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Research paper

Iron prevents demyelination of frog sciatic nerves

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ABSTRACT

Metal ions are of particular importance in nervous system function, notably iron. However, very little has been done to investigate its physiological role in frog peripheral nervous system. The present research aim to evaluate i) the time-effect of sciatic nerve ligation and/or ii) iron sulphate (1.50 mg/kg, *in lymphatic sac*) on frog myelin sheaths. Histological sections following ligation shows degeneration of some fibres with axonal and myelin breakdown associated to a decrease of Schwann cells number following 2 h (45.00 ± 0.30, $p < 0.0001$), 24 h (28.00 ± 0.020, $p < 0.0001$). Interestingly, iron administration reduces the degeneration of myelin sheaths classically observed in frog ligated sciatic nerve associated with an increase of Schwann cells number (139.00 ± 0.50, $p < 0.0001$). Thus, iron could prevent degeneration or promote regeneration induced by ligation in frog sciatic nerve.

1. Introduction

Frog nervous system was considered as an important tool in electrophysiological studies (Banasr et al., 2010; Mbainaibeye et al., 2012; Li-Hua et al., 2015; Das et al., 2016). The myelin sheath is a specialized membranous organelle of the nervous system elaborated by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system (Lemke, 1992). This organelle consists of a large sheet of plasma membrane that is repeatedly wrapped and very tightly compacted around axons (Lemke, 1992). Myelin is essential for rapid conduction of action potentials in vertebrates (Das et al., 2016). Connor and Benkovic (1992) suggested that in the central nervous system, myelin is a lipid rich membrane composed of oligodendrocytes. These oligodendrocytes are enriched in iron and transferrin; furthermore, transferrin has been identified as an essential factor for myelination (Tran et al., 2012; Polin et al., 2013; Radlowski and Johnson, 2013; Rao et al., 2013). Wallerian degeneration (WD) can be induced by experimental nerve crush or section, which promotes a cascade of events described by Waller (1850). The sequence of characteristic events of WD starts with Ca^{+2} influx followed by axonal cytoskeleton disintegration and myelin sheath breakdown. After that, macrophages are recruited in the lesion area in order to phagocyte axon and myelin sheath debris (Stoll et al., 2002). The process of regeneration starts with axon sprouting at the nearest node of Ranvier in the nerve proximal stump. Moreover, when ligatures were used to constrict tibial nerve in rabbits, nerve distal to the site of constriction underwent a reduction in axonal and external fibre diameter and in conduction velocity (Baba et al., 1982, 1983). Distal changes could be detected within 7–12 days of the onset of constriction; removal of the ligatures was followed by partial recovery.

Previous investigations showed the importance of iron in myelin production has been demonstrated by studies showing that decreased availability of iron in the diet is associated with hypomyelination (Greminger et al., 2014; Jougleux et al., 2014). Iron is involved in many central nervous system processes, playing a key role in myelin formation maintenance (Rao et al., 2013; Levi and Taveggia, 2014). In addition, iron requirements are expected to exceed iron intake during the first 6–18 months of postnatal life (Greminger and Mayer-Pröschel, 2015).

The present study aims firstly to study the effect of sciatic nerve ligation on myelin sheaths and secondly, to evaluate the involvement of iron in nerve regeneration.

2. Material and methods

2.1. Animals

Frogs (*Rana esculenta*) were cared for under the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes and the experimental protocols were approved by the Faculty Ethics Committee

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of *Faculté des Sciences de Bizerte*, Tunisia. Animals were subjected to regular monitoring on the zoo technical and health. Frogs (*Rana esculenta*) weighing 15–20 g were randomly divided into three groups: control group (n = 6), ligated group (n = 6) and ligated + treated with iron sulphate ($FeSO_4$) group (n = 6). The sciatic nerves were ligated during 2, 24, and 72 h by the application of two nylon ligatures tightly tied around the sciatic nerve 0.50 cm apart, ~1 cm distal to the tendon of the obturatorinternus muscle. Following ligatures, frogs received a solution of iron sulphate a dose of 1.50 mg of FeSO4 per Kg of body weight (ferrous sulphate dissolved in sodium chloride 0.9%) by injection *in lymphatic sac*. At the third day, the proximal segments of sciatic nerve samples (n = 6) were obtained after sacrifice of frogs (*Rana esculenta*) then harvested in order to study histological sections.

2.2. Histological study on sciatic nerve

For histological analysis, 4 µm-thick sections of paraffin embedded organs were stained with hematoxylin and eosin (Hanini et al., 2011). Tissue sections were examined and images were captured with automatic image analyzer microscope (Leica Qwin) (Hanini et al., 2011; Trabelsi et al., 2013; Ferchichi et al., 2016). Image processing was carried out with ImageJ software (Trabelsi et al., 2013; Ferchichi et al., 2016) in the purpose to evaluate the rate of myelin degeneration after a sciatic nerve ligation and the rate of regeneration after iron injection.

2.3. Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) for comparison between groups. Values for (***) $p \leq 0.0001$ were considered statistically significant. The data are shown as a mean ± Standard Error of the Mean (SEM).

3. Results

In control frog, myelin fibres stained with Hematoxylin and eosin showed homogeny distribution (Fig. 1). The Schwann cell's nucleus was represented in black (Fig. 1A). Following ligation (2 h, 24 h and 72 h) we observe degeneration of some fibres with axonal and myelin breakdown (Fig. 1B–D). The qualitative evaluation demonstrates that degeneration was proportional to the time of ligation in frog sciatic nerves until 24 h post-ligation. In control group, the average of Schwann cells was about (68.00 ± 0.30). The ligation induced a decrease of Schwann cells number following 2 h (−33.82%, ***$p < 0.0001$), 24 h (−58.82%, ***$p < 0.0001$), and 72 h (−26.47%, ***$p < 0.0001$) Compared to control group (Fig. 2).

3.1. Impact of iron treatment on frog ligated sciatic nerves

Fig. 3 shows representative histological sections of frog sciatic nerves following ligation or ligation and iron treatment. Seventy two hours after ligation, degeneration of some fibres with axonal and myelin breakdown was seen (Fig. 3B). Interestingly, iron administration reduces the degeneration of myelin sheaths classically observed in ligated frog sciatic nerve (Fig. 3C).

Following ligation (72 h), we observe a decrease of Schwann cells number (−26.47%, ***$p < 0.0001$). Interestingly, following ligation and iron treatment we note an increase of Schwann cells number compared to control (+104.41%, ***$p < 0.0001$) (Fig. 4).

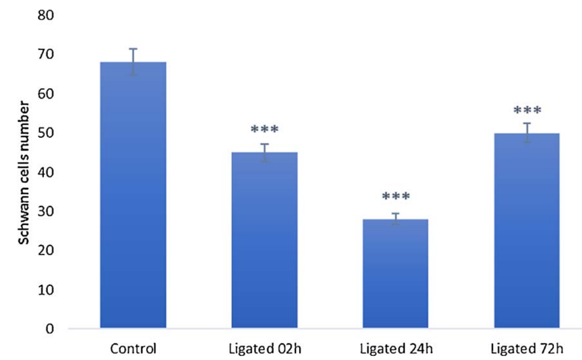

Fig. 2. Quantitative analysis of Schwann cells number following ligation (2 h; 24 h and 72 h). Values are given as the mean ± SEM, for groups of 6 animals each. Schwann cells number of ligated group was compared with control group $p < 0.0001$.

4. Discussion

The present investigation indicated that ligation (2 h, 24 h and 72 h) of frog sciatic nerve caused proportional myelin degeneration. In addition, iron administration (*in lymphatic sac*) reduces the degeneration of frog sciatic myelin sheaths classically observed following nerve ligation. Thus, our data point that iron prevents degenerative process and/or probably contribute to nerve recovery (Fig. 5).

Previous investigations point that iron can play a great role in nerve regeneration through its neuroprotective role and the activation of some neurotrophic factors such as Brain-Derived Neurotrophic Factor (BNDF) (Radlowski and Johnson, 2013; Ziv-Polat et al., 2014). The results from the present experiments based on frog sciatic nerve histology indicated that the rate of Schwann cells decreased following 2 h, 24 h and 72 h; indicating degenerative process proportional to time of ligation. Interestingly, we note that following frog sciatic nerve ligation paranodal myelin damage and cytoarchitecture disruption occurred in nerves and it showed typical non-random distribution of secondary demyelination. All these finding emphasize the role of axon in the genesis of paranodal demyelinisation (Baba et al., 1983; Jack et al., 2013). Yet, depending on the nature and the severity of the initial impact of ligature on frog sciatic nerve, the endogenous and

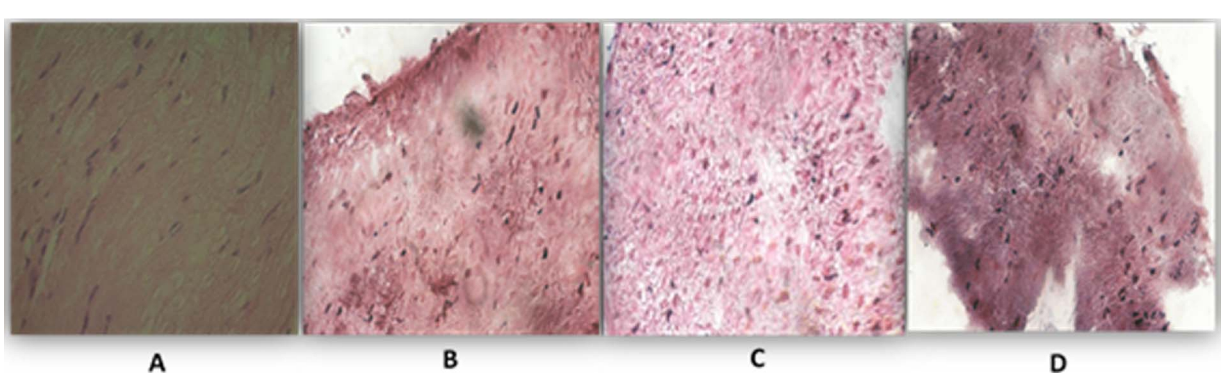

Fig. 1. Cross section through frog sciatic nerve (Hematoxylin-Eosin X 640). A: Sham control, B: ligation for two hours on myelin sheaths and, **C:** ligation for 24 h h on myelin sheaths, D: following 72 h.