EVALUATION OF DICLOFENAC SODIUM INJECTION INDUCED NEUROPATHY

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ABSTRACT

Background: *Injection neuropathy is becoming a widespread health problem with increasing incidence in Turkey as well as in developing countries and causing high morbidity. It is seen that every day, the number of files sent by the judicial authorities for evaluation with the claim of medical malpractice is increasing. The aim of the present study is to investigate neuropathic damage or drug-induced neurotoxic effects that may occur due to injection applied to the gluteal region.*

Materials and methods: *In this study, with the aim of investigating the neuropathic damage or drug-induced neurotoxic effects due to injection applied to the gluteal region; no injection was administered to the control group, intramuscular and intraneuronal injections were made to the sham and drug groups, and all mice were subjected to the Rotarod, Tail-flick, Cold Plate and Von Frey tests.*

Results: *In all four test models, it was observed that there was a significant difference between the groups that received intraneuronal administration and the groups that received intramuscular administration (P<0.001). In almost all tests performed at the end of 24 hours, it was observed that the test results of the control and intramuscular administration groups were similar among themselves, and the intraneuronal administration groups were similar to each other.*

Conclusions: *Sciatic neuropathy can cause an extensive range of damage, from the minor motor and sensory abnormalities to complete paralysis. The pathological effects of injection-related injuries vary with the injected agent. Intramuscular administrations usually do not cause nerve damage or nerve damage is minimally limited. Intraneuronal injection applications cause minimal to severe nerve damage depending on various factors. For the prevention of injection neuropathy, it should be ensured to develop proper health education policies and to conduct studies to determine the effects of the drugs found to cause injection neuropathy on the sciatic nerve toxicity.*

Keywords: *Injection injury, injection neuropathy, nerve injury.*

DOI: 10.19193/0393-6384_2022_1_114

Received *March 15, 2021;* ***Accepted*** *December 20, 2021*

Introduction

Injection neuropathy is becoming a widespread health problem with increasing incidence in Turkey as well as in developing countries and causing high morbidity. Today, intramuscular drugs are widely administered outside the hospital as well as in the hospital setting. Although it is considered a fairly simple technique among the people, it can cause very serious complications if it is not applied with appropriate methods[(1)]. According to the World Health Organization, 16 billion injections are given each year, of which 5% are administered for immunizing children and adults, and 5% for blood transfusions and injectable contraceptives. The remaining 90% of injection applications are made for intramuscular and subcutaneous administration of drugs[(2)]. Most of these injections are made due to unnecessary drug use or can be replaced by oral medications. Administration of drugs by injection can cause both infections (HIV, Hepatitis B-C, etc.) and various complications (abscess, cyst, necrosis, etc.) in the

injection site. While the dorsogluteal region is the most commonly used intramuscular injection site in adults, the lateral femoral, deltoid, and dorsogluteal regions are frequently used in infants and toddlers[3]. The use of the dorsogluteal region in adults tends to decline in recent years due to resulting complications and difficulty in localization[4].

The complications associated with injection in the dorsogluteal region are due to the presence of the sciatic nerve, superior gluteal nerve and artery in this region, and injuries that occur during injection, especially in the sciatic nerve[5]. Intramuscular injection is performed by injecting about 2-5 ml of drug into the muscle. Muscle tissue is highly vascularized and contains fewer nerves compared to subcutaneous tissue. Because of its high vascularization, muscle tissue allows the irritant drug to pass into the circulation more quickly, as well as the drug to be administered at a higher volume.

It is seen that every day, the number of files sent by the judicial authorities for evaluation with the claim of medical malpractice is increasing. The aim of the present study is to investigate neuropathic damage or drug-induced neurotoxic effects that may occur due to injection applied to the gluteal region.

Materials and methods

For sciatic nerve injection in mice, intraperitoneal injection of Ketamine (80 mg/kg) + Xylazine (10 mg/kg) was performed for anaesthesia before the procedure.

Anesthetized Mice were exposed under aseptic conditions in order to reach the mouse right leg sciatic nerve, after an incision below the right hipbone, of approximately 1 cm was applied to the biceps femoris and after muscle dissection, the sciatic nerve was exposed. While the sham group animals were used as controls and had only injected SF (20 µl) intraneural (Group IV), In the Intraneuronal Diclofenac Group, 15 mg/kg Diclofenac Sodium was injected intraneuronal (Group V). Then, the incision in the muscle layers and skin layer was closed with stitches (4.0 silk). The general condition will be followed up in terms of infection at 12-hour intervals after the operation.

SF (20 µl) was injected intramuscular in Group II and Diclofenac Sodium (15mg/kg) was injected intramuscular in Group III (in the gluteal muscles). Group I (control) is the untreated group of intact mice. Cukurova University Health Sciences Experimental Application and Research Center (Cu-Sabidam).

Behavioural tests

Animals

In our study, Swiss albino male mice, 8 weeks old (25-30 g), obtained from Çukurova University Health Sciences Experimental Application and Research Center (ÇÜ-SABİDAM) were used. Before starting the experiments, C.U. experimental animals' ethics committee Approval was obtained on March 18, 2021(permit number 7/2021).

In order to Care and minimize animal suffering and to limit the number of animals used in experiments (3R policy). All of the experiments were performed according to the guidelines of the International Association for the Study of Pain (IASP)[6] and the ethical rules specified in the ÇÜ-SABİDAM directive. The mice used in the experiments were placed 8 in each cage, it was housed in standard laboratory conditions and Surgical operations on mice were performed under aseptic conditions in the Behavioural Pharmacology Laboratory of ÇÜ-SABİDAM. The mice were housed on a 12-hour light/12-hour dark cycle (lights on at 07:00 A.M.), in a soundproofed, temperature (22-24 °C) and humidity-controlled (55% and 65%) laboratory, with food and water to eat and drink were available ad-libitum. The latency (in seconds) for the first fall was recorded at 30 min, 1 h and 2 h after administration of DS. The cut-off time was set at 300 s.

Rota-rod test

The injection Influence on motor coordination was evaluated by assessing mice ability to walk on a rotating rod. The rotarod test apparatus (Rota-Rod Treadmill For Mice, Ugo Basile 7600, Varese, Italy) was used according to Kayser V. described previously[7]. The latency for the first fall was recorded before (Pretest) (0) and 30 min, 1, 3, 6 h and 24 h after administration of Diclofenac Sodium. The cut-off time was set at 180 s. the mice were placed on the rotating rod for 3 min at a fixed speed of 18 rotations per minute (rpm).

Tail-flick test

Acute nociception induced by a thermal stimulus which measured by tail-flick test was carried out according to a previously described method[8] The test were performed before (Pretest) and 1, 3, 6, 12 and 24h after the injection of DS (Tail Flick Analgesia Meter; Harvard, Edenbridge, Kent, UK). the tails of the animals were exposed for a short time to the Radiant heat for a maximum cut-off time

was set to 9 s to prevent tissue damage. The mice flicked its tail as a response to a thermal stimulus and time was recorded.

Cold plate test

Chronic nociceptive tested in order to possible sciatic nerve injury that induced by DS injection. Cold allodynia was evaluated with the cold plate apparatus (Ugo Basile, Comerio, Italy) as previously described method[9] before (Pretest) and at different time points (0.25, 0.5, 1, and 2 h) after the administration of DS. The temperature of the plate was kept at 4°C, and the cut-off latency was 30 s. The mice were placed on the cold plate, and the time until lifting up the paw and licking on the toes was recorded.

Von frey test

Determination of Mechanical allodynia was measured by using the Von Frey test that applied punctate stimulus on the paw and assessed the paw withdrawal with the up-down parameter according to a previously described method by Dixon, W.J. et al.[10]. Mechanical allodynia was measured Pretest and 1, 4, 8, 12 and 24 h after the DS injection.

The animals were habituated for 1 h in individual clear boxes on an elevated wire mesh-bottomed platform for access to the plantar surface of the mice hindpaws.

The range of calibrated von Frey monofilaments with bending forces was 0.008g to 300g (Aesthesio, User Manual, USA). Withdrawal threshold measured by applied filaments to the between the third and fourth metatarsus or lateral plantar vertically with enough pressure to bend the filament.

If paw reflex was not exist after each filament tested five times, at an interval of at least 3s, the response to the filament was considered negative and led to use of a stiffer filaments. Paw lifting, licking, biting, flinching and rapid withdrawal of the stimulated paw appearing more than three times were record as a positive response[11].

Results

In this study, with the aim of investigating the neuropathic damage or drug-induced neurotoxic effects due to injection applied to the gluteal region; no injection was administered to the control group, intramuscular and intraneuronal injections were made to the sham and drug groups, and all mice were subjected to the Rotarod, Tail-flick, Cold Plate and Von Frey tests.

Assessment of Injection-Induced Neuropathy with the Rota-rod Test

The motor deficits occurring due to neuropathy at the injection site caused by diclofenac sodium injected were evaluated according to the performance time of the mice on the rod.

In the comparison of the control group with the sham group that was subjected to intramuscular injection, it was determined that the decrease in performance time starting at the 30th minute almost completely returned to the normal at the end of 24 hours [$F(4, 35) = 2237.913$; $p<0.001$]. The test performed at the 30th minute after the injection revealed that the time was significantly lower in all treatment groups compared to the control group [$F(4, 35) = 1069.605$; $p<0.001$]. It was found that there was a significant difference ($p<0.001$) in all time frames between the sham and diclofenac sodium groups in which intramuscular administration was performed, and the test times decreased by 7.63% after the injection application. The rota-rod test data are shown in Table 1 and Figure 1.

Group	Injected	Dose	Average of falling times (seconds)						Percent Decrease in time (Prettest-24h)
			Pretest	30min	1h	3h	6h	24h	
Control Group	None	None	180	180	180	180	180	180	%0
Sham (Intamuscular)	SF	20 µl	180	180	180	180	180	180	%0
Sham (Intraneuronal)	SF	20 µl	180	59.12± 6.46*	67.62± 2.34*	69.37± 6.96*	69.87± 9.96*	72± 5.23*	%60
Diclofenac Sodium (Intramuscular)	Diclofenac Sodium	15mg/kg	180	120.12± 7.43*	109.62± 6.96*	120± 7.52*	143.25± 13.4*	166.25± 6.15*	%7.63
Diclofenac Sodium (Intraneuronal)	Diclofenac Sodium	15mg/kg	180	30.75± 8.81*	33.87± 5.30*	34.12± 6.08*	35.62± 3.54*	38.5± 3.89*	%78.6

Table 1: Observation table for nerve injury by rota-rod apparatus.
*Values are the mean ± SEM for 8 mice. *P<0.001, Compared to control, bonferroni test.*

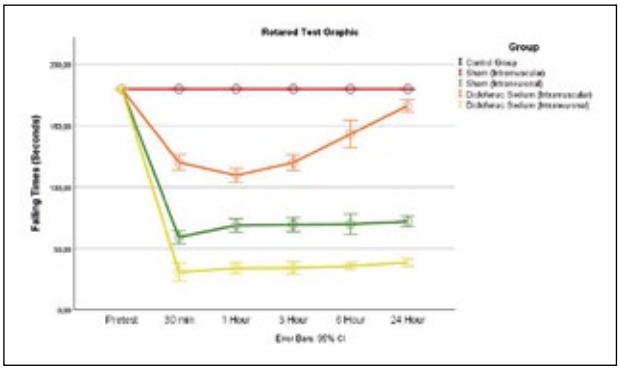

Figure 1: Rota-rod test findings.

Assessment of injection-induced neuropathy with the tail-flick test

Acute nociception occurring due to neuropathy at the injection site caused by diclofenac sodium injected was determined by measuring the tail flick

time of the mice in response to painful stimulus. It was determined that there was no significant difference between the control group and the sham group that received intramuscular administration at all time intervals ($p<0.001$). There was a significant difference in tail-flick time, especially at the first hour, between the sham and diclofenac sodium groups that were administered intramuscularly [$F(4, 35) = 265.101$; $p<0.001$], on the other hand, no significant difference in all time intervals was detected between the diclofenac sodium and sham groups that received intraneuronal injection. Figure 2 illustrates the tail flick times of the groups.

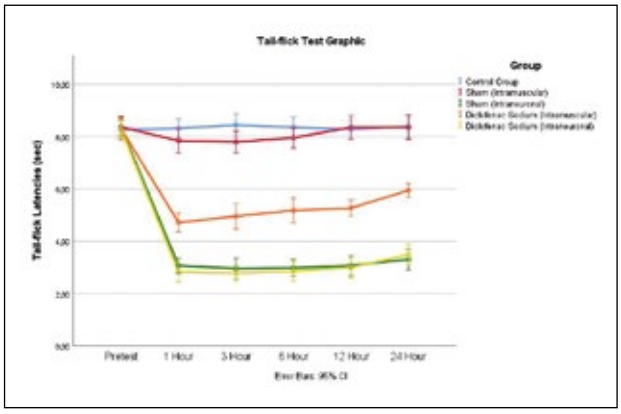

Figure 2: Tail-flick test findings.

Assessment of injection-induced neuropathy with the cold-plate test

The heat allodynia occurring due to the neuropathy at the injection site caused by the injected content was assessed by the measurement of paw licking time of the mice.

The results showed that there was no significant difference at all test time points between the control group and the sham group that received intramuscular injection. In addition, no significant difference in terms of paw licking times was found between the sham and diclofenac sodium groups at the end of the 24th hours in intramuscular injection, while among the mice subjected to intraneuronal injection, the difference between the sham and diclofenac sodium groups was statistically significant at all test time points starting from the first hour of application [$F(4, 35) = 134.721$; $p<0.001$]. The cold plate test results are presented in Table 2 and Figure 3.

Assessment of injection-induced neuropathy with the von frey test

The change in mechanical allodynia occurring due the neuropathy at the injection site caused by the injected content was evaluated according to paw withdrawal latency time. No significant difference was detected between the control group and the sham group receiving intramuscular injection at all test time points.

The Von Frey test results revealed that there was no significant difference at the end of the 24th hour between the sham and diclofenac sodium groups that received intramuscular injection, in addition, among the mice that were subjected to intraneuronal injection, the difference between the sham and diclofenac sodium groups at all time intervals was not significant. The Von Frey test results are shown in Figure 4.

Group	Injected	Dose	Average of Paw-Licking times (seconds)						Percent Decrease in time (Prettest-24h)
			Pretest	1h	4h	8h	12h	24h	
Control Group	None	None	17.31± 0.80	17.33± 0.54	17.38± 0.52	17.37 ±0.54	17.30± 0.47	17.37 ±0.44	%≅0
Sham (Intamuscular)	SF	20 µl	17.15± 0.54	16.67± 0.68	16.98± 0.40	16.98 ±0.44	17.02± 0.40	17.03 ±0.44	%0.6
Sham (Intraneuronal)	SF	20 µl	17.32± 0.58	11.71± 1.27*	11.51± 0.95	11.37± 1.11	11.81± 1.07	11.72± 0.85	%32.3
Diclofenac Sodium (Intramuscular)	Diclofenac Sodium	15mg/kg	17.30± 0.45	14.96± 0.87	14.88± 0.59	15.12± 0.68	15.97± 0.57	16.73± 0.65	%3.2
Diclofenac Sodium (Intraneuronal)	Diclofenac Sodium	15mg/kg	17.27± 0.63	7.86± 1.18	7.88± 0.92	7.37± 0.54	7.42± 1.09	7.83± 0.69	%54.6

Table 2: Observation table for nerve injury by cold plate apparatus.
Values are the mean ± SEM for 8 mice.

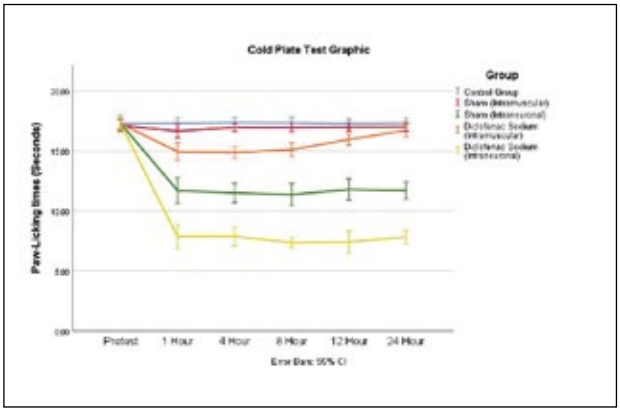

Figure 3: Cold plate test findings.

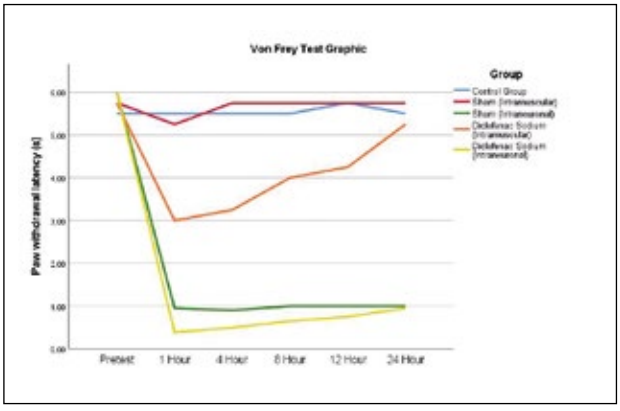

Figure 4: Von frey test findings.

Discussion

While sciatic nerve neuropathy is the most important complication that develops after injection into the gluteal region, complications including abscess, necrosis, infection, contracture, hematoma, chronic pain and periostitis can also develop. The sciatic nerve, on the other hand, can be damaged due to surgical procedures, exposure to toxic substances, infection, penetrating injuries, fractures of the femoral head, and drug injections made directly in or near nerves[12].

Sciatic neuropathy can cause an extensive range of damage, from the minor motor and sensory abnormalities to complete paralysis[13]. Its typical symptoms include low foot, loss of finger flexion and extension, dysesthesia, chronic leg and foot pain, and loss of foot sensation. In our study, after the injections were made, various tests were conducted to evaluate the neuropathy-related response differences in sensitivities such as motor, mechanical sensation, heat sensation, and pain sensation. It was determined in the Rotarod test performed for the evaluation of motor deficit that there was no difference in all time periods between the control group and the sham group receiving intramuscular saline. The performance time at the rotating rod in the intramuscular diclofenac sodium group was significantly lower than the control and the intramuscular saline group [$F(4, 35) = 1069.605$; $p<0.001$], however, in the test repeated at the end of 24 hours, it was observed that the mean times of the group that received intramuscular diclofenac sodium injection approached that of the control group and the group that received intramuscular saline injection, and the difference decreased down to 7.63%. The time-dependent decrease of the difference is thought to be related to the reduction in the neuronal blocking effect of diclofenac sodium administered. Among the mice that received intraneuronal injection, a significant difference was detected between the group with saline solution and the group with diclofenac sodium at all time intervals starting from the 30th minute ($p < 0.001$) up to the 24th hour ($p < 0.001$). It is considered that this difference could be attributed to the chemically induced neuropathic effect of diclofenac sodium. It was reported by Selander et al. that the pathological effects of injection injuries vary depending on the agent injected, extrafascicular injection usually cause no or minimal nerve damage, on the other hand, intrafascicular injection can lead to nerve damage ranging from minimal to severe depending on the drug and dose used[14]. In a study conducted by Gentile et al., intrafascicular and extrafascicular injections of drugs were administered to the rats, they stated that not all extrafascicular injections are harmless, and that various drugs such as benzyl penicillin, diazepam and chlorpromazine cause nerve fiber damage also in extrafascicular administration[15].

The results of the Tail-flick test conducted to assess the painful stimulus sensory deficit indicated that there was no significant difference at all time intervals between the sham group in which the mice received intramuscular saline and the control group, whereas in general the response time to painful stimulus was significantly shortened in the other experimental groups. The decrease in the response time to painful stimulus is considered to occur due to neuropathy-induced hypersensitivity. In the study in which ropivacaine induced peripheral nerve injection injury in the rodent model was investigated, intrafascicular injections of saline, phenol and ropivacaine were made, and it was reported that in saline injection, there was only intraneural edema without demyelination or wallerian degeneration, in phenol injection, all fibers were damaged without measurable regeneration, and in ropivacaine injection, similar to extrafascicular injection, a permanent wallerian degeneration and focal axonal destruction occurred[16].

In the Cold Plate test performed for the assessment of the heat-induced sensory deficit, it was observed that there was no significant difference at all time intervals between the sham group receiving intramuscular saline and the control group, at the 1st hour the group given intramuscular diclofenac sodium injections was significantly different than the other groups, but at the test performed at the 24th hour there was no significant difference among the control group, as well as the saline and diclofenac sodium groups that intramuscular injection was applied. The response times of the saline and diclofenac sodium groups that received intraneuronal injection were observed to be significantly shortened starting from the first hour and continued to be the same in all time points with no prominent change. On the other hand, in the mice subjected to intraneuronal injection, the difference between the saline group and the diclofenac sodium group was significant, and this significant difference continued through all time points. The difference in response time is thought to be attributed to the toxic effect of the drug. The results obtained from the Von Frey

test for the evaluation of the mechanical stimulus sensory deficit showed that there was no significant difference at all time intervals between the group receiving intramuscular saline and the control group. In the test performed at the end of the 24th hour, the mechanical stimulus response time of the group administered intramuscular diclofenac sodium was almost alike to those of the control group and the group administered intramuscular saline, similar to the results of the Rotarod test. It is thought that this situation could be related to the change in the response time due to the toxic effect of the drug in intramuscular administration and to the length of the effect of the damage caused by the toxic effect of the drug on the sciatic nerve after the effect of the drug wears off. It is considered that in intramuscular administration, a change in response time occurs depending on the toxic effect of the drug, and as the effect of the drug wears off, the damage caused by the toxic effect of the drug on the sciatic nerve is related to the length of the duration of action.

For the prevention of injection neuropathy, it should be ensured to develop proper health education policies and to conduct studies to determine the effects of the drugs found to cause injection neuropathy on the sciatic nerve toxicity. Obtaining informed consent from the patients about the toxic effect of injection neuropathy and giving physicians the habit of treating non-consenting patients orally will contribute to the legal and ethical resolution of injection neuropathy, which is the most common medical malpractice that nurses encounter. Informing physicians and nurses that injection neuropathy may develop due to the toxic effect of certain drugs such as diclofenac sodium-containing drugs, administrating orally taken drugs unless contraindicated for oral use and reducing the frequency of intramuscular injection use would help prevent victimization of patients and nurses.

Iatrogenic causes, primarily injection, constitute an important place in the formation of sciatic nerve lesions in Turkey. Although the intramuscular injection appears to be a very simple procedure, to minimize complications, the practitioner having sufficient training should know the anatomy of the region, the advantages/disadvantages of the injection sites, the borders of the injection sites, the injection techniques and constantly update this knowledge.

In a study conducted with the participation of 58 second-year nursing students, the students were asked to mark the injection site on the dorsogluteal region, but it was observed that 33.3% of those marked a region other than the upper-outer quadrant[17]. Considering that millions of injections are administered every year, all efforts should be made to resolve this serious problem. Thus, for injection neuropathy, which returns to health workers as a malpractice lawsuit, first of all, complication/malpractice discrimination should be made, and it should be ensured that healthcare professionals receive the required training and show the necessary sensitivity. The absolute language of law about this condition, which causes the wrongful accusation of health workers who are the target of such lawsuits, which is the nightmare of both patients and healthcare workers, and which is always presented in the press with headlines such as 'paralyzed as a result of faulty injection' is that there is a fault occurred after the injection, so there is a 'crime' and therefore there is also a 'criminal'.

However, sometimes there may not be a criminal; since the factors such as variations mentioned above, the primary effect of the drug, the oedema and/or scar tissue that occurs after injection, individual anatomical differences, the uncertainty of the practitioner in repeated injections, and the lack of a definitive method for diagnosis would be sufficient to eliminate the concept of 'criminal'.

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Ethics approval and consent to participate
- Ethical approval for this study was obtained. The purpose of the study was explained to the participants/their families and informed consent was obtained.

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