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Effects of hydrocortisone on the sciatic nerve crush injury in adult rat - a light microscopic study

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Abstract

A wide range of agents including corticosteroids & vitamin-C have been shown to possess some neuro-regenerative effect in peripheral nerve injury, but histopathological support for such claims remains scanty. Therefore, the present study was planned to assess the role of local administration of hydorcortisone in the healing of experimentally induced sciatic nerve crush injury. 30 rats obtained from the central animal facility were divided into one control and four experimental groups of 6 rats each. Walking track prints of each animal was taken before induction of injury and at weekly intervals postoperatively. Under general anaesthesia and aseptic condition the sciatic nerve crush-injury was induced in the mid-thigh region with Kocher's forceps. 0.5 ml of cortisol was instilled at the site of injury and wound was closed. The 2nd dose of cortisol was given after two weeks. Sciatic nerve function was assessed from the walking track analyses. At the end of the study period (3 to 6 weeks), animals were sacrificed and nerves were immersion fixed in Karnovsky's fixative. Tissue samples were either stained en-block with Osmic acid or 07-10 µm thick paraffin sections were stained with H& E, Cresyl violet & Luxol fast blue and Van Gieson's stain. It was observed that the animal on the operated side developed weakness, had altered gait and revealed lengthening of foot prints. The sciatic nerve function index ranged from -70 to -55 at 1 and 6 weeks post-operatively. Light microscopy- as compared to control, after one week of injury the nerve fibres were swollen, showed fragmentation by 3 weeks and complete degeneration along with marked removal of debris of degeneration by six weeks. Macrophages were associated with degenerating myelin sheath and Schwann cells along with the endoneurial fibroblasts showed remarkable proliferation and were visible as prominent fascicles alternating with the degenerating nerve fibres. However, during the period of study no intact nerve fibre was seen to cross the actual site of nerve injury. It was concluded, that though the local administration of hydrocorticsone appears to perceptibly improve the functional recovery, absence of concomitant supportive histopathological findings remains to be explained and therefore, the topical use of steroids to either reduce the post-injury nerve dysfunction or improve nerve regeneration warrants further study.

Keywords: Sciatic nerve, Crush-nerve injury, Hydrocortisone, Nerve regeneration, histopathology.

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Introduction

The peripheral nerve trauma remains a major cause of morbidity and even with optimal surgical repair, clinically the sensory outcome remains very poor [1] and poor sensory function adversely affects motor function, particularly fine manipulative movements [2]**.** After primary nerve injury the secondary injury to the nerve which leads to autodestruction is caused by reactive oxygen species which is promoted by lactic acidosis [3**,** 4, 5, 6, 7] while ascorbic acid (Vitamin-C), an anti oxidant has been shown to protect the nerve from secondary injury and thus promotes recovery [8, 9].

From the functional recovery of peripheral nerves following defined acute crush injuries in rat sciatic nerve [10] it was concluded that the rate of recovery was directly related to the initial load responsible for the injury. Moreover, the speed of recovery from injury is affected by both the physical and pharmacologically active agents. For example, swimming exercise applied during the acute or late phase of crush-nerve injury accelerates nerve regen-

eration [11] and synaptic elimination after axonotmesis, thereby suggesting that exercise may be initiated immediately after injury. Experimental study on regeneration of sciatic nerve crush injury evaluated the role of certain physical therapies (e.g., electrical stimulation, and combined decimeter and infrared) and concluded that physical therapies can improve the regeneration of the injured sciatic nerve of rats [12]. Study on the effect of hyperbaric oxygen on the regeneration of experimental crush injuries of nerves [13] concluded that hyperbaric oxygen therapy has no influence on functional recovery. Role of topical steroids (dexamethasone) in reducing dysfunction after experimental axonotmesis in rat sciatic nerve [14] showed a trend toward superior recovery for the steroid group compared with controls (90% *vs*. 73%), but this difference did not reach statistical significance. Effect of corticosteroid usage combined with multidrug therapy on nerve damage assessed by using nerve conduction studies [15] concluded that corticosteroids is not very efficacious in the prevention or reversal of nerve damage. Comparison between the effects of local and systemic dexamethasone on the rat traumatic sciatic nerve model [16, 17] concluded that recovery in the group treated with local dexamethasone was more remarkable than that in the group treated with systemic dexamethasone**.** A review of the application of "high-dose" steroid therapy for CNS injury from a pharmacological perspective [18] revealed that dose and dosing regimen remain a controversial issue because of their poor clinical record and also because of reduction of safety margin with increasing dose of glucocoticoids. High-dose steroid treatment given for acute brain and spinal cord injuries [19] needs careful consideration of its risks because it may induce acute tumor lysis syndrome.

Androgen has also been shown to reduce the recovery time of crush-nerve injury [20]. Improved sciatic nerve regeneration by local thyroid hormone treatment [21] in adult rat is accompanied by increased expression of SCG10. The stimulating effect of T3 on SCG10 expression could provide a mechanism by which T3 enhances peripheral nerve regeneration. Experimental immunological demyelination has been shown to improve regeneration in acute crush injury of sciatic nerve [22]. However, literature providing histopathological support for many such claims of clinical recovery remains scanty, therefore the present study was planned to assess the role of topical corticosteroid in the healing of experimentally induced peripheral nerve injury in adult rat.

Material and Methods

After IAEC-clearance a total number of 30 rats were obtained from the central animal facility of JN Medical College, AMU, Aligarh and were divided into one control and four experimental groups of 6 rats each. Control-without crush, crush without corticosteroid (3 week & 6 weeks), crush with corticosteroid (3 weeks & 6 weeks). Walking track analysis for sciatic function index [23] of each experimental animal was performed before induction of injury and subsequently at weekly interval. From the hind limb foot prints, print length factor (PLF), toe spread factor (TSF) and intermediary toe spread factor (ITF) were calculated. Incorporating these factors in the following equation, the Sciatic Function Index (SFI) was calculated [SFI = -38.3 x PLF + 109.5 x TSF + 13.3 x ITF – 8.8]. Under general anaesthesia and aseptic condition the sciatic nerve crush-injury was induced in the mid-thigh region with Kocher's forceps pressed and locked for 5 minutes. 0.5 ml of cortisol (Primacort-100 containing Hydrocortisone sodium succinate inj. IP; from Macleods Pharmaceutical Ltd, Mumbai) was instilled locally at the site of injury and wounds were closed with 3-0 Vicryl (2 metric – NW2401)-absorbable sterilized surgical needled suture USP (synthetic; braided coated polyglactin 910 violet; from Ethicon, manufactured in India by Johnson Johnson Ltd, Aurangabad). Animals were given 0.5 ml injection of analgesic Voveran/Rumagesic (Diclofenac sodium containing 25 mg DS/3ml from Zee Drugs, New Delhi) and antibiotic Monocef (containing Ceftriaxone 1gm, injection, IP from Aristo Pharmaceuticals, Mumbai) – and wounds were dressed with solution Betadine (10% Providone-Iodine solution IP, from Win Medicare, Pvt. Ltd, New Delhi). The second dose of cortisol was given after two weeks. At the end of the study period (3 to 6 weeks), animals were sacrificed and nerves were procured and immersion fixed in Karnovsky's fixative. The whole nerve segments from some samples were treated with Osmic acid [24] for a couple of days and subsequently processed for paraffin embedding and sectioning. These sections generally did not require counter stain. Other nerve samples were processed for paraffin embedding. 07-10 µm thick sections were stained with H& E, Cresyl violet & Luxol fast blue and Van Gieson stain. All sections were examined under trinocular light microscope (Olympus: BX40, Japan) both under low and high magnification objectives and representative pictures of relevant findings were recorded.

Observations

Gross

All animals from both control and experimental groups remained healthy throughout the period of study and none of the rats showed signs of infection or ulceration of the foot. However, in some of the animals the skin wounds tend to gape after receiving 2nd dose of corticosteroids. The animals from experimental groups showed weakness in the hind limb, altered gait, dragged the dorsum of their foot and showed reduced response to pinch test. However, with the passage of time, they showed gradual improvement in their gait as compared to one observed on post-injury day-1. Walking track analysis for assessment of

sciatic nerve function revealed that animal developed weakness of limb on the injured side, altered gait and lengthening of footprints. However, from day 1 to day 4, on the operated side, the animals dragged their limb without casting discernible foot print and therefore in absence of foot print recordings the assessment of the sciatic nerve function index (SFI) was not possible. The SFI ranged from -70 to -55 at 1 and 6 weeks post-operatively indicating thereby that functional recovery was taking place in both groups (without & with corticoids). However, the one with corticoisteroids showed a bit better SFI.

Microscopic

It was observed that in control group the sciatic nerve fascicles had densely packed parallel running nerve fbres [Fig. 1A]. In Osmic acid stain samples [*image not included*] nodes and internodes could easily be made out. The myelin sheaths were smooth, uniform and their overall thickness varied with the diameter of the nerve fibres. The connective tissue fibres associated with perineurium and endoneurium were minimal and could be just identified. In every field the number of cells was remarkably less as compared to those seen in the experimental groups. Crush-injury lead to degeneration at the site of injury while part proximal to it remained intact [Fig 1B].The experimental group showed degeneration in different stages. In early stage the nerve fibres were swollen followed by fragmentation [Fig. 1C] and by six weeks complete degeneration was seen in the vicinity of injury. The debris of degeneration was marked in early stage while in later stages major portion of degeneration product was removed. Macrophages were associated with degenerating myelin sheath and Schwann cells showed remarkable proliferation and along with the endoneurial fibroblasts and appeared as prominent fascicles alternating with the degenerating nerve fibres [Fig. 1D]. However, during the entire period of study no intact nerve fibre was seen to cross the actual point of nerve injury.

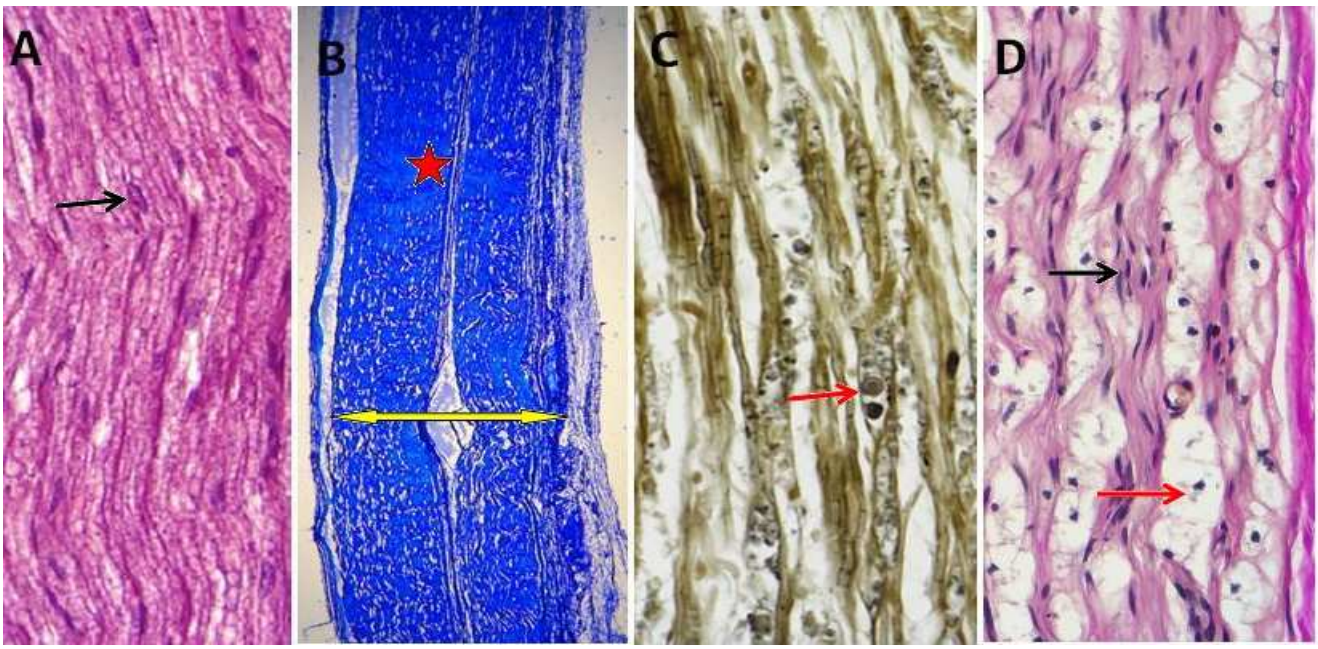

Figure 1. *Representative photomicrographs of longitudinal section of sciatic nerve from control [**A**], showing parallel running closely packed nerve fibres which is associated with Schwann cell (arrow), H & E stain. Experimental group[**B**] under lower magnification, showing region of crush-injury (yellow, double horizontal arrow) and part of the nerve proximal to it (red star); Cresyl violet & Luxol fast blue stain. Experimental group: 3 week post-crush [**C**], showing degenerating nerve with myelin fragments (red arrow), endoneurium stained light yellow; Osmic acid stain. Experimental group: 6 week post crush [**D**] showing degenerating nerve fibres having foamy or cloudy appearance, and macrophages (red arrow) and pink region (black arrow) represent hyperplastic endoneurium and Schwann cells; extreme right (red border) is epineurium; van Gieson's stain.*

Discussions

A broad spectrum of traumatic injuries, diseases, tumors and some iatrogenic lesions are commonly included under peripheral nerve injury. Experimental nerve crush is an accepted model for peripheral nerve injury and its subsequent regeneration. In the present study neuroprotective effect of topical corticosteroids have been assessed using both functional and histopathlogic parameters. Up to the 4th day post operatively, the animals dragged the hind limb of the operated side with only mild gait improvement. By the end of 1 week they start taking some support

on the operated limb and thus also help in finding out the SFI. With apparent improvement in gait and improving SFI from around -70 to -55 over the period of 6 weeks suggests an appreciable improvement. This present observation on the functional recovery of crush-nerve injury is in agreement with the observation of many previous workers [14, 16**,** 17 and 25]. However, certain workers could not find significant improvement when corticosteroid was used with multidrug therapy [15] where improvement was assessed by conduction study. In fact some workers [18] reviewed the application of "high-dose" steroid therapy for CNS injury from a pharmacological perspective and noticed that dose and dosing regimen remained a controversial issue because of their poor clinical record and also because of reduction of safety margin with increasing dose of glucocoticoids. Some researchers [19] have gone even one step further by suggesting that a high-dose steroid treatment given for acute brain and spinal cord injuries needs careful consideration of its risks because it may induced acute tumor lysis syndrome which is a constellation of metabolic crises that results from massive tumour cell destruction especially with hematological malignancies. Recently, some studies have shown the neuroregenerative effects of certain other hormones as well, for example androgen reduces the recovery time of crush-nerve injury [20] and local thyroid hormone treatment improved sciatic nerve regeneration in adult rat [21].

Microscopic examination in the present study revealed that as compared to control sciatic nerve the crushed area of nerve shows increased cellularity. In the initial stage it was primarily due to influx of inflammatory cells, while in the later stages it shows further increase because of proliferation of resident cells of connective tissue, namely endoneurial fibroblasts and Schwann cells. The crushed sciatic nerve showed changes very similar to that described for Wallerian degeneration and in fact in the present study, subtle degenerative changes were seen as early as by just 24h post operatively similar to one as described earlier in the optic nerve after ocular enucleation in adult rabbit [26**]**. The changes seen in the sciatic nerve after nerve-crush injury were in the form of swelling, oedema, fragmentation and condensation of myelin. Many macrophages were cited in the vicinity of myelin fragments. Endoneurial fibroblasts and Schwann cells showed both hypertrophy and hyperplasia. In longitudinal sections they were seen in the form of quite robust fascicles alternating with the degenerating nerve fibres. They were in fact associated with formation of endoneurial tube for the passage of future growing nerve fibres from the proximal nerve stump. In the present study in addition to H & E staining, many other staining techniques (Osmic acid, Cresyl violet & Luxol fast blue and Van Gieson stain) have been used to demonstrate nervous and connective tissue element separately. And it was interesting to note that none of the sample stained by different techniques from experimental group at 3rd and 6th week revealed completely intact nerve fibre crossing the actual site of nerve injury. On the face of such histopathological finding it was difficult to ascertain the possible reason for the obvious functional improvement with increasing postoperative time from 1 to 6 weeks as revealed by improvement in the gait and SFI.

The secondary nerve injury which is believed to follow the primary one is likely to cause deterioration with increasing post- injury time. The reason for autodestruction during secondary nerve injury is believed to be caused by reactive oxygen species which is due to neutrophil infiltration and in addition promoted by lactic acidosis leading to free radical-induced lipid peroxidation [3, 4, 5, 6, 7]. On the other hand ascorbic acid (vitamin-C) a known anti oxidant has been shown to protect the nerve from secondary injury and thus has been demonstrated in many studies to promote recovery [8, 9]. In the present study both sets of experimental groups (crush-without corticosteroid and crush with corticosteroid) show functional improvement with a bit faster in the group treated with corticosteroid. This suggests that possibly there is an inbuilt mechanism in place to check the possible harmful effects of secondary injury and the corticosteroid somehow appears to support this mechanism and thereby producing a bit faster functional improvement as compared to one without corticosteroid. It is believed that because free radicals are very short-lived and usually present at low concentrations, it is difficult to measure them in the biological samples [3]. Study on the protective effects of alpha-lipoic acid on crush injury in rats [27] assessed with functional, molecular and o electromicroscopic analyses concluded that it is a neuroprotective agent for peripheral nerve injury and promoted peripheral nerve regeneration via its anti-inflammatory and antiapoptotic effects. In the present study the anti-inflammatory potential of corticosteroid must also be playing a significant role in faster functional recovery.

The relative significant role of chronic denervation of distal nerve stump and muscle in regeneration process remains debatable. According to one [28] chronic denervation of the distal stump plays a key role in reduced nerve regeneration, but the denervated muscle is also a contributing factor. It is believed that at the lesion site, reactive Schwann cells provide trophic support and guidance for outgrowing axons. In the present study though reactive Schwann cells are seen but not a single growing axon/neurite from the proximal stump was seen to cross the actual site of injury which could serve as a physical basis for functional recovery. It is possible that some additional time was required to observe such thing to happen. But according other [29] chronic Schwann cell denervation, chronic neuronal axotomy, and misdirection of regenerating axons into wrong endoneurial tubes are primarily responsible for poor functional recovery. The effect of muscle denervation atrophy is secondary. From

the techniques/staining used in the present study, even the misdirected fibres would have been identified if they were there at the site of nerve injury. Many other factors that either regulates Schwann cell or myelination also affect regeneration for example endogenously synthesized FGF-2 [30] influences early peripheral nerve regeneration by regulating Schwann cell proliferation, axonal regrowth, and remyelination. Interestingly, recently a novel experimental immunological demyelination method [22] has been used to enhance nerve regeneration in the adult rat sciatic nerve. Thus, from the present study it was concluded that though the local administration of corticosteroid appears to promote functional recovery the histopathological findings at the actual site of injury remains incongruent and therefore the use of topical steroids to either reduce post-injury nerve dysfunction or improve nerve regeneration warrants further study.

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