An Experimental Study for Improving the Regeneration of the Injured Sciatic Nerve by Utilization of Acetyl Salicylic Acid.

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ABSTRACT

A total of thirty two adult rats (Sprague Dawely) of both sexes weighing 250-350g were used in this study. These were classified equally and randomly into two groups Group (A) and group (B). All the animals were exposed to induction of left sciatic nerve crush injury by using fine forceps after application of surgical procedures. Rats in group (A) were left to be surviving for 15, 30, 90 and 180 days post crush (four animals per each period) and considered as control animal. Group (B) were injected by Acetyl Salicylic acid (ASA) 25mg/kg as anti- inflammatory drug for successive 14 days and left for successive 14days post crush(PC) and left to be survive for 15, 30, 90 and 180 days pc (Four animals per each period). Histological examination and statistical analysis of the nerve specimens for all survival periods of both groups indicated that application of ASA in case of group (B) gave longer internodes and diameter of the regenerated nerve fibers after 180 day pc when compared with that of control group at the same period, the mean internodal length of the nerve fibers of group (B) after 180 day was 396um, and its mean diameter was 8.4uM while the mean internodal length of the nerve fibers of group (A) after 180 day was 277um and its mean diameter was 7.2um. The result of the present study demonstrated that application of ASA for 14 day intramuscularly and continuously in group (B) improved the regeneration of the injured nerve fibers better than the untreated group and this may reflect the role of ASA to suppress the signs and complication of inflammatory process at the site of sciatic nerve injury, so enhance the re growth of new nerve fibers faster and better than the control group (A).

Keywords: Healing, Injury, Acetyl salicylic acid, Sciatic nerve

1. Introduction

Peripheral nerve injuries were first described by Waller (1974) who describes the degenerative changed occurring in the distal stump of the crushed peripheral nerve. Inflammation is a process of tissue damaging, and this process represents a nonspecific

responses of tissue to immunological and non- immunological reaction (Hughes, 1981) this process requires therapeutic intervention. Ranisford (1984) showed that the ability of acetyl salicylic acid to suppress the inflammation may develops during the repair of injury in case of experimental rat which can be obtain by repeated doses of ASA in the process of nerve repair. Lubinska (1970) demonstrated that in the rat phrenic nerve, Wallerian degeneration (WD) begins near the lesion and then spread progressively distal to the lesion, this degeneration advance in less than 5 hours. Twenty four hours after the injury schwann cell are hyperactive, the cell become discrete their nuclei, enlarge and develop prominent nucleoli (Bradley, 1970). Seitz et al. (1989) reported that the site of crush injury associated will breaking down of blood nerve barrier immediately in the distal stump and lead to leakage of serum protein will phagocytic invasion until day 4 after crush, Olsson and Sjosterd (1969) observed that a ten fold increase of mast cell on the 4th day at the site of injury of nerve crush was occur. Therapeutic effect of ASA are mainly causes by inhibition of prostaglandin synthesis, this effects are analgesic antipyretic and anti inflammatory action. This action and the effect achieved with 30 minutes after I/M administration of ASA (Kwo and Tremaine, 1995).

2. Materials and Methods

Thirty- two adult healthy rats (Sprague - Dawely) of both sexes weighing 250-350 gm were used. All were allowed free access to food and water before the experiment, and were divided equally into two groups A and B. The two groups were housed in steel mesh cages at room temperature. The left sciatic nerve of all animals was subjected to a mechanical crush injury. Group (A) were used to induce nerve crush injury and survived for (15, 30, 90 and 180 day) post crush injury, (Lubinska, 1970) animals per period. Anti inflammatory non- steroidal drug (ASA) was used 25mg/kg intra muscularly of the group (B) for the first day post crush and left to be survive for (15, 30, 90 and 180 day)PC (Lubinska, 1970) animal per period . Animals were anaesthetized with intra peritoneal injection of pentobarbital sodium 2.5mg/100gm (90). An incision was made on the skin over the biceps of the left hind limb. A moderate pressure was applied on the sciatic nerve using sterilized fine forceps for 10 seconds at the level of hip. The muscles were approximated, and the incision was closed carefully by interrupted silk suture. The rats were killed with over dose of ether after different survival periods (15, 30, 90 and 180 day PC). The incision of 1cm was made, muscles were separated to expose the left sciatic nerve 5mm long of sciatic nerve was removed distal to the site of injury. Immediately fixed with aldehyde fixative Karnovisky (Karnovisky, 1965) and post fixed in osmic acid for 4 hours. Each segment of the nerve was subdivided into two segments, one for nerve teasing and the other for histological procedures (Hildebrand and Jahansson, 1991). Measurements of the teased nerve fibers included length and fiber diameter measured with 10X eye piece ocular micrometer. The mean of four diameters for each internodal segment was done, 180-200 internode were measured for each specimen (Thopson, 1976). Statistical analysis for each internode space of nerve fiber was carried out.

3. Results & Discussion

Group (A) included animals were subjected to crush of sciatic nerve and survived for 15,30, 90 and 180 day PC left without treatment as control. 15 day PC showed numerous , ovoid debris of myelin and axon fragmentation in the engorged endoneurial tubes which infiltered by leukocytes and macrophages , the perineurium was also having these types of cell , signs of regenerative fibers were detected which appeared as tortous , but measuring of length or diameter of nerve was not possible. The nerve specimens of 30 day PC demonstrated degenerative changes of nerve fibers were still present, leakage and infiltration of macrophages, mast cells and other inflammatory cells were detectable (fig. 1). The mean length of internodal length was 223 um, and the diameter was 3.5 um. The nerve specimen 90 day PC myelin debris was still occurred with the macrophages in the endonerium with mast cell, other inflammatory cells were also observed (fig. 2). Regenerative, remyelinated nerve fibers of variable size were examined. The mean internodal length was 253um and the mean diameter was 5.9um. The nerve specimens 180 day PC included myelin debris ,macrophages , mast cells and other inflammatory cells markedly decreased in comparison with the former period of this group, and the perineurial connective tissue sheath was investing regenerated remyelinated nerve fibers which appeared irregular (fig. 3). The mean diameter was 7.2 um. The group (B) was the treated group. The nerve specimens of 15 day PC showed myelin degradation products were observed, myelin debris was still contained in phagocytes in the endonerium (fig. 4) regenerative sprouts were found. The nerve specimens 30 day PC. Debris of myelin sheath and axon were present in a little amount. The mast cells were rarely seen and numerous new nerve fibers were present, exhibited smooth myelin. The mean of internodal length was 276um. the mean diameter was 6.3um. The nerve specimens 90 day PC. There was little debris and few macrophages in between nerve fibers. Myelinated nerve fibers of variable sizes with regular smooth outline of myelin sheath were observed. The mean length of nerve fibers was 283um. The mean diameter was 6.9um. The nerve specimen 180 day PC. Neither debris nor macrophages were seen; nodes of Ranvier were easily recognized. Fibers were present with regular outlines and thick myelin around it (fig. 5). The variable sizes of myelinated nerve fibers were invested by a thick connective tissue of epineurium. The mean diameter of nerve fibers was 396um. The mean diameter of nerve fibers were 8.4um.

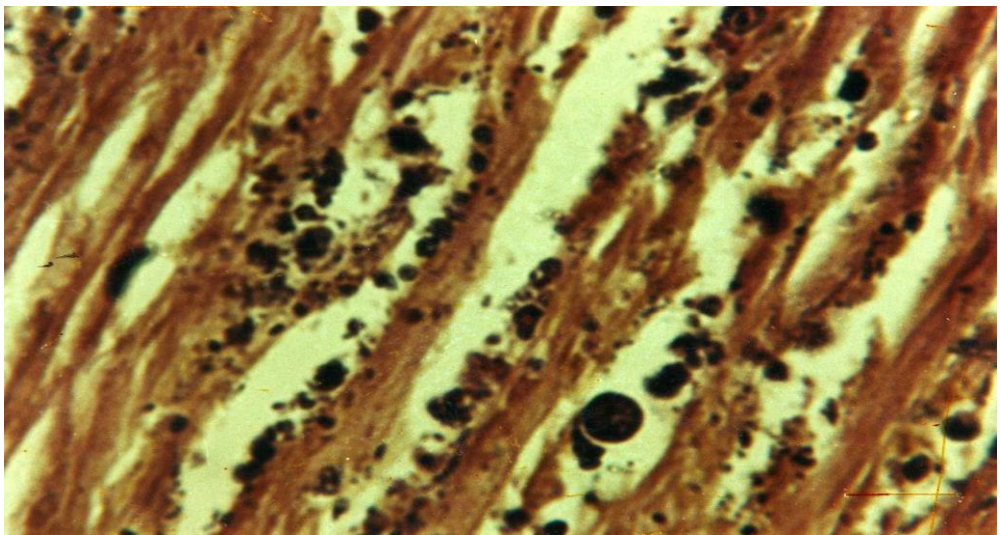

Fig. 1: Showing the degenerative changes of nerve fibers and the presence of the inflammatory cells. Control group (A), 30 day PC. (Osmic acid stains X40).

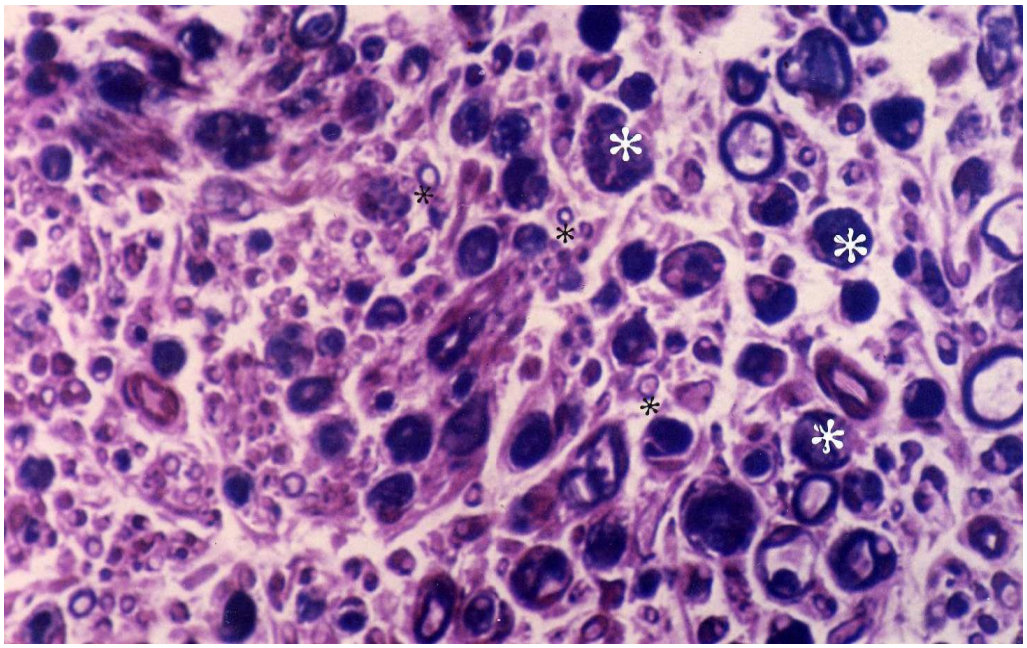

Fig. 2: Demonstrating myelin debris in the macrophages of endoneurium, also showing regenerative nerve fibers of variable size after 90 day PC. Group (A). (Zimmerman stain, X40).

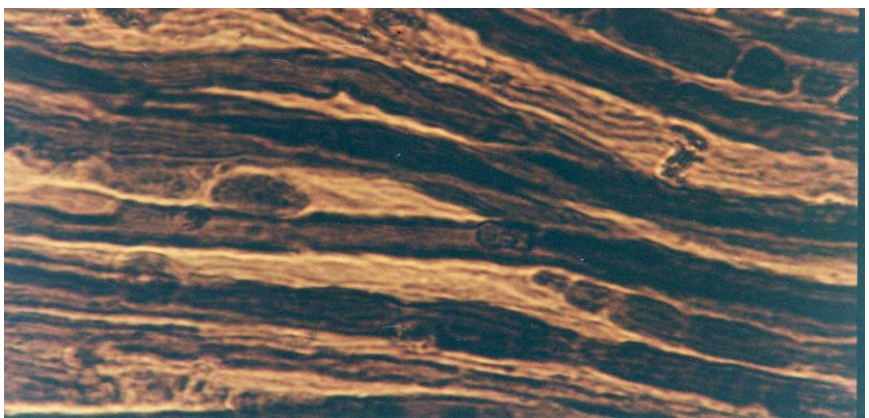

Fig. 3: nerve fibers after 180 day PC of control group, showing irregular nerve fibers ensheathed by myelin (Osmic acid. X40).

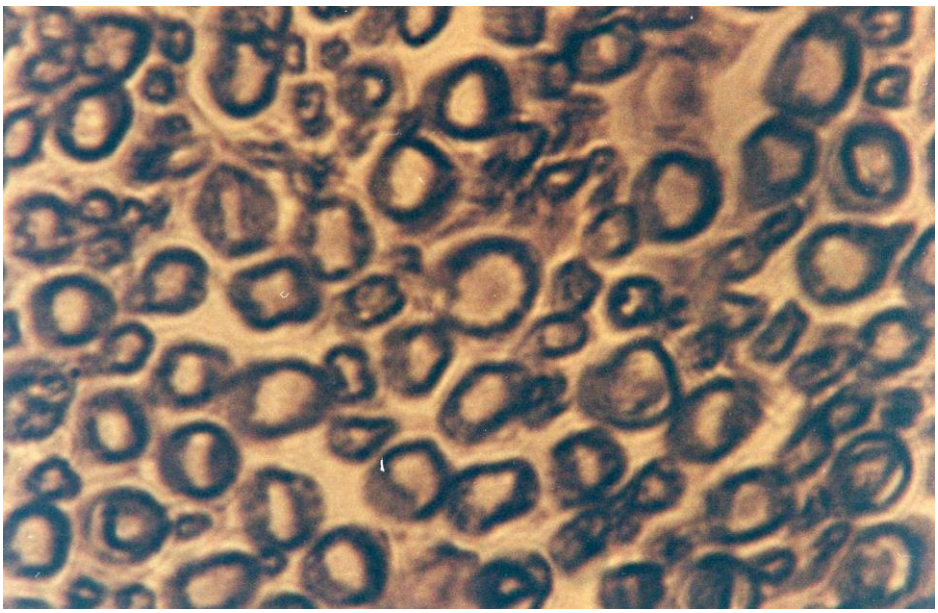

Fig. 4: nerve fibers, demonstrating the nerve fibers after 180 day PC,

Also presence of smooth outlines of myelin sheath (Osmic acid. X10).

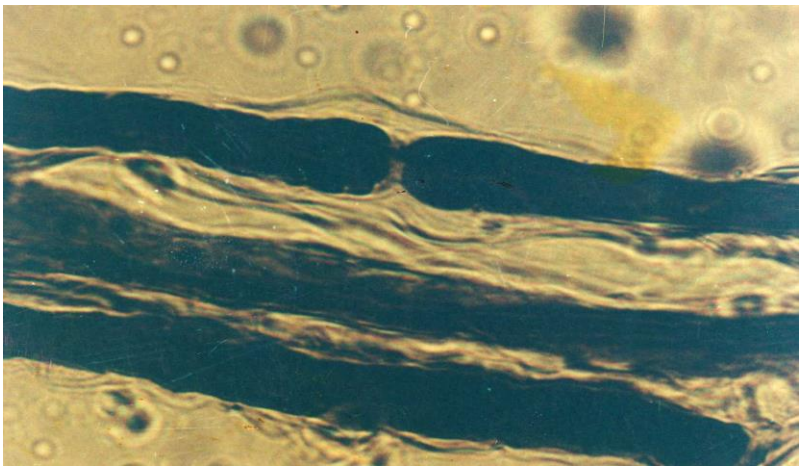

Fig. 5: Myelinated nerve fibers at 180 day PC indicating full myelination of the nerve fibers with nodes of Ranvier (Osmic acid X40).

4. Discussion

Localized nerve injuries resulting in wallerian degeneration, were those produced experimentally by compressing the nerve fibers (Rainsford, 1984). The present study demonstrated that the crush nerve injury of sciatic nerve was associated with the paralysis of left leg of the rat. The signs of paralysis were disappeared from 10-12 day PC. Animals were treated with ASA 25mg/kg bw I/M appeared healthy after injection. There were no signs of loss of weight or appetite PC, and after treatment. Haftek and Thomas (1968) observed that the first signs of inflammation were appeared after 1-2 hour post injury, characterized by increased of the permeability of damaged capillaries and presence of the endoneurial edema. William and Hall (William and Hall, 1971) was demonstrated that after (12-48) hour PC there was degenerative changes of axons, otherwise Gaster and Rand (1971) showed that mainly, there was an inflammatory cell of all types seen at the site of injury within 2-3 days. The present study showed that the presence of inflammatory process was detected in groups, but the treated group with ASA, the signs of inflammation, aggregation of inflammatory cells mainly at the first 14 days PC was lesser than that which is not exposed to any treatment. Although after 15 days of injury, there was new re-growth of nerve sprouts at the site of injury. The inter-nodal length of nerve fibers and its diameter of group (B) was better than of group (A) (control) untreated group , this reflect the reaction of ASA to suppress the inflammation when administered to the rat for 14 days continuously PC and this showed the continuous regenerative process which was ultimately included the elimination from inflammatory cells which consider as suppressive factor to elongate the nerve fibers more and at the same time arrest the remyelination process and this confirm the concept put forward by Hildebrand (Hildebrand et al., 1987) in which it was stated that regenerated myelinated fibers show increase in the nodal spacing with the diameter after the complication of the site of the nerve injury by inflammation.

5. Conclusion

Application of acetyl salicylic acid for 14 day intramuscularly and continuously in experimental rat improves the regeneration of the injured nerve fibers. This may reflect the

role of ASA to suppress the signs and complication of inflammatory process at the site of nerve injury and enhance the regrowth of new nerve fibers faster.

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