributions not passing the normality test, comparisons were performed with Kruskal-Wallis One-Way ANOVA and Dunn's post-hoc test for individual pairs. Confidence interval for statistical significance was chosen as statistical probability p<0.05.

RESULTS

In vivo Excitability Results

As a general reference, stimulus strengths required to obtain 50% of maximum compound muscle action potential (CMAP) maximum for each group are given in figure 2C. Comparative 40% TE curves belonging to depolarizing and hyperpolarizing conditioning pulse for CIS and OXA are given in Figure 1C. During 100 ms depolarizing conditioning current, TE curve for OXA follows the same path with CON while CIS deviates from CON significantly (p<0.05). TE curves associated with 20% of maximal response for CIS and OXA compared to CON group are also given in Figure 1C. The excitability for OXA and CIS during 100 ms depolarizing conditioning current also follows the same path with CON.

Rheobase and SDTC values derived from threshold charge-stimulus duration curve for each group, were computed from the slope and t-intercept, respectively (Figure 1A), revealing a significant increase in rheobase and decrease in SDTC values, only for CIS (Figure 2A, 2B). I-V relationship, analogous to a current-voltage plot, reflecting a measure of axonal rectifying properties, are given as %change in Figure 1D. The steepening of the curve in upper right quadrant reflects outward rectification accommodating to depolarizing current, and in the bottom left quadrant, gradual steeping reflects inward rectification accommodating to hyperpolarization. Neither OXA nor CIS had significant difference compared to CON. Lastly, the RC curve, pattern of excitability changes following impulse conduction was plotted in the same axis for CON, OXA and CIS in Figure 1B. Mean absolute refractory period values (ms) are also given as a bar graph in Figure 2D.

In vitro Conduction Results

The conduction and excitability parameters of isolated nerve derived from the analyzed CAPs are given in Table 1. No significant difference for either OXA or CIS as compared to CON were found for the CV (m/s) calculated from stimulus artifact to the onset latencies of CAPs. Similarly for the other parameters such as time to maximal derivative ($t_{\dot{v}_{max}}$, ms), rheobase (mV) and chronaxie (ms) values, no significant difference were found between the groups, either. However, the area under CAPs was found to be significantly different for only CIS as compared to CON (p<0.05, Table 1).

Table 1: Conduction and excitability parameters derived from compound action potential (CAP) recording of isolated nerve by suction technique for each group (CON, control; OXA, oxaliplatin; CIS, cisplatin). Values are given as mean ± SEM and * represents the significant levels (p<0.05) as compared with CON.

	CON (N=9)	OXA (N=9)	CIS (N=9)
CV (m/s)	43.59±1.44	41.82±1.96	42.54±1.18
$t_{\dot{v}_{max}}$ (ms)	0.60±0.03	0.55±0.03	0.56±0.02
Area (mV.ms)	4.38±1.86	2.46±0.63	1.01±0.13*
Rheobase (mV)	2.78±0.09	2.80±0.10	2.89±0.05
Chronaxie (µs)	18.70±0.00	16.00±0.00	17.00±0.00

DISCUSSION

In this study, the effects of CIS and OXA on excitability and conduction parameters of rats' caudal and sciatic nerves were investigated by using TT and conventional suction CAP recording methods. The TT method provides a practical means to non-invasively obtain information about excitability of the axons and functions of ion channels. While, the analyses of CAPs recorded from isolated nerves provide direct information related to morphology of a nerve such as demyelination or regeneration.

In our study, Wistar rat model was used; it has similar findings with the clinical application in humans. Kidney pH level, liver and dorsal root ganglion platinum levels and morphometric changes were found to be affected from platinum compound toxicity as well as general parameters in that study by intraperitoneal injection (2).

In vivo Excitability Findings

The average strength values of stimulus for 50% maximal response for rat caudal nerve (Figure 2C) revealed a significant increase for only CIS compared to CON (p<0.05). This result may indicate that the influence of cisplatin on excitability is more than oxaliplatin.

Despite there was no information pertaining to Na^+ and K^+ channel conductance in our study, the gathered and analyzed data using non-invasive threshold tracking technique and its idiosyncratic analysing methods, may provide information about function of ion channels and passive membrane properties of axons (8).

The mean depolarizing TE curves of 40% for CIS significantly deviates to collapsing-in commonly referred to as fanning-in compared to CON curve (p<0.05, Figure 1C). Similar effect can be seen on fast conducting fibers, but it is not significant (20% curves of depolarizing TE, Figure 1C) So, one can say that 4 mg/kg/week dose of cisplatin may have relatively powerful effect on excitability of middle conducting fibers (represented by 40% curves of TE) rather than fast conducting fibers (represented by 20% curves of TE) (24). On the other hand 8 mg/kg/week dose of oxaliplatin has similar fanning-in effect on 40% and 20% curves of depolarizing TE, but it is not significant (p<0.05, Figure 1C). A change in excitability of nerve during depolarizing conditioning current is determined mainly by passive membrane properties, such as resistance and capacitance (20), and by the functions of fast and slow K^+ channels (7, 11). During depolarizing conditioning current, a decrease in K^+ current results in fanning-out of TE waveform; resistivity of axon is decreased in depolarized axons, resulting in a fanning-out. Demyelinization also resulting in a fanning-out. However, our findings show fanning-in property, meaning the excitability for CIS tends to decrease (Figure 1C). These results may be due to adverse changes in Na^+/K^+ pump function (8,16).

The mean hyperpolarizing TE curves of 20% for both CIS and OXA do not deviate significantly from mean CON curve. Although not significant, there is funning-in through end of the curve (S3 fase) of 40% cisplatin curve. This effect may arise from the increment in activity of inward rectifier K^+ channel.

Both reobase and the SDTC are nodal properties and, related to the activity of the persistent Na^+ channel conductance (16). The significant increment in rheobase ($p=0.0047$; Figure 2A) and the significant decrement in SDTC ($p<0.0001$; Figure 2B) due to cisplatin shows that cisplatin may have an effect on persistent Na^+ channel activity. Persistent Na^+ channels are comprised of less than 5% of total, and allow persistent influx of sodium ions (25). So, since the persistent sodium channels determine the degree of membrane polarization and excitability of the axon, cisplatin may decrease membrane excitability by reducing the persistent Na^+ conductance. Oxaliplatin also showed similar behaviour, but it is not significant.

The current-voltage (I-V) relationship provides a measure of axonal rectifying properties (16, 26). The mean curves of outward rectification (in the upper right quadrant of Figure 1D) for OXA and CIS were found to be similar as compared to CON. This finding is consistent with 40% TE curve findings (Figure 1C). Though not meaningful, there is inclination of the curves of inward rectification for CIS towards to right, so it diverges from the CON. This finding may indicate that cisplatin increases the activity of inward rectification K^+ channel (24). These findings are consistent with literature (8). The effect of oxaliplatin on the activity of inward rectifying K^+ channels is not meaningful which is consistent with the findings of Tomaszewski and Busselberg (2007) (27). Inward rectifier channel is located mostly on internodal region of the axon (16, 28). In normal axon, inward rectifier channels are mostly inactive. With demyelinization, inward rectifier channels' activities begin to become evident (16). So, cisplatin may damage the myelin sheaths.

The recovery cycle of excitability results (Figure 1B) imply that the effect of cisplatin and oxaliplatin on the recovery of transient Na^+ channels from the refractory periods are not significant (Figure 2D), yet it also might not be negligible.

In vitro Conduction Findings

Conduction velocity findings imply that the chemotherapeutic agents with specified dosages do not have significant effects on passive conduction properties of fastest fibers (Table 1). Nerve conduction can provide evidence of myelinated damage. This finding supports our 20% TE findings that the effect of administered dosages of oxaliplatin (8mg/kg/week) and cisplatin (4 mg/kg/week) on fastest fibers are limited. This is consistent with previous study (29).

Our results showed that there was no significant difference in CAP area (mV.ms) for oxaliplatin and a significant decrease was observed for cisplatin ($p=0.0338$; Table 1). The area provides information about the number of conducting axons including slowest ones (18). Background studies (30) showed that oxaliplatin affects large sensory axons but not motor axons. Another study demonstrated that oxaliplatin affects cold-sensing nociceptors by changing ion channel expression (31). Based on Descouer et al. (2011) and New et al. (1993) research findings, it was found that oxaliplatin had a significant effect on sensory axons ($p<0.05$). In our findings, we demonstrated that there was a non-significant reduction ($p>0.05$) in oxaliplatin in the CAP area and a significant reduction in cisplatin in the CAP area ($p<0.05$). According to these findings, we may suggest that oxaliplatin affects only sensory fibers while cisplatin affects both sensory and motor fibers because, CAP area consists of both sensory and motor fibers. Several factors such as membrane depolarization or hyperpolarization, axonal shrinkage, or selective loss of large fibers, can cause a significant slowing of impulse conduction (32).

No significant differences exist between groups in terms of rheobase and chronaxie values supporting our findings relating to CV and $t_{\dot{v}_{max}}$ (Table 1). These findings support our SDTC findings (Figure 2B).

This study has investigated the effect of the level of neurotoxicity of oxaliplatin and cisplatin over peripheral nerves in terms of excitability and conduction. Cisplatin is more neurotoxic than oxaliplatin, which is consistant with literature (29, 33). It is found that cisplatin is more effective on the fibers having small radius (slowly conducting).

The partial blockade of Na^+ channels (mostly persistent) and an increment in the activity of inward rectifier K^+ conductance due to cisplatin are noteworthy. The increment in the activity of inward rectifier K^+ channel may arise from that of demolition of myelin sheath, so histological investigation study will be useful in this context. Finally, in terms of neurotoxicity, oxaliplatin may be more preferable compared to cisplatin clinically.

Conflict of interest

The authors declare that they have no conflicts of interest.

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