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Therapeutic Effect of Progesterone on Experimental Peripheral Nerve Injury

C.R. Kayaoglu, Sedat Binler, Mehmet Kizilkaya and Goksin Sengul
Department of Neurosurgery and Anesthesiology, Ataturk University,
Medical School, Erzurum, Turkey

Abstract: This study aims to investigate the therapeutic effect of progesterone on experimental peripheral nerve injury. Methods: A total of 20 male Albino rats weighing 250-300 g were anaesthetized and subjected to sciatic nerve injury model. Rats were then seperated into two groups; one group was treated with progesterone and the other with saline for five time up to 21 days after injury. Both groups were evaluated fuctionally for 21 days and then killed. Sciatic nerves were examined histopathologically. There was no statistically significant difference between these two groups for up to 4 days. But evaluations after 4th day revealed a better improvement in progesterone treatment group. Our data indicate that progesterone has a beneficial effect in peripheral nerve injury.

Key words: Progesterone, peripheral nerve injury, rat

INTRODUCTION

Progesterone is synthesized in the nervous system by neurons and glial cells[1]. Progesterone has been proposed to have neurotrophic role for development and regeneration of the peripheral nervous system[2]. Because of their simple structure, plasticity and capacity of regeneration, peripheral nerves are particularly well suited for studying effects of hormone. In peripheral nerves, the local synthesis of progesterone plays an important role in the formation of myelin sheats.

Based on peripheral nerve injury, this study focuses, experimentally, on the effect of progesteron, on axonal regeneration and remyelination functionally and histopathologically.

MATERIALS AND METHODS

Animals: All procedure of this study were performed in accordance with the Ethical Guidelines of the International Association for the Study of Pain[3] and were approved by the Ethical Comittee on Animal Experiments of the University of Ataturk, Turkey. A total of 20 male Albino rats weighing 250-300 g were used. Male rats were preferred to prevent cyclic changes on progesterone levels that could affect the results. Surgical procedures were performed under the anesthesia with ketamine hydrochloride (Ketalar, Parke-Davis GmbH, Berlin, Germany, 10 mg kg^{-1}, im). The skin of the lateral surface of the thigh was incised and a section made directly through the biceps femoris muscle exposing the sciatic nevre and its terminal three branches: sural, common peroneal and tibial nerves[4]. Sciatic nerve was crushed at the proximal end of its bifurcation for 20 sec by using a watchmaker's forceps.

Treatment protocols: Rats were randomly divided into two groups: progesterone treatment group and the saline control group. These subgroups received 1, 5, 9, 13 and 17th days injections of either progesterone 1 mg intraperitoneally (Progesterone ampullen, Eifelfango Nevanati) or 1 mg kg^{-1} saline intraperitoneally, respectively, post-injury. Rats were killed on the 22th day and their sciatic nerves were removed.

Functional assessment: Both groups were evaluated on the 4, 7, 14 and 21th days by using Sciatic Functional Index (SFI) scala described by Bain J.R. *et al.*[5]. SFI score is accepted to be 0 in normal sciatic nevre and it is supposed to be -100 in complete nevre transsection.

Histopathological assessment: Sections were prepared haematoxylin and eosin and luxol fast blue. Changes due to degeneration in myelinated fibers, Schwann cell proliferation and new myelin sheath formation were evaluated histopathologically.

Data analysis: Data analysis was performed using SPSS version 10.0 for Windows (SPSS Inc., Chicago, IL, USA). The SFI scores were analysed using the Mann-Whitney U-test and p value <0.05 was considered to be significant.

Corresponding Author: C.R Kayaoglu, Department of Neurosurgery and Anesthesiology, Ataturk University, Medical School, Erzurum, Turkey

RESULTS

Functional results: SFI scores in both progesterone treatment and saline control groups showed no significant improvement on 1st and 4th days. There was no statistically significant difference between these two groups. But 7, 14 and 21th days evaluation revealed a better improvement in progesterone treatment group ($p<0.01$). SFI scores of two groups are shown in Fig. 1.

Histopathological results: Degeneration of myelinated fibers, surrounding tissue bleeding and oedema were observed in saline controls. There was no increase in the number of Schwann cells. There were fewer young Schwann cells in the saline controls when compared with the progesterone treatment group. Myelin sheath had partially slimmed along the nerve trace. Axonal atrophy and slim and irregular myelin sheath of regenerated axons were also observed Fig. 2.

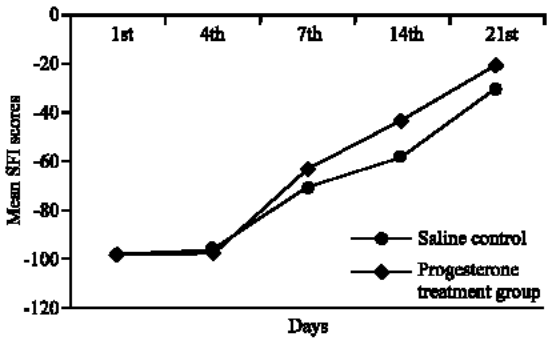

Fig. 1: Profile of SFI scores for all rats treated with either progesterone or saline

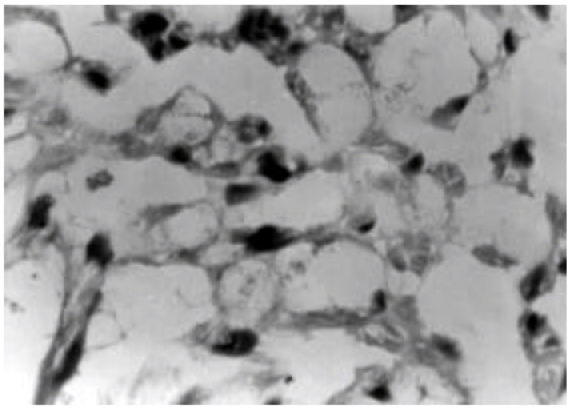

Fig. 2: Photomicrograph of injured sciatic nerve in saline control group. Luxol Fast Blue, magnification×1000

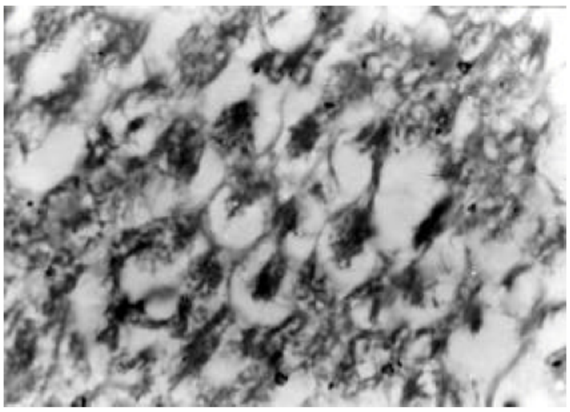

Fig. 3: Photomicrograph of injured sciatic nerve in progesterone treatment group. Luxol Fast Blue, magnification×1000

New myelin sheath formation and more young Schwann cells were observed in progesterone treatment group. More regenerated axons and thick and regular myelin sheath were also observed Fig. 3.

DISCUSSION

Simply structure and easy preparation of pure culture of Schwann cells renders peripheral nerves suitable to explore the synthesis and functions of steroids. The most important feature of peripheral nerves is their remarkable regenerative capacity. Following injury, axons and their myelin sheats distal to the lesion degenerate by a process known as Wallerian degeneration, leaving behind the dividing Schwann cells, which produce neurotrophic factors and hormones[6]. Schwann cells play a crucial role during the regeneration of nerve fibres, which begins already a few hours after lesion of the nerve by local crush or freezing. In addition, their multiplication is critical for allowing axons to regenerate across gaps. Later Schwann cells remyelinate the regenerating axons and under favorable circumstances, the appropriate neuro-muscular connections and functions may be restored in rats within 4 to 6 weeks[6,7]. Whereas nerve fibers easily regenerate after crush or cryolesion, there is no regenerating after nerve transection. However, even after this type of lesion, Schwann cells proliferate and survive within their basal lamina tubes for months[8].

Progesterone can be locally synthesized by peripheral nerves either de novo from cholesterol or or from blood-derived pregnanolone[9]. Progesterone is an important signalling molecule in the nervous system, regulating vital neuron and glial functions by multiple mechanisms of action. Progesterone activates

the transcription of hormone-sensitive genes after binding to an intracellular receptor, modulate the activity of neurotransmitter receptors by acting directly on the neuronal membrane. Progesterone also has a signalling and regulating effects in the spesific steps of myelin synthesis as demonstrated in the peripheral nervous system[1].

Koening HL *et al.*[2] formed axonal degenaration and demyelination with cold injury model in a rat sciatic nerve. They indicated that progesterone concentration were higher in the plasma than in the injured cell on the 7th and 15th days. Progesterone can easily pass blood-brain and blood-nerve barrier with its lipid solution. For this purpose, in order to obtain high plasma concentrations, we administered progesterone systematically.

Melcangi *et al.*[10] injected 1mg progesterone subcutaneously with certain intervals to investigate the myelinating effect of progesterone in 22-24 week rats. They found that myelin sheat had regular borders and was thick in rats which had been treated with progesterone. In our study we administered 1 mg progesterone systemically following crushing injury in sciatic nerve. We observed therapeutic effect of progesterone on spontaneous axonal regeneration and remyelination in the basal lamina tube histopathologically and functionally. We found that the difference of improvement starting from the 4th day between the progesterone treatment and the saline control groups lasted till the 21st day. The significant difference starting from the 7th day in the SFI results of the progesterone treatment group indicates that progesterone positively affects active myelination.

Thomas AJ *et al.*[11] reported that progesterone significantly improves neurologic recovery after spinal cord injury in rats histopathologically and functionally.

In our study,we histopahtologically determined that young Schwann cells are more and the myelin sheath surrounding the axons are wider and more reguler in the progesterone treatment group than in the saline control group. We found that Schwann cell formation was less in the saline controls. We also found that a thin and irreguler myelin sheat formed in axonal regeneration. We found that retraction and fragmentation occured as well as the thinness and irregularity in the myelin sheath.

Our histopatological and functional results prove that progesterone, is effective on both axonal regeneration and myelination. The synthesis of progesterone in the nervous system suggests that this hormone can be administered systemically in nerve injuries and demiyelinating diseases and their synthesis can be increased locally. When the effect of progesterone on axonal growth and new myelin sheat formation is taken into consideration in peripheral nervous system with regeneration ability, it can be suggested that progesterone can be used for treatment in peripheral nerve injuries.

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