The histological effect of the injection of nonsteroidal anti-inflammatory drugs on sciatic nerve of rats

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Abstract

The ongoing work aims to compare the effect of extraneural and intraneural injection of therapeutic doses of meloxicam and diclofenac sodium on the sciatic nerve of rats. Six groups of adult albino rats were used with five animals per group. Control group (A), group (B), and group (C) received a single extraneural injection of normal saline (NS) 0.25 ml/kg/rat, meloxicam (M) 0.11 mg/kg/0.25ml/rat and diclofenac sodium (V) 1.1 mg/kg/0.25ml/rat respectively. In contrast, control group (D), group (E), and group (F) received a single intraneural injection of the same doses of normal saline, meloxicam, and diclofenac sodium, respectively. Histological evaluation reveals an increased thickening of epineurium, dilatation, congestion of epineurial blood vessels, intrafascicular edema, axonal degeneration, myelin degeneration, and vacuolization in group (C) which was higher than those in the group (B). These changes were also greater in group (F) compared to groups (D) and (E). The histopathological changes of the sciatic nerve were greatest in the case of intraneural injection of saline, meloxicam, and diclofenac sodium, respectively. Histological evaluation reveals an increased thickening of epineurium, dilatation, congestion of epineurial blood vessels, intrafascicular edema, axonal degeneration, myelin degeneration, and vacuolization in group (C) which was higher than those in the group (B). These changes were also greater in group (F) compared to groups (D) and (E). The histopathological changes of the sciatic nerve were greatest in the case of intraneural injection of saline, meloxicam, and diclofenac sodium, which means that the damaging effect of intraneural injection of the drug was greater than the extraneural injection of the same drug. It is concluded that extraneural and intraneural injections of therapeutic doses of meloxicam cause less damage to the sciatic nerve compared to diclofenac sodium. Thus, it is considered to be more secure than diclofenac sodium after intramuscular injection. Sciatic nerve injury can occur following intramuscular injection into the gluteal region, particularly if the needle hits the nerve.

Keywords: Axonal degeneration, Diclofenac sodium, Intraneural injection, Meloxicam, Sciatic nerve

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life, especially in children, underweight patients, and the elderly, thus it represents a widely used subject for preclinical histopathological research (9,10).

The present study compares the effect of extraneural and intraneural injection of therapeutic doses of meloxicam and diclofenac sodium on sciatic nerve tissue of adult rats.

Materials and methods

Ethical approve

The research was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul. Ref. no.: UOM/COM/MREC/20-21. Date: 30/4/2021.

Animals

Thirty healthy adult male Wistar albino rats close to 13-15 weeks old and weighing 250-300 grams were collected from the Animal House of College of Veterinary Medicine, University of Mosul, Mosul, Iraq. Animals were kept in specially designed cages (1 rat/ cage) in the laboratory under a suitable environment for seven days before the experiment. The rats were fed a typical diet and water ad-libitum.

Drugs

Meloxicam (M) (Mobic®, Boehringer Ingelheim, Germany) of 15mg/1.5ml Ampule and Diclofenac sodium (V) (Voltex®, PIONEER Co. for Pharmaceutical Industries, Sulaymaniyah - Iraq) of 75mg/3ml Ampule, were purchased from a standard drug store. The drugs were diluted with normal saline in order to estimate the doses. The dose of injection for each drug was similar to the human therapeutic dose (11).

Groups and experimental design

The animals were randomly separated into six equal groups, with five rats for each. Subsequently, all of the rats were weighed before starting the treatment to calculate their doses.

Extraneural injection groups

Rats received a single injection of medication to the area surrounding the sciatic nerve (8). Group A control rats received normal saline (NS) in the dose of 0.25ml/kg/rat. Group B received meloxicam (M) in the dose of 0.11mg /kg /0.25ml/rat. Group C received diclofenac sodium (V) in the dose of 1.1mg /kg /0.25ml/rat.

Intraneural injection groups

Rats received a single injection of medication into the sciatic nerve (12). Group D control rats received normal saline in the dose of 0.25 ml/kg/rat. Group E received meloxicam in the dose of 0.11mg /kg /0.25ml/rat. Group F received diclofenac sodium in the dose of 1.1mg /kg /0.25ml/rat.

Protocol

The animals were anesthetized using ketamine and xylazine. The sciatic nerve was exposed after dissecting the gluteal muscles. The saline or drugs were extraneural and intraneural injected at the point just above the branching of the sciatic nerve in all groups (Figure 1). The muscles and skin were sutured. The rats were kept for one week in cages. Then, all rats were decapitated after deep anesthesia with ketamine and xylazine, and the sciatic nerves were dissected and removed.

Figure 1: A picture illustrates the site of injection in the sciatic nerve.

Histological preparation and evaluation

The sciatic nerve specimens were fixed for more than 24 hours in neutral buffered formalin (10%), dehydrated with ethanol, cleared by xylene, embedded in paraffin, and cross-sectioned into five μm sections (13). The sections were stained using Hematoxylin and Eosin, in addition to Masson’s Trichrome. Qualitatively all sections obtained from the six groups were examined by light microscope (Novex, Holland) to detect the thickening of epineurium of the sciatic nerve, congestion, and/or dilatation of blood vessels within epineurium and intrafascicular edema. A quantitative study of the sciatic nerve was performed using simple counting method by examination of the abnormal cells after taking a digital picture for the section by 48 Megapixels digital camera with unique microscope fixer and using (ACDSee Photo Studio Ultimate 2019) program for counting the following: Axonal degeneration, myelin degeneration, and vacuolization within the sciatic nerve sections. The counting unit of abnormal cells was (nerve cell / 400X field) (14).

Statistical analysis

A computer package (Sigma plot V12.0 / SYSTAT software) was applied to manage the histomorphometric analysis. Data were displayed as means±SE (Standard error) and were analyzed using Duncan’s test with a significant level set on P <0.05 (15).
Results

Group A (control group)

The sciatic nerve in the control group histologically appears to be expected. Epineurium was normal with no increase in thickening (Figure 2). Within the epineurium, the blood vessels look normal without any dilatation or congestion (Figure 3). There was no intrafascicular edema, though few changes in the histological structure of the sciatic nerve were observed, such as axonal degeneration in the average of 1.2±0.1, myelin degeneration 1.5±0.23, and vacuolization 0.3±0.1 per 400X field. (Figure 4, Table 1).

Group B (extraneural meloxicam injection)

The epineurium mildly increases in thickening. The blood vessels within the epineurium show mild dilatation without congestion (Figures 5 and 6). There was no intrafascicular edema. In the fascicle, there were axonal degeneration 5.3±0.3, myelin degeneration 7.6±0.35, and vacuolization 0.9±0.19 per 400X field significantly higher than the group treated with normal saline (Figure 7, Table 1).

Group C (extraneural diclofenac sodium injection)

There was a mild to moderate increase in the thickening of epineurium after staining with Masson’s Trichrome (Figure 8). Also, there was mild to moderate dilatation and congestion of blood vessels within the epineurium (Figure 9). There was intrafascicular edema. Additionally, many histopathological changes appeared within the neural cells compared with groups A and B; axonal degeneration

Table 1: Histopathological changes in the sciatic nerve following extraneural injections in different groups

<table>
<thead>
<tr>
<th>Changes</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>An (NS)</td>
</tr>
<tr>
<td>Axonal degeneration</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Myelin degeneration</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

The different letters in rows mean a significant difference at P≤0.05.
8.2±0.360, myelin degeneration 12±0.62, and vacuolization 2.5±0.29 per 400X field (Figures 10 and 11, Table 1).

Figure 6: A photomicrograph of group B’s sciatic nerve section showing epineurium (ep) and mild blood vessels dilatation (arrows). H&E, X400.

Figure 7: A photomicrograph of a section of the sciatic nerve of group B showing normal axons (white arrows), degenerated axons (arrowheads), and myelin degeneration (black arrows). H&E, X400.

Figure 8: A photomicrograph of a section of the sciatic nerve of group C showing mild to moderate increase in thickening of epineurium (arrow). Masson’s Trichrome, X400.

Figure 9: A photomicrograph of group C’s sciatic nerve section showing epineurium (ep) and mild to moderate blood vessels dilatation and congestion (arrow). H&E, X400.

Figure 10: A photomicrograph of a section of the sciatic nerve of group C showing normal axons (white arrows), degenerated axons (arrowheads), myelin degeneration (black arrows), vacuolization (green arrows), and edema (e). H&E, X400.

Figure 11: A histogram showing the histopathological changes in the sciatic nerve after extraneural injection of the drugs in control and treated groups.
**Group D (control group)**

The histological examination of the sciatic nerve of group D appears normal, except a few histopathological changes occur within axons. The epineurium appears normal (Figure 12). The blood vessels in the epineurium appear normal without dilatation or congestion (Figure 13). There was intrafascicular edema, and there were few histological changes inside the fascicle, such as axonal degeneration 2.8±0.2, myelin degeneration 5.5±0.29, and vacuolization 0.6±0.13 per 400X field (Figure 14, Table 2).

### Table 2: Histopathological changes in the sciatic nerve following intraneural injections in different groups

<table>
<thead>
<tr>
<th>Changes</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D (NS)</td>
</tr>
<tr>
<td>Axonal degeneration</td>
<td>2.8±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Myelin degeneration</td>
<td>5.5±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>0.6±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The different letters in rows mean a significant difference at \( P \leq 0.05 \).

**Group E (intraneural meloxicam injection)**

The epineurial layer of the sciatic nerve mildly increased in thickening. The epineurium contains mildly dilated and congested blood vessels (Figures 15 and 16). There was edema inside the fascicle. Moreover, there were axonal degeneration 9.6±0.7, myelin degeneration 10.8±0.81, and vacuolization 1.3±0.23 per 400X field, all of which were considered higher than the control group (Figure 17, Table 2).

**Group F (intraneural diclofenac sodium injection)**

There was a moderate increase in the thickening of epineurium (Figure 18). The blood vessels revealed mild to moderate dilatation and congestion (Figure 19). There was edema inside the nerve fascicle, axonal degeneration in an average of 20.4±0.8, myelin degeneration 16.6±0.75, and vacuolization 4.3±0.57 per 400X field (Figures 20 and 21, Table 2).

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[Figure 12](#): A photomicrograph of group D's sciatic nerve section showing normal epineurium (arrow). Masson’s Trichrome, X400.

[Figure 13](#): A photomicrograph of group D's sciatic nerve section showing epineurium (ep) and normal blood vessels (arrows). H&E, X400.

[Figure 14](#): A photomicrograph of a section of the sciatic nerve of group D showing normal axons (white arrows), degenerated axons (arrowheads), myelin degeneration (black arrows), vacuolization (green arrow), and edema (e). H&E, X400.

[Figure 15](#): A photomicrograph of a section of the sciatic nerve of group E showing a mild increase in the thickening of epineurium (arrow). Masson’s Trichrome, X400.

[Figure 16](#): A photomicrograph of a section of the sciatic nerve of group E showing edema inside the nerve fascicle. Masson’s Trichrome, X400.
Figure 16: A photomicrograph of group E's sciatic nerve section showing epineurium (ep) and mild blood vessels dilatation and congestion (arrows). H&E, X400.

Figure 17: A photomicrograph of a section of the sciatic nerve of group E showing normal axons (white arrows), degenerated axons (arrowheads), myelin degeneration (black arrows), vacuolization (green arrow), and edema (e). H&E, X400.

Figure 18: A photomicrograph of a section of the sciatic nerve of group F showing a moderate increase in the thickening of epineurium (arrow). Masson's Trichrome, X400.

Figure 19: A photomicrograph of group F's sciatic nerve section showing epineurium (ep) with mild to moderate blood vessels dilatation and congestion (arrows). H&E, X400.

Figure 20: A photomicrograph of a section of the sciatic nerve of group F showing normal axons (white arrows), degenerated axons (arrowheads), myelin degeneration (black arrows), vacuolization (green arrows), and edema (e). H&E, X400.

Figure 21: A histogram showing the histopathological changes in the sciatic nerve after intraneural injection of the drugs in control and treated groups.
Comparison between the histopathological changes of extraneural and intraneural injections of each drug in different treated groups is shown in Table 3.

Table 3: Comparison between histopathological changes of extraneural and intraneural injections of each drug

<table>
<thead>
<tr>
<th>Drugs</th>
<th>NS</th>
<th>M</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axonal degeneration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraneural</td>
<td>1.2±0.1a</td>
<td>5.3±0.3a</td>
<td>8.2±1.6a</td>
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<tr>
<td>Intraneural</td>
<td>2.8±0.2b</td>
<td>9.6±3.1b</td>
<td>20.4±0.8b</td>
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<tr>
<td>Myelin degeneration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraneural</td>
<td>1.5±0.2a</td>
<td>7.6±0.3a</td>
<td>12±0.6a</td>
</tr>
<tr>
<td>Intraneural</td>
<td>5.5±0.2b</td>
<td>10.8±0.8b</td>
<td>16.6±0.7b</td>
</tr>
<tr>
<td>Vacuolization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraneural</td>
<td>0.3±0.1a</td>
<td>0.9±0.1a</td>
<td>2.5±0.2a</td>
</tr>
<tr>
<td>Intraneural</td>
<td>0.6±0.1a</td>
<td>1.3±0.1a</td>
<td>4.3±0.5b</td>
</tr>
</tbody>
</table>

The different letters in columns mean a significant difference at P≤0.05.

Discussion

Due to the difficulty in dissecting and examining the nervous tissue in humans, histological examination of the sciatic nerve injury after drug injection into the gluteal region of rats was performed in this study. The sciatic nerve appears to be normal in the extraneural control-treated group, but few changes like axonal degeneration, myelin degeneration, and vacuolization were observed. Many studies agreed that normal saline has minimal damage or no damage to the sciatic nerve (8).

In the meloxicam treated group, the epineurium thickening was mildly increased, possibly because of the excessive fibrin formation by the epineurial fibroblast in order to replace the damaged tissue (16). Meloxicam causes mild intraneural damage within the nerve fiber axons, including axon degeneration, myelin degeneration, and vacuolization. Compared with the control group, there was a significant difference between histopathological changes at (p ≤ 0.05) except vacuolization, which indicates that both normal saline and meloxicam had minimal damage. This damage was probably not enough to produce cell death of myelinated axons and thus minimize vacuolization. Unfortunately, there were no available articles that discuss that, but Bostan et al. (17) support this explanation. They mentioned that lornoxicam (same family of meloxicam) had a mild effect when injected into the sciatic nerve’s neighboring tissue.

In the diclofenac sodium treated group, there was a mild to moderate increase in the epineurial thickening, which means more damage to the area surrounding the fascicle due to the toxic effect of diclofenac sodium, which was more than that induced by saline and meloxicam. Similarly, Bostan et al. (17) found that all rats injected extraneural with diclofenac had epineurium thickening. More than that, there was mild to moderate dilatation and congestion of blood vessels in the extraneural epineurial layer. Qassim et al. (18) noticed congestion of blood vessels when diclofenac was injected into the gluteal muscles. This bolsters the present study and leads to the fact that the irritation and toxicity of the drug will create congestion of the epineurial blood vessels. The presence of intraneuricular edema after extraneural injection of diclofenac sodium which was absent in the meloxicam and saline groups, was in agreement with Bostan et al. (17), who found that injection of diclofenac sodium into the area surrounding the sciatic nerve will cause edema inside the nerve fascicle. This is perhaps due to the damage of blood vessels by the toxicity of diclofenac sodium after extraneural injection, which will result in increased vascular permeability with subsequent accumulation of fluid inside the nerve (19).

Furthermore, there were axonal degeneration, myelin degeneration, and vacuolization. Compared with sections of control and meloxicam groups, there was a significant difference between histopathological changes (P ≤ 0.05). This occurs due to increased damage produced by diclofenac sodium as approved by Bostan et al. (17). Also, Canan et al. (20) considered that axon’s defect was significantly prominent after diclofenac sodium injection. As diclofenac is a cyclo-oxygenase inhibitor, it inhibits prostaglandin synthesis, thus creating vasoconstriction, which would cause ischemia and necrosis of deep tissue or muscle or even skin after intramuscular injection (21). This explains the extent of damage formed by diclofenac sodium in the sciatic nerve tissue, as observed in the current study.

The findings which were mentioned above summarized that meloxicam had lower toxicity and damage than diclofenac. This is because meloxicam had a slower release rate compared to diclofenac. It reaches the maximum concentration in the plasma 1 to 2 hours after injection (22), whereas diclofenac reaches its maximum concentration after 20-40 minutes (23). This explanation was supported by Sutton et al. (24), who mentioned that following intramuscular injection, local tissue damage was in relation with the local concentration of the drug besides its release from the formulation, as the slower drug release leads to lower tissue damage since the drug is diluted in the interstitial fluid and cleared by lymphatics.

In the intraneural control-treated group, the histological appearance of the sciatic nerve was similar to that described by other authors (8,25). There was intraneuricular edema and several intraneuricular histopathological changes involving axonal degeneration, myelin degeneration, and vacuolization. These changes were considered to be less than those in other treated groups, and this may be due to the non-toxic and non-irritant behavior of normal saline that was in agreement with other studies (17, 26).
In the meloxicam treated group, the sciatic nerve was mildly affected, and there was edema inside the fascicle. It is suggested that intraneural injection of the drug will cause pressure on the blood vessels, thus causing inhibition of microvascular blood flow, producing an increase in the vascular permeability leading to edema (27). The histopathological changes involving the nerve axons were significantly different from the control group (P ≤0.05) except for vacuolization. It seems that meloxicam causes minimal tissue damage, supported by Bostan et al. (17), who said that intraneural injection of lornoxicam had minimal damage on nerve tissue.

In the diclofenac sodium treated group, there was moderate damage to the sciatic nerve. This result was congruent with Bostan et al. (17). There were significant differences between histopathological changes of nerve axons compared with saline and meloxicam groups at (P ≤0.05) and non-significant difference of myelin degeneration between meloxicam and diclofenac at (P ≤0.05). Alabdaly et al. (28) mentioned that NSAIDs could cause oxidative stress. Oxidative stress and mitochondrial dysfunction will lead to nerve cell damage represented by axonal degeneration, myelin degeneration, and vacuolization (29). In the present study, there was intrafascicular edema which was also described by Emir et al. (8), perhaps because of the increased perineurial permeability induced by the drug (30).

Diclofenac in this work shows more significant damage than meloxicam on the neural tissue, probably because it is a preferential COX-II inhibitor (31). The inhibition of COX-II will interrupt homeostatic function, resulting in vasoconstriction and ischemic necrosis, causing more damage to the tissue (6), while meloxicam has minimal damage on the neural tissue because it is a preferential selective COX-II inhibitor (31).

Generally, from the results mentioned before, apart from vacuolization, all the histological changes of the sciatic nerve were so evident in the case of intraneural injection of saline, meloxicam, and diclofenac sodium, which means that the damaging effect of intraneural injection of the drug was more significant than the extraneural injection of the same drug.

The mechanical trauma to the sciatic nerve fibers which is exerted by the sharp edge of the needle (32), as well as, the injection of the drug by the needle into the nerve which will bypass the protective barrier of the epineurium and perineurium (12), in addition to the increased intraneurial volume and concentration of the drug after intraneurial injection (8); these hypotheses may be the reason for this significant damage induced by intraneurial injection in comparison to extraneurial one.

Conclusion

Extraneural and intraneurial injections of therapeutic doses of meloxicam cause minor damage to the sciatic nerve compared to diclofenac sodium. Thus, intramuscular injection of meloxicam seems to be more secure than that of diclofenac sodium. Sciatic nerve injury can occur following intramuscular injection when the needle hits the nerve, so precaution is mandatory during intramuscular injection, and selection of the correct size is crucial to prevent this injury.

Acknowledgments

The authors would like to thank the College of Medicine, the University of Mosul for supporting this work. Our appreciation to the staff members of Animal House in the College of Veterinary Medicine, the University of Mosul, for the help they gave us to accomplish the work.

Conflict of interest

There is no conflict of interest as declared by the authors.

References


