Original Article

Does Intramuscular Injection into Paralyzed Tissue in Rats Alter the Absorption of Drugs? An Experimental Multidisciplinary Study

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Abstract

Aim: This study examined if there was altered absorption of a drug administered by intramuscular injection into paralyzed tissue in rats with sciatic nerve injury.
Materials and Method: In this experimental study 16 female Spraque Dawley rats (250–300 gr) and diclofenac sodium as drug were used. The rats were randomly divided into two groups. Axonotmesis was caused by clamping the rats' sciatic nerves. Seven days after sciatic nerve trauma, drug were injected into the biceps femoris muscles of the two groups of rats. In the experimental groups of rats the paralyzed muscles were used. At the end of the experiment, blood samples were collected from the rats' heart and the maximum plasma concentration levels of drug were measured.
Results: The mean drug plasma concentrations levels of the experimental group was 8.84 µg/ml and the control group was 9.46 µg/ml. There was no significant difference between experimental group and control group terms of drug levels in plasma.
Conclusion: Intramuscular injection into paralyzed tissue in rats does not alter the absorption of a drug.

Keywords: intramuscular injection, nursing, paralysis, sciatic nerve injury, drug absorption.

Introduction

Nurses are responsible for the intramuscular (IM) administration of drugs (Rodger & King, 2000; Suhrabi & Taghinejad, 2014). IM injections are not just a routine practice for nurses, but also a complex procedure requiring knowledge of anatomy, physiology, pathology, and pharmacology (Harkreader, 2007; Hunter, 2008; Ismail et al., 2007, Nicoll & Hesby, 2002; Potter & Perry 2009; Small, 2004).

In clinical areas, nurses often encounter patients with paralysis and administering drugs to them via the IM route. Most nurses think that injecting into paralyzed tissue causes the patient less pain, therefore they prefer this site (Yurdagul, 2015). The literature (Harkreader, 2007) suggests that IM injections should not be given into paralyzed tissues; however, the reasons for this recommendation are unclear.

In order for drugs to have an impact on any organ or tissue, they should reach an amount that exceeds a certain concentration in an affected area. The first step in reaching the affected area is the absorption of the drug from the point of administration (Kayaalp, 2012). Absorption of a drug is affected by the solubility of the drug, the type of solvent, drug volume, molecular size of the drug, vascular perfusion of the region where the drug is administered, and the breadth and permeability of the surface of absorption (Whiteneck & Jawad, 1993; Zuidema et al., 1994). Absorption of a drug being administered by intramuscular injection is affected more by the blood flow at the site of administration of the drug than the physicochemical structure of the drug molecule. The speed of a blood flow passing through a muscle at rest is similar to the speed of a blood flow in a subcutaneous tissue. A muscular movement increases the blood flow passing through the muscle leading to an increase in drug absorption (Kayaalp, 2012).

It has been reported that decreased muscular movement and vascular perfusion in paralyzed tissues affects drug absorption and bioavailability when the drug is delivered to these tissues intramuscularly (Whiteneck & Jawad, 1993). There are a limited number of studies reporting the bioavailability of drugs administered intramuscularly to a paralyzed tissue and the results obtained from these studies are inconsistent (Lopez & Salasa, 1999; Segal, 1988).

This experimental study was conducted to investigate whether difference drug absorption between normal tissue and paralyzed tissue after intramuscular drug injection in rats made subject to sciatic nerve injury.

Materials and Methods

Sixteen female Spraque-Dawley rats weighing 250–300 g were used in the study. The rats were randomly divided into two groups of 8, an experimental group comprising rats that were made subject to a sciatic nerve injury and a control group with no sciatic nerve injury.

Surgical procedure for sciatic nerve injury

The rats were fasted for 6 hours prior to the onset of the surgical procedure; they then received an intraperitoneal general anesthetic with thiopental sodium in a dose appropriate for their weights (50 mg kg-1).

The rats were placed in a prone position so that their right femurs were visible and the operation area was disinfected with 10% povidone-iodine. An incision was made along the sciatic nerve track in the right gluteal area using a number 10 lancet to expose the nerve and retract skin and subcutaneous area.

The muscle was dissected with the help of the lancet and clamps to explore the sciatic nerve. To create an axonotmesis-type injury (an injury when there is a complete incision in the myelin sheath at and around the nerve axon keeping the mesenchymal structures, perineurium, and epineurium, protected), a 12.5 cm long clamp was applied on the surgically explored sciatic nerve for one minute (Sezer et al., 2014).

After completing the experimental trauma, the layers were duly closed and the wound was disinfected. All the rats in the experimental group were observed to develop paralysis affecting their right hind limb flexor muscle groups. The rats in both the experimental and control groups were kept in closed cages, housing 4 rats each, in a laboratory environment at 21–24 °C, leaving the rats free to access water and food.

Since the entire motor, sensory and sympathetic functions disappear within 24–72 hours in axonotmesis-type nerve injuries, the rats were kept in a laboratory environment for 7 days for the effects of paralysis to emerge (Koyuncu & Cetinus, 2009).

Procedure for IM injection

Seven days after creating paralysis, IM drug (diclofenac sodium 10 mg/kg) was administered to the rats in both the control and experimental groups into their right biceps femoris muscles. The injections were made into the paralyzed extremities of the experimental group rats.

Analysis of Drug Levels in Rats

The blood samples that were collected from the hearts of rats were transferred in 2 ml EDTA vacuum glass tubes to determine the concentrations of diclofenac 40 minutes after the injections.

The plasma was immediately separated by centrifugation at 4000 rpm for 10 minutes. Extraction procedure was applied to 1.0 mL of each plasma sample. Plasma samples was added to a test tube, 0.1 mL of internal standard was spiked into the plasma samples and 0.5 mL H_3PO_4 solutions were added.

After vortex mixing for 5 s, 3 mL of ethylacetate and hexane (2:3, v/v) was added as organic layer. The mixture was vortexed for 2 min and then centrifuged at 3000 × g for 3 min. The organic layer was transferred into another 5 mL test tube and evaporated to dryness under a stream of nitrogen gas at 40 °C.

The residue was reconstituted in 1.0 mL methanol, and a 20 µL aliquot was injected into the HPLC system.

Chromatographic conditions

Chromatographic analysis was performed by an Agilent Technologies 1200 series HPLC system (Agilent, USA) equipped with a solvent degassing module (G1322A), quaternary gradient pump (G1312A), autosampler (G1313A), thermostated column compartment (G1316A) and Agilent HPLC Chamstation software.

The reversed-phase ACE C18 analytical column (250 mm × 4.6 mm, 5 µm particle size) at variable temperature and the isocratic mobile phase consisted of 20 mM phosphate buffer (pH 7) containing 0.1% TFA-acetonitrile (38:62 v/v). The injection volume and the flow rate were 20 µl and 1 ml/minute, respectively. The eluent was monitored by UV detection at 225 nm.

Method validation

Calibration curves were plotted by spiking a known amount of diclofenac (0.25, 0.75, 1, 2, 6, 8, 16 and 32 µg/mL) and internal standard solutions into 8 different blank plasma samples. The correlation coefficient was greater than 0.9996.

The LOD and LOQ of the proposed method were determined at signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD and LOQ were 0.05 µg/mL and 0.25 µg/mL, respectively. Average recovery value of HPLC-UV method was found as 96.0%.

Statistical analysis

The Mann-Whitney U test was used for the statistical analysis of the data. The statistical level of significance was set at $p < 0.05$.

Ethical Consideration

The Central Medical Experimental Research and Application of Ataturk University provided the animals. In addition, the experimental protocol was submitted to and approved by the Animal Research and Ethics Committee of Ataturk University.

Results

After administering IM drug to the rats paralyzed by an experimental sciatic nerve injury and to the rats in the control group, the concentration levels of the drug that appeared in plasma are given in Table 1.

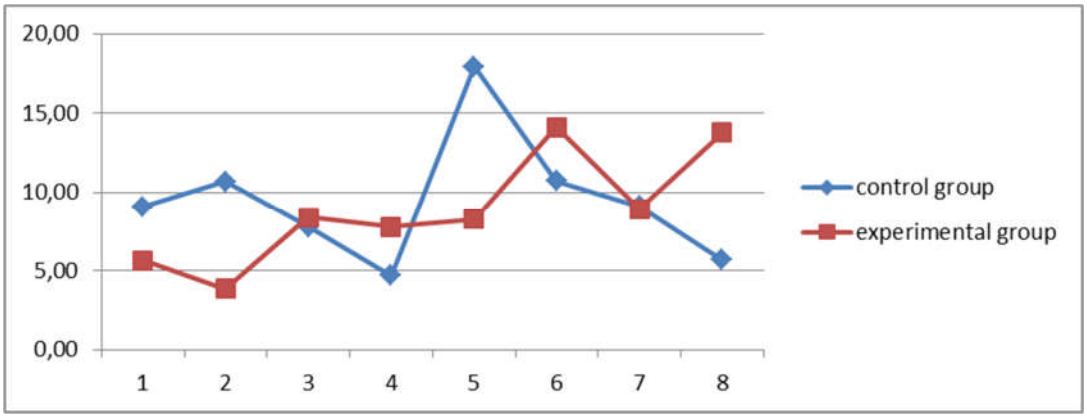
The mean drug levels of the rats in the experimental group was 8.84 µg/mL and that of the control group rats was 9.46 µg/mL (Figüre 1). Although the plasma concentration level of the drug was lower in the paralyzed rats than in the healthy rats, the difference between the two groups was not statistically significant ($p>0.05$; Mann-Whitney U test value = 26.000).

Table 1. Diclofenac sodium levels of plasma

Rats No	Diclofenac sodium levels (µg/mL) experimental group	Rats No	Diclofenac sodium levels (µg/mL) control group
1	5.64	9	9.08
2	3.84	10	10.68
3	8.39	11	7.80
4	7.78	12	4.71
5	8.29	13	17.93
6	14.11	14	10.72
7	8.92	15	9.11
8	13.77	16	5.67
Mean	8.84		9.46

p>0.05; Mann-Whitney U test value = 26.000

Figure 1: Drug plasma levels of control and experimental groups



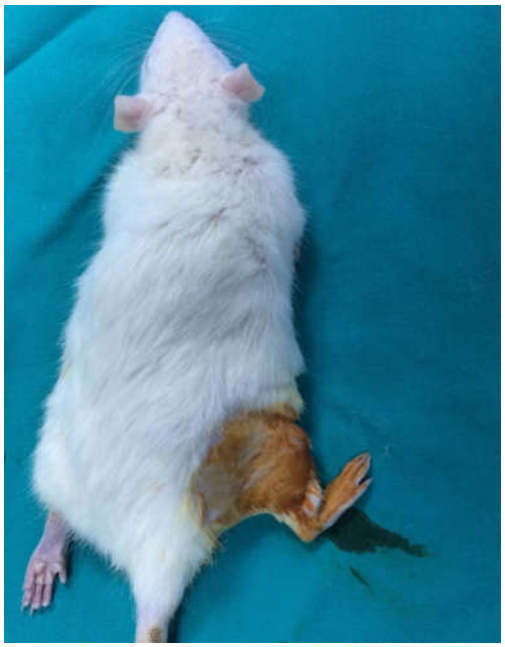


Figure 1: Prone position before operation

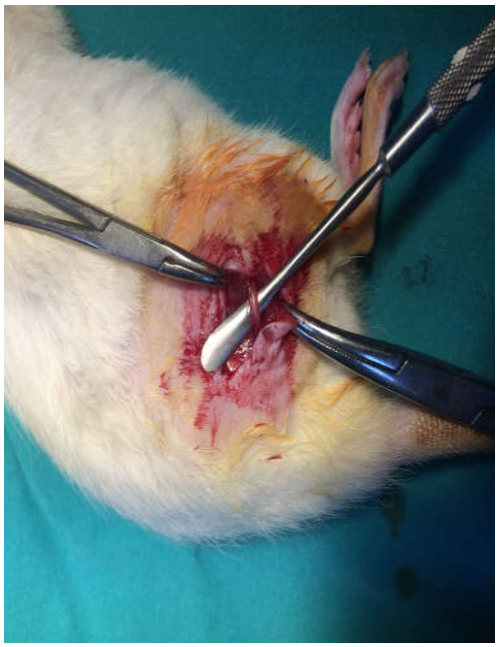


Figure 2: Sciatic nerve exploration

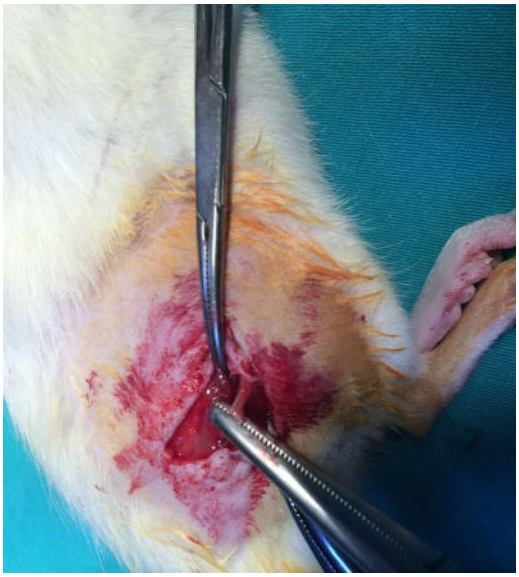


Figure 3: Injury caused by tightening a 12.5 cm long clamp by 1 tooth on the rat's sciatic nerve for 1 minute

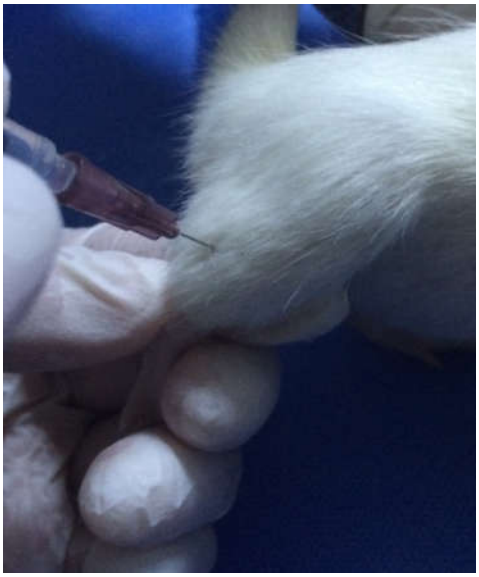


Figure 4: IM injection to biceps femoris

Discussion

No statistically significant difference was found between the experimental and control group rats in terms of the plasma concentration level of drug after the IM injection was administered to the rats. However, the experimental group rats had a lower mean drug concentration level in their plasma as compared to the control group rats.

In their study examining the bioavailability of the IM diclofenac sodium administered 24 hours after creating an experimental spinal cord injury in rats, Lopez and Salasa (1999) found that there was no statistically significant difference between the experimental and control groups in terms of maximum concentration levels of diclofenac sodium in blood. Our study supports the results of this study in this respect. In their study, Segal et al. (1988) stated that among patients who had a spinal cord injury for at least a year the bioavailability of gentamicin deteriorated in this group as compared to a control group.

A paralysis that occurs in muscles for any reason causes a decrease in distal blood flow and an impairment in distal perfusion (Judge, 2007; Malik et al., 2009; Whiteneck & Jawad, 1993). These findings indicate that the blood flow in the paraplegic tissue deteriorates progressively over time. The results of our study indicate that tissue perfusion was not impaired at a level that affects drug absorption seven days after sciatic nerve injury.

In the study by Sankaranarayanan et al. (1989), the blood levels at the 15th, 30th and 45th minutes of administering gentamicin to the paralyzed deltoid muscles of patients were found to be lower than the blood levels of patients in a control group that had no paralysis, but no difference was found between the groups at the 60th, 90th and 120th minutes. This result suggests that drug absorption from paralyzed tissues is slower within the first 45 minutes of administration. Finding no difference between the groups in our study may be because the blood samples were taken 40 min after the injections or perhaps because the sample consisted of rats.

Segal (1988) and Sankaranarayanan et al. (1989) have reported in their studies of patients who had longstanding spinal cord injuries that other factors besides absorption (distribution, metabolism and elimination) also have an impact on the blood plasma level of the drug. Since no injury was created in the present study that would impair the distribution, metabolism or elimination of the drug, we assume that only drug absorption had an impact on plasma drug concentration. We suggest that the differences between the results of our study and those of Segal (1988) and Sankaranarayanan et al. (1989) were due to the reasons mentioned above.

Limitations

The blood samples were taken from the heart of the rats, as insufficient plasma could be obtained for analysis from the blood drawn using other methods. Therefore, this is a limitation of this study in that recurring measurements could not be made and the results of plasma drug levels could not be obtained at different times.

Conclusion

It was concluded in the present study that IM injection into paralyzed tissue in rats does not alter the absorption of a drug. However, we believe that this study will shed light on the advanced clinical and experimental researches.

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