Iraqi Journal of Veterinary Sciences

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Comparison between two medical drugs' effect on the crushed sciatic nerve healing in dogs

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Article information

Article history:
Received 06 March, 2023
Accepted 13 June, 2023
Available online 12 December, 2023

Keywords:
Nerve injury
Nerve regeneration
Methylprednisolone
Therankeron

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Abstract

This article was conducted to evaluate the role of therankeron and methylprednisolone on the healing and regeneration process of the damaged sciatic nerve by crushing in dogs. Eighteen adult dogs were used. The animals were divided into three equal groups. The sciatic nerve of the left hind limb in all experimental animals was damaged by crushing forceps for two minutes. In group one, the damaged nerve was left without any medical therapy, while in groups two and three, methylprednisolone and therankeron were used to treat the crushed sciatic nerve, respectively. The Clinical signs and histopathological changes depended on the 30th and 60th postoperative days to evaluate the degree of nerve repair. In group one, all animals did not use their affected limb with loss of sensation completely, while early and pronounced enhancement in the hind limb function was shown, especially in group three than in group two. In group one, the histopathological changes were characterized by the presence of areas of nerve fiber necrosis and disconnection of it, inflammatory cell infiltration with blood vessel congestion, and edema formation between the nerve fibers in both following periods. The same changes were shown in groups two and three, in addition to the presence of nerve fibers' regeneration, especially in group three, superior to group two. In conclusion, the results of this article revealed positive clinical and histopathological effects of both theranekron and methylprednisolone on the healing and regeneration of the crushed sciatic nerve, with priority for therankeron in dogs.

DOI: 10.33899/ijvs.2023.138795.2842,

Introduction

Damage to the peripheral nerves is a serious injury that may result in debilitating sensorimotor dysfunction and a prolonged period of pain (1). Injuries of peripheral nerves may be shown as isolated from other nervous systems or accompanied by the trauma of the central nervous system (2). Different animals, such as dogs, cats, sheep, and rats, were used to study the damaged nerves' function, injury pathway, treatment, and regeneration (3). Numerous causes, such as crushing and compression trauma, injuries by sharp objects, fractures, and electrical shock, may lead to damage to the nerve (4). Injury of the sciatic nerve occurs commonly and is used widely as a model to understand the nerve's ability for regeneration. The injury of the central nervous system differed from peripheral nerve injury because the peripheral nerves can regenerate through axons rather than the central nervous system (5). In dogs, repairing the damaged sciatic nerve was studied using several methods, such as autotransplantation of bone marrow (6) and tendons with bone marrow (7). Homeopathy is an effective medical system used in veterinary medicine without any complications or side effects to treat some pathological conditions (8). *Tarantula cubensis* extract (Theranekron) is considered a homeopathic medicine (9). Theranekron is defined as an alcoholic extract of the venom of *Tarantula*

cubensis, which keep its activity in pharmaceutical compounds for a considerable time. Theranekron is regarded as an antiphlogistic, demarcating action and improvement of wound healing in animals. In cows, theranekron was used successfully for necrotic tissue demarcation and treatment of infected and necrotic wounds (10). On the other hand, theranekron was used in dogs as conservative therapeutics in some cases of mammary tumors (11), treatment of oral papillomatosis (12), and enhancement of the healing process of intestinal anastomosis (13). In addition, theranekron is used to treat many pathological conditions, such as abscesses and harmful diseases (14). Methylprednisolone is an anti-inflammatory agent that has been widely used in the treatment of different pathological conditions in the body (15-20). It has been used in experimental studies to enhance and repair peripheral nerve damage because it is a neuroprotective substance (21). Generally, glucocorticoids are anti-inflammatory agents used to resolve the development of edema and reduce the inflammatory response by regulating the expression of pro-inflammatory factors. Also, glucocorticoids have anti-inflammatory effects in both peripheral and central nervous systems, such as the inhibition of infiltration of inflammatory cells (22). This study aimed to evaluate the effect of *Tarantula cubensis* extract and methylprednisolone on the regeneration of crushed sciatic nerves in dogs.

Materials and methods

Experimental animals

Eighteen adult healthy male dogs weighing 17±1.7 kg was used. The animals were divided into three equal groups, six for each group. Physical and clinical examinations confirmed that the animals were healthy and free from diseases and affections. All animals were kept in specific cages in the animal house at the College of veterinary medicine, university of Mosul.

Ethical approve

The research was approved by the Ethics Committee of the Faculty of the College of the Veterinary Medicine / University of Mosul. No. UM. VET.2021.067.

Anesthesia

The surgical operations were performed after induction of general anesthesia using a mixture of ketamine HCL and xylazine at doses 10 mg/kg and 2mg/kg, BW, respectively, with atropine sulfate as a pre-anesthetic agent at a dose of 0.05 mg/kg.

Surgical operation

Animals were placed in lateral recumbency after induction of general anesthesia. A surgical wound was performed about 10 cm. length on the lateral aspect of the femur. Then, the muscles of the thigh region were blunt dissection to expose the sciatic nerve. Crushing forceps were used and left for two minutes to damage the sciatic nerve. Then, the dissected muscles and incised skin were closed separately using polyglactin (Vicryl®) and silk sutures. The crushed nerve of the group one animal (control group) was left without any treatment, while in groups two and three, the animals were injected intramuscularly with 1mg/kg of methylprednisolone (23) and 3 ml/animal of theranekron (13), respectively.

Assessment of wound healing

In all groups, clinical signs observation has been performed daily to evaluate the functional use of the hind limb, especially monitoring the presence of movement and sensation with histopathological changes performed on the 30th and 60th postoperatively.

Results

Clinical observations

In all animals, the surgical operation site was characterized by inflammatory signs. All animals have not used the affected hindlimb during the first postoperative week, in addition to the absence of animals' sensation to pain during external stimuli due to paralysis of the limb. There was no clinical improvement of the hindlimb function in the animals of group one, which is manifested by paralysis of the affected limb, subsequent foot skin ulcer, loss of sensation, and atrophy of the limb muscles until the end the study. For group two, the experimental animals showed sensation and motor activity loss during the 30th postoperative day. The movement and sensation of the affected hindlimb were improved slightly and gradually at the end of the study. In group three, there was slight and gradual clinical improvement in the gait during the following third and fourth weeks, and the animals regained relatively both normal gait and sensation at 60th postoperatively.

Histopathological examination

The histopathological finding revealed at the 30th day after the operation, the section of the injured nerve showed in group one the presence of fibers necrosis, Schwann cells swelling, inflammatory cells infiltration, and blood vessel congestion. In addition, formation of edema between the nerve fibers (Figure 1). On the 60th day postoperatively, some areas showed disconnection of nerve fibers, inflammation of blood vessels, and inflammatory cell infiltration with congestion of blood vessels (Figure 2). In general, there was a disconnection of nerves during the study.

In group two, the histopathological features on the 30th postoperative day showed the presence of interruption between fiber nerves with inflammatory cell infiltration, development of edema, vasculitis, and congestion of blood vessels (Figure 3). While, at 60th postoperative day, the

histopathological section revealed that there were inflammatory cells, edema formation, and collagen deposition between fibers. Pyknosis and Schwann nuclei hypertrophy were also observed. Other sections showed regenerative areas of nerve fibers (Figure 4).

In group three, the histopathological sections on the 30th postoperative day accelerated the damaged nerve's healing process compared with groups one and two. The presence of regeneration areas of nerve fibers characterized the section. Very few inflammatory cells and the formation of edema were shown. In addition, congestion and inflammation of blood vessels with reduced nerve disconnection were shown (Figure 5). On the 60th postoperative day, there was more nerve regeneration and normal Schwann cells. The nerve loss rate and edema development reduction were also shown (Figure 6). Generally, good binding appeared between the fibers of nerves.

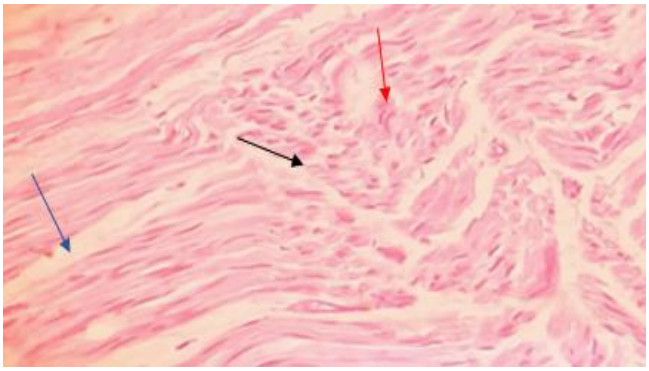

Figure 1: A histopathological section of the injured nerve on the 30th day after the operation shows nerve fibers necrosis (black arrow), swelling of nuclei of Schwann cells (red arrow) and edema between nerve fibers (blue arrow). (H&E, 100X).

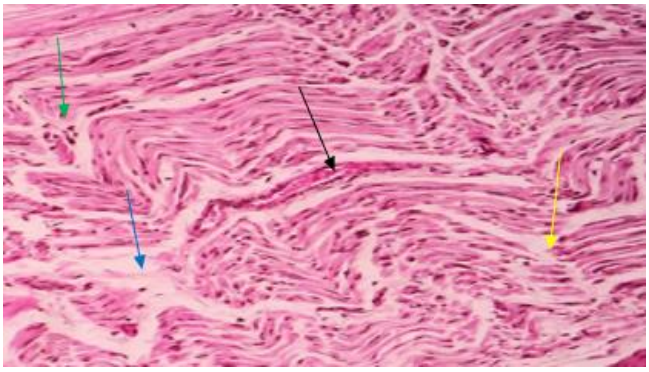

Figure 2: A histopathological section of the injured nerve at the 60th day after the operation shows vasculitis with blood vessels congestion (black arrow), inflammatory cells infiltrations (green arrow), edema (blue arrow) and nerve fiber disconnection (yellow arrow) (H&E, 100X).

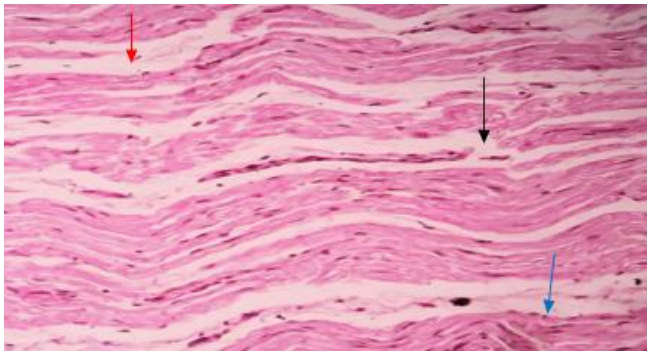

Figure 3: A histopathological section of the injured nerve on the 30th day after the operation shows the area of nerve fibers loss (black arrow), edema (red arrow) and infiltration of inflammatory cells (blue arrow). (H&E, 100X).

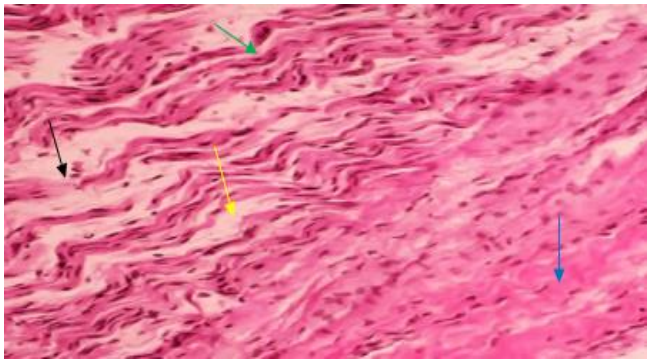

Figure 4: A histopathological section of the injured nerve at the 60th day after the operation shows the area of nerve regeneration (Black arrow), hypertrophy and pyknosis of Schwann nuclei (green arrow), deposition of collagen fibers (blue arrow) and edema (yellow arrow). (H&E, 100X).

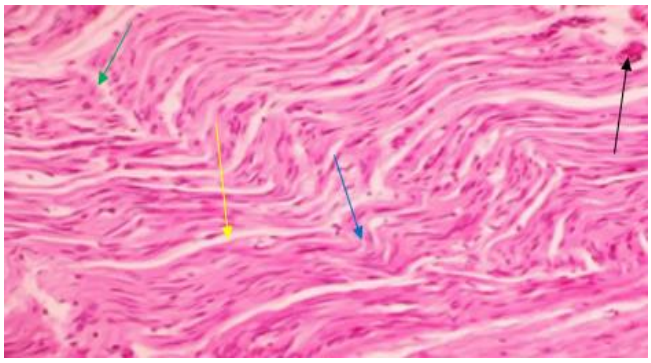

Figure 5: A histopathological section of the injured nerve at the 30th day after the operation shows blood vessels congestion (black arrow), nerve fibers regeneration (blue arrow), nerve fiber disconnection (green arrow) and edema (yellow arrow). (H&E, 100X).

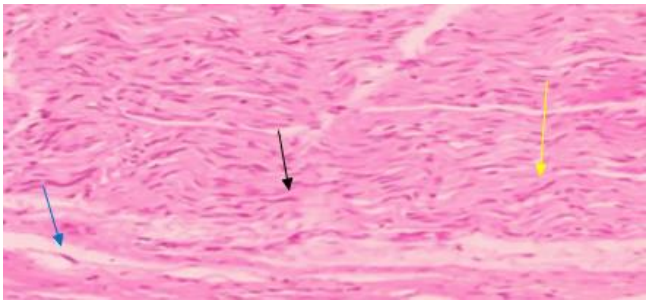

Figure 6: A histopathological section of the injured nerve on the 60th day after the operation shows normal Schwann cell (black arrow), edema (blue arrow) and nerve fiber regeneration (yellow arrow). (H&W, 100X).

Discussion

Peripheral nerve injuries are considered common types of limb trauma that lead to extreme dysfunction of the affected part. Inside the body, after peripheral nerve injury, several complex regulated events occur to remove the damaged tissue and begin the repair process (24). Generally, nerve injuries can be classified into neuropraxia, axonotmesis, and neurotmesis. In the first type, the injury occurred to the myelin sheath but it's still preserved and results from stretching or compression without Wallerian degeneration. In the second type, the axon was affected by the occurrence of Wallerian degeneration, while in the last type, there is complete disruption of a nerve fiber (25). Induced nerve damage by crushing as a model to know the mechanism of nerve regeneration was used to avoid the formation of a gap between nerve fibers which usually plays a role in preventing or delaying nerve regeneration where Sahar *et al.* (26) reported that the main goal of each nerve gap repair technique is to bind the transected ends of the nerve fibers together to facilitate nerve continuity. In this study, a complete functional loss of the hind limb was noticed in all animals after induced nerve damage by crushing. This is most likely due to the effect of compression by crushing forceps that induce damage to the sciatic nerve. The trauma of peripheral nerves causes limb dysfunction with sensory and motor loss and neuropathic pain (27). The authors Seddon (28), Kaya and Sarikcioglu (29), Sunderland (30) mentioned different types of peripheral nerve injuries. The mild form is neurapraxia which is characterized by a localized area of demyelination or ischemia, block of nerve impulses conduction with partial or complete loss of motor and sensory conduction and has a good prognosis if treated with some medical therapy with an expected period of recovery about three months from the occurrence of injury when the nerve fibers are remyelination completely (31,32). The improvement of the hindlimb function and histopathological results in group two due to methylprednisolone's anti-inflammatory and neuroprotective effects, where the authors Ozsoy *et al.* (33), Serdar *et al.* (34), Oztruk *et al.* (35) concluded that methylprednisolone has a positive effect on the enhancement of sciatic nerve regeneration. Generally, the effect of glucocorticoids as an anti-inflammatory agent was used to reduce the development of tissue edema resulting from the injury. This medication can reduce pro-inflammatory factors production, such as interleukin-1β (36).

Additionally, it plays an important role in reducing axonal and myelin degeneration, developing edema, and improving neural function (37). In group three, there is an acceleration in the period needed for return to the limb function, and histopathological results were more advanced than those reported in group two due to the effect of *Tarantula cubensis* extract. The current results were supported by Kizilay *et al.* (38), who reported that therankeron lead to a decrease in the degeneration in the axon and myelin sheath of nerve fiber after damaged by injury because of the protective action of therankeron to nerves as an anti-inflammatory agent and on the levels of pro-inflammatory cytokine. However, using therankeron in our study led to acceleration and enhancement of the nerve healing process, which was in agreement with Adib-Hashemi *et al.* (39), who concluded that therankeron could improve the wound healing process by reducing the infiltration of the inflammatory cells. In addition, the authors Corum *et al.* (40), and Albay *et al.* (41) said the *Tarantula cubensis* alcoholic extract is used to improve and accelerate the wound's healing process and to relieve edema formation in many animal species.

Conclusion

Both *Tarantula cubensis* extract and methylprednisolone were releveled histopathological and clinical improvements in the motor and sensation activity of sciatic nerve damage of the hindlimb with priority for *Tarantula cubensis* extract.

Acknowledgments

The authors thank the College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Conflict of interest

The authors declare that there is no conflict of interest.

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مقارنة بين تأثير عقارين طبيين على التئام العصب الوركي المتهتك في الكلاب

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الخلاصة

صمم هذا البحث لغرض تقييم دور الثيرانكيرون والمثيل بريدنيسولون على عملية تجدد والتئام العصب الوركي التالف بواسطة السحق في الكلاب. تم استخدام ثمانية عشر من الكلاب البالغة. قسمت حيوانات التجربة الى ثلاثة مجاميع متساوية. تمت عملية إتلاف العصب الوركي للقائمة الخلفية اليسرى في جميع الحيوانات بواسطة السحق ولمدة دقيقتين. في المجموعة الأولى ترك العصب الوركي التالف بدون أي علاج طبي في حين تم علاج العصب التالف في حيوانات المجموعة الثانية والثالثة بمادتي المثيل بريدنيسولون والثيرانكيرون على التوالي. تم اعتماد العلامات السريرية والتغيرات المرضية النسيجية بعد ٣٠ و٦٠ يوما من إجراء العملية لتقييم درجة إصلاح العصب. تمثلت العلامات السريرية بظهور فقدان الإحساس والحركة في القائمة الخلفية في المجموعة الأولى وبشكل كامل بينما لوحظ حدوث تحسن واضح وأسرع في الأداء الوظيفي للقائمة الخلفية في المجموعة الثالثة مقارنة بالمجموعة الثانية. تميزت التغيرات النسجية المرضية في المجموعة الأولى بوجود مناطق تنخر في الألياف العصبية وانفصالها وارتشاح للخلايا الالتهابية واحتقان للأوعية الدموية مع تكون الوذمة بين الألياف العصبية طيلة فترة الدراسة. ظهرت نفس العلامات النسيجية في المجموعتين الثانية والثالثة بالإضافة الى وجود مناطق لتجدد الألياف العصبية وبشكل خاص في المجموعة الثالثة مقارنة عن المجموعة الثانية. نستنتج مما سبق وجود تأثير إيجابي لكل من الثيرانكيرون والمثيل بريدنيسولون على عملية تجدد والتئام العصب الوركي المتهتك مع الأفضلية للثيرانكيرون في الكلاب.