

Effect of Plaferon LB on the Injured Sciatic Nerve Regeneration

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ABSTRACT

One of the most common problems in the fields of orthopaedic surgery and neurology is that of the injured peripheral nerve. Injured nerve fibers in the adult mammalian peripheral nervous system can and often do regenerate, thereby restoring at least some lost functions. The surgical repair technique per se plays a minor role for the functional outcome. Critical role is played by Schwann cells as well as macrophages and inflammatory cells. The importance of neurotrophic factors has also been elucidated. Yet, functional recovery after peripheral nerve injuries is frequently poor despite the capacity for axonal degeneration. Nowadays the model of pharmacological stimulation of rat sciatic nerve regeneration is the most popular. The aim of our study was to determine the influence of PLB treatment on the regeneration processes of the injured peripheral nerve. Experiment was conducted on twelve white rats. Animals were daily treated by PLB. Treatment was begun three days before operation. Six animals were treated with PLB, another six were undergone a course of saline treatment. Operation was carried out by surgeon. Sewed sciatic nerves were obtained and used for morphological observation. Preparation was stained by hematoxylin-eosin methods, neurohistological method-Nils and immunocytochemical method. These findings suggest that PLB may play an important role in the regeneration of the injured peripheral nerve. PLB enhances proliferation of Schwann cells and mast cells, decreases the amount of lymphocytes. The present observations point to PLB as stimulator of regenerative process in injured peripheral nerve.

KEYWORDS: PNS, injured peripheral nerve, plaferon LB, Schwann cells, mast cells, lymphocytes

One of the most common problems in the fields of orthopaedic surgery and neurology is that of the injured peripheral nerve. These injuries cause extensive disability, both immediate and long-term, represent a constant medical and socioeconomic problem, and remain a complex challenge to surgeons because the quality of recovery is considerably varied, despite significant developments in anatomic nerve reconstructive with the use of microsurgical technique [1] During the past decade enormous progress has been made in the understanding of the cellular events and molecular changes during degeneration and regeneration of peripheral nerves. However, our knowledge of the regulatory mechanisms and signaling cascades underlying the complex molecular regeneration program is still very limited [2]. despite an enormous amount of new experimental data [3]. The surgical repair technique per se plays a minor role for the functional outcome, it has been realized that treatment of nerve injuries is not a mechanical problem but an extremely complex biological problem. In the laboratory environment, interest has shifted from primarily focusing on surgical repair techniques to basic biological mechanisms regulating and influencing key factors [4] Critical roles are played by Schwann cells as well as macrophages and inflammatory cells. The importance of neurotrophic factors has also been elucidated [5]. Nowadays the model of pharmacological stimulation of rat sciatic nerve regeneration is the most popular. A comparative study of the effects of Xymedone, Riboxin, and Piracetam upon the regeneration of sciatic nerve transection in rats [6] It has been found that the nerve regeneration can be fostered pharmacologically [2].

The aim of our study was to determine the influence of PLB treatment on the regeneration processes of the injured peripheral nerve.

MATERIALS AND METHODS

Twelve adult male white rats weighing 200 g were used for this study. Animals had free access to food and water. Rats were anesthetized with Calipsol (0,5mg/kg). The left

sciatic nerve was separated from the surrounding tissue, was transected in its mid-thigh portion and sewed up. Experimental animals were divided into two groups. The first group of rats were daily injected intraperitoneally with 0,5 mg/kg PLB. Treatment was started 7 days before operation and was being carried out till the end of observation. Saline injections were administrated in the second (control) group of animals in the same manner. All rats were killed with an overdose of anesthetic 1 week or 1 month after trans-section. Sewed sciatic nerves were obtained and used for morphological observation.

Peripheral nerve segments were fixed in 10% formalin solution and was embedded in paraffin (paraplast, SHADON). Paraffin section were stained by hematoxylin-eosin method, neurohistological method-Nils and immunocytochemical method by using monoclonal antibody S100, visualization system LSAB (secondary antibodies labeled by streptavidin-biotin). Substrate diaminobenzidine-DAB (f. DAKO, cytomatic, Denmark).

RESULTS

Results of our investigation showed significant morphological changes as in the first so in the second groups.

In the injured site of peripheral nerve the quantity of cells are significantly increased in comparison with the undamaged site (Fig.1, Fig.2) as in the first so in the second groups. (The undamaged site width 0,4nm, the injured site width 1nm). In the both groups in the injured site of peripheral nerve intense increase of the quantity of Schwann cells was observed (Fig.3, Fig.4). Quantitative analysis of the above mentioned parameters showed that in the first group their increase was statistically reliably higher than in the second group (Tab.1). Particularly, in the preparations stained by hematoxylin-eosin under the influence of PLB the quantity of Schwann cells was 2,9 times more than in the undamaged site. In the second group this parameter was only 2,17 times more than in the intact nerve fiber. In the preparations stained by Nils under the influence of PLB the quantity of Schwann cells

was 3,53 times more than in the intact nerve fiber and in the control group only - 2,97 times. Immunohistochemical method obtained increase of Schwann cells correspondingly 2,57- and 2,08 times in the first and the second groups (Fig.5, Fig.6).

It is remarkable that, in the first group infiltration of lymphocytes was less expressed. Fig.7, Fig.8. The quantity

of lymphocytes in the preparations stained by hematoxylin-eosin 2-times and in the preparations stained by Nils 1,2-times decreased after PLB treatment (Tab.1).

Quantitative analysis of mast cells showed that this parameter significantly increased as in the first so in the second groups. Mast cells most intensively enhanced in the first group (2,57- and 2,08 times accordingly) (Tab.1).

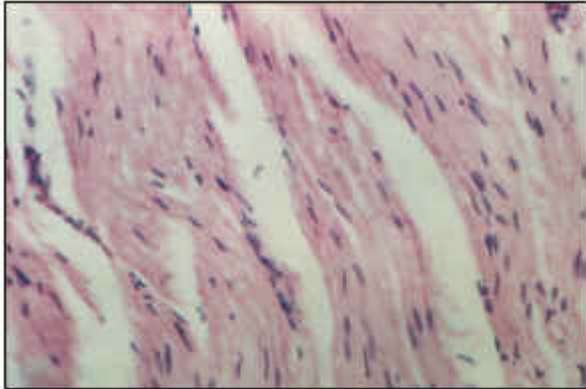


Fig.1 Undamaged site of peripheral nerve.

H & E X 200

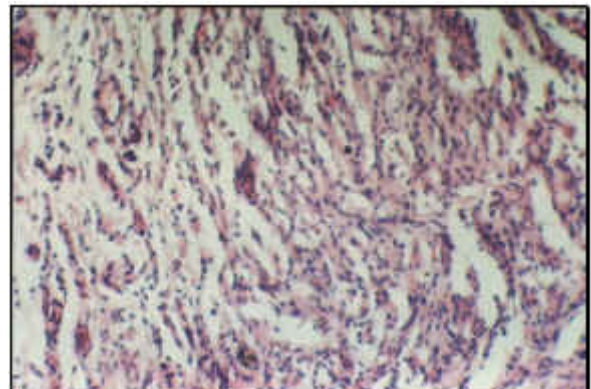


Fig.2 Injured site of peripheral nerve.

H & E X 200

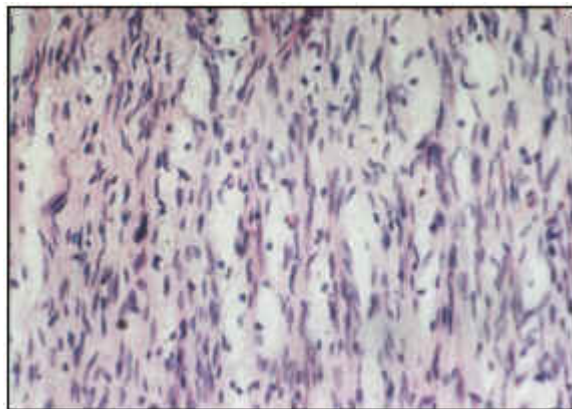


Fig.3 Injured site of peripheral nerve. Intense increase of the quantity of Schwann cells.

H & E, x200

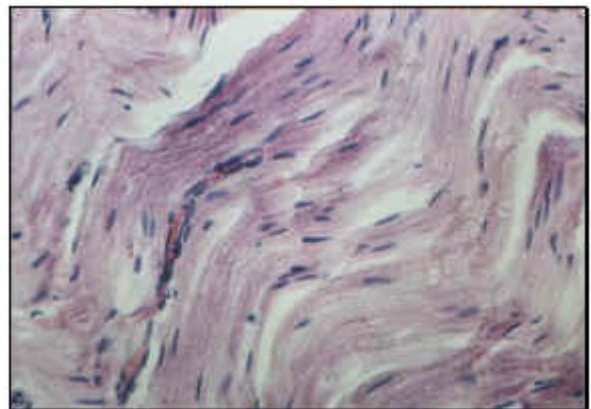


Fig.4 Intact nerve.

H & E, x200

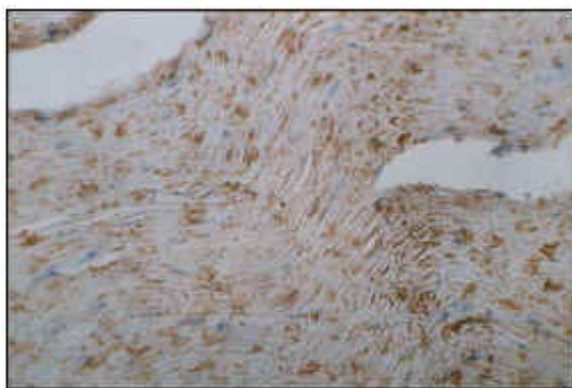


Fig.5 Injured nerve. Intense increase of quantity of S100 positive Schwann cells. IHC, visualization system LSAB.

Subs. DAB, x200

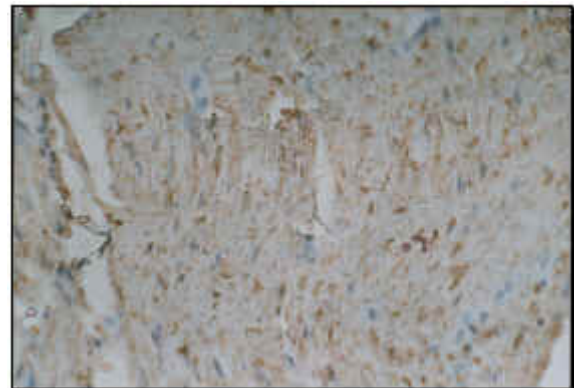


Fig.6 Injured nerve. Moderate quantity of S100 positive Schwann cells. IHC, visualization system LSAB.

Subs. DAB, x200

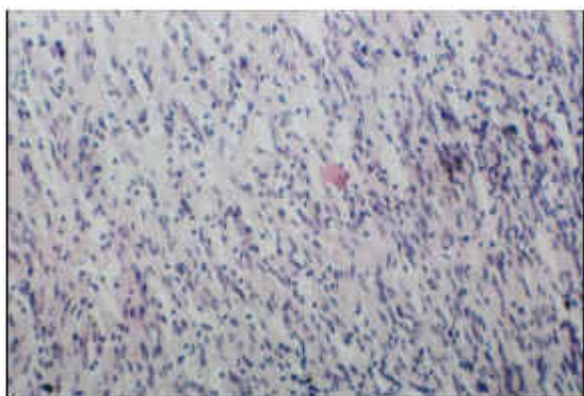


Fig.7 Perineural infiltration. Abundance of lymphocytes and plasmocytes.

H & E x150

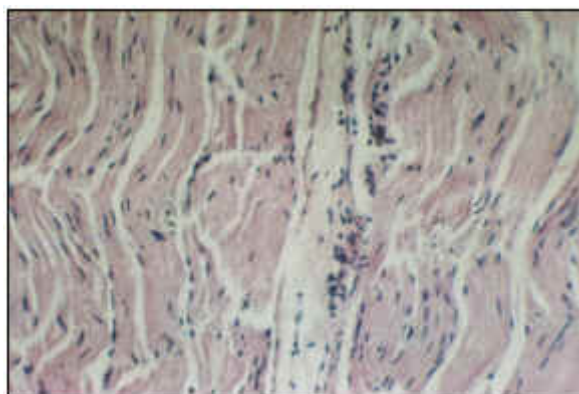


Fig.8 Injured nerve. Significant decrease of the quantity of lymphocytes.

H & E x200

METHODS	SHWANN CELLS				LYMPHOCYTES		MAST CELLS			
	P-LB		Placebo		P-LB	Placebo	P-LB		Placebo	
	Injured nerve fibers	Intact nerve fibers	Injured nerve fibers	Intact nerve fibers	Injured nerve fibers	Injured nerve fibers	Injured nerve fibers	Intact nerve fibers	Injured nerve fibers	Intact nerve fibers
H&E	480,9±4,8	164,5±3,4	344,6±5,7	158,8±1,3	37,4±1,8	74,3±2,3	----	----	----	----
NILS	498,9±5,7	140,7±2,2	357,7±4,3	120,5±1,3	64,5±1,8	78,4±1,5	5,4±0,5	2,1±0,3	2,5±0,3	1,2±0,1
IMMUNO-HISTOCHEMICAL S100	375,4±3,3	125,6±3,1	254,6±3,1	194,7±2,4	----	----	----	----	----	----

Tab.1 Quantitative analysis of cellular elements that are responsible for the regeneration processes of the injured peripheral nerve.

DISCUSSION

Thus, injured nerve fibers in the adult mammalian peripheral nervous system can and often do regenerate, thereby restoring at least some lost functions [7]. The peripheral nerve response to injury is unique [5]. Injury initiates a complex cascade of signals involving neurons, glia, and cells of the immune system that leads to Wallerian degeneration [8] after which myelin debris is removed and a suitable environment for growing axons is generated [9]. Schwann cells play a key role in Wallerian degeneration [5] by regulating macrophage infiltration [10]. After peripheral nerve injury the Schwann cells produce cytokines [10]: TNF- α , IL-1 α and IL-2 β . First, they could initiate the cytokine network of WD as they do in other networks of inflammation. Second, they contribute to macrophage recruitment to inflammatory sites through endothelial cell activation and chemokine production [11]. Likewise they could contribute to macrophage recruitment in WD and consequently to macrophage-dependent functions (e.g., myelin removal by phagocytosis) [12]. We also provide evidence for an autocrine-signaling cascade involving IL-6, LIF, and MCP-1 in Schwann cells that could result in a gradual amplification of the macrophage attracting activity secreted by these cells. Cytokines induced in Schwann cells after peripheral nerve injury could play a key role in

the interactions between Schwann cells and macrophages [10]. An initial Schwann cell role is to help remove the degenerated axonal and myelin debris and then pass it on to macrophages [5]. Macrophages form an important part of the cellular response to peripheral nerve injury and they cause phagocytosis of myelin. Macrophages may also be required for Schwann cell proliferation [13]. Macrophages are known to secrete a variety of proteins that initiate or enhance proliferation in nonneuronal cells [14,15]. Previous studies have led to the suggestion that a disturbance in the axon-myelin-Schwann cell unit is sufficient to induce macrophage recruitment, and it is widely accepted that this is the initiating signal for the inflammatory reaction in peripheral nerve injury [10]. It is of interest that the function of Schwann cells in the regeneration process of injured nerve is not only the recruitment of macrophages. Crushing of the peripheral nerve may also induce MHC class II molecules on Schwann cells. This strengthens the possibility that in living nerves Schwann cells are able to function as accessory cells in the initiation or augmentation of T cell-mediated immune responses [16]. Therefore it is thought that Schwann cells are also active regulators of the early inflammatory response, rather than simply passive targets of extrinsic signals [10].

Endoneurial mast cells also play a pivotal role in this process. They release histamine and serotonin, which enhance capillary permeability and facilitate macrophage migration [5]. Except these mast cells are capable of synthesis and responding to NGF, that is target-derived factor for survival and maintenance of peripheral and central neurons, has been implicated in inflammatory processes [17].

It is of interest that the contact of axons to Schwann cells based upon the structural and molecular linkages seems to be indispensable for stable and successful regeneration. In addition to cell adhesion molecules, Schwann cells utilize short focal tight junctions to provide morphological stabilization of the contact with the elongating axon, as well as small scale gap junctions to facilitate traffic of substances between them. Thus, nerve regeneration is not a simple phenomenon of axonal elongation on the part of the Schwann cell membrane, but is based on direct and dynamic communication between the axon and the neighboring Schwann cell, which may be partly associated with the mechanisms of neural regeneration. [18].

Yet, functional recovery after peripheral nerve injuries is frequently poor despite the capacity for axonal degeneration [19]. The several experimental strategies to promote axonal regeneration and functional recovery have been proposed: nerve regeneration stimulation by cytokines [19], cyclosporine [20], xymedone, riboxin, and piracetam [6] and so on.

PLB has been used in our study, for the pharmacological stimulation of rat sciatic nerve regeneration. PLB is a new "natural" immunomodulatory drug with minimal side effects. It has immunomodulatory, anti-oxidant, anti-ischemic, hepatoprotective and neuroprotective activities [21]. All this let us to pass an opinion that PLB can positively affect process of regeneration of the injured nerve.

Thus the results demonstrate that the quantity of Schwann cells in group undergo PLB treatment was more, than in control group. That confirms the positive effect of the preparation on the process of regeneration of the injured nerve. As, Schwann cells are the "key" of regeneration, increase of their quantity probably will affect cellular mechanisms discussed above. Besides the second group, we observed decrease in lymphocytes quantity in comparison with first group, which indicates the completion of WD, because the Wallerian degeneration (WD) is the inflammatory response of the nervous system to axonal injury. The rate of WD points to the rate of regeneration, e.g. Schwann cells are activated during rapid-WD but not slow-WD [22], which is in agreement with the efficient production of GM-CSF that activates Schwann cells during rapid-WD but not slow-WD [23]. Macrophages are recruited and activated during rapid-WD but not slow-WD [22], which is in accord with the role of TNF- α , IL-1 α , and IL-1 β in the recruitment of macrophages to sites of inflammation [24]. Deficient macrophages recruitment and deficient Schwann cells activation result, in turn, in delayed myelin removal by phagocytosis during slow-WD [25]. NGF production is upregulated during rapid-WD but not slow-WD [26]. Regarding mast cells, their quantity in the second group is more than in the first group, which indicates the positive effect on the regeneration process of the injured peripheral nerve. Increase in mast cells quantity induces migration of macrophages and activation of NGF synthesis.

Summary: These findings suggest that PLB may play an important role in the regeneration of the injured peripheral nerve. PLB enhances proliferation of Schwann cells and mast cells, decreases amount of lymphocyte. The present observations point on PLB as stimulator of regenerative process in the injured peripheral nerve.

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Влияние препарата плаферон-ЛБ на процесс регенерации поврежденного периферического нерва

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Р Е З Ю М Е

Повреждение периферических нервов является одной из важнейших проблем неврологии и ортопедической хирургии. Поврежденные волокна нерва обычно восстанавливаются, но их полное функциональное восстановление происходит весьма редко. Цель исследования - изучение влияния плаферона ЛБ (PLB) на регенерацию травмированного седалищного нерва у белых крыс. В ходе операции седалищный нерв перерезывали и восстанавливали микрохирургическим методом. PLB вводили животным за 3 дня до операции интродуперитонеально, ежедневно. Контрольным крысам в те же сроки вводили физиологический раствор. Материал для исследования забирался на 30-й день эксперимента. Препараты окрашивали гематоксилин-эозином по Нильсу и иммуноцитохимическим методом. Морфологический анализ показал, что PLB действует благотворно на регенерацию травмированного периферического нерва путем увеличения числа клеток Швана и мастоцитов и уменьшения количества лимфоцитов, вследствие чего достигаются оптимальные условия для функциональной регенерации седалищного нерва.

Ключевые слова: ПНС, седалищный нерв, плаферон ЛБ, клетки Швана, мастоциты, лимфоциты