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Phototherapy in Peripheral Nerve Injury: From Basic Science to Clinical Study

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Summary. Laser phototherapy accelerates and enhances axonal growth and regeneration after injury or reconstructive peripheral nerve procedure. Animal and clinical studies show the promoting action of phototherapy on peripheral nerve regeneration, which makes it possible to suggest that the time for broader clinical trials has come.

Key words: *peripheral nerve, phototherapy.*

Object. Posttraumatic nerve repair and prevention of muscle atrophy represent a major challenge of restorative medicine. Considerable interest exists in the potential therapeutic value of laser phototherapy for enhancing regeneration of severely injured peripheral nerve and restoring or temporarily preventing denervated muscle atrophy. This work summarizes our experience and work in the field since the late seventies until this day.

Methods. Low power laser irradiation was applied for treatment of peripheral nerve injury using a rat sciatic nerve model after crush injury, nerve anastomosis or artificial neurotube reconstruction, as well as for treatment of rat denervated muscle. The animal outcome allowed clinical double-blind, placebo-controlled randomized study which measured the effectiveness of 780nm laser phototherapy on patients suffering from incomplete peripheral nerve injuries for a period of at least 6 months up to several years.

Results:

I. Peripheral Nerve Injury

Animal studies show that laser phototherapy has a protective immediate effect, maintains functional activity of the injured nerve, decreases scar tissue formation at the injury site (Fig.1), decreases degeneration in corresponding motor neurons of the spinal cord (Fig. 2), significantly increases axonal growth and myelination (Fig. 3) and accelerates regeneration through direct anastomosis (Fig.4) and artificial neurotube (Fig.5,6).

A pilot clinical double-blind, placebo-controlled randomized study shows that in patients with incomplete long-term peripheral nerve injury 780nm laser irradiation can progressively improve peripheral nerve function, which leads to significant functional recovery (Fig.7, Fig.8).

II. Denervated Muscle

In denervated muscle, animal study suggests that the function of denervated muscles can be partially preserved by temporary prevention of denervation-induced biochemical changes. The function of denervated muscles can be restored, not completely but to a very substantial degree, by laser treatment, initiated at the earliest possible stage post-injury (Fig.9, Fig.10).

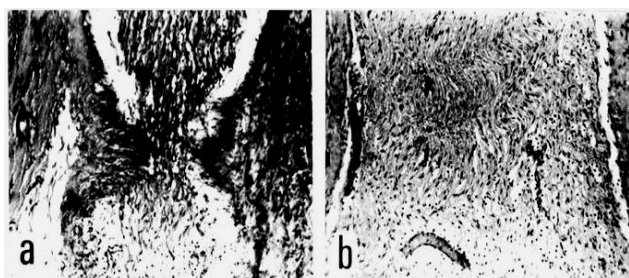


Fig 1. Histological section of the crushed area of a rat sciatic nerve showing the response of the nerve to laser phototherapy. a: Nonirradiated nerve. Note the ring of prominent scar tissue. b: Laser treated nerve shows no visible scar. H& E, original magnification x 150. (Rochkind et al, *Laser Surgery and Medicine* 7: 441-443, 1987)

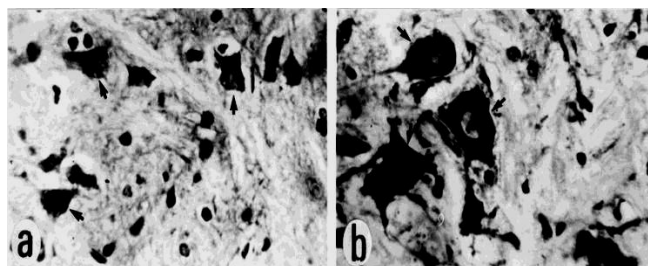


Fig 2: Paraffin section from the anterior horn of corresponding segments of the rat spinal cord 14 days after crush injury to the sciatic nerve, showing the spinal cord response to laser treatment of the injured peripheral nerve. a: Section from a control animal shows extensive chromatolysis and cytoplasmic atrophy found in 40% of the motor neurons (arrows). b: Section from a laser-treated animal shows minimal degenerative changes found in 20% of the motor neurons (arrows). Stained by cresyl fast violet, magnification X800. (Rochkind et al, *Spine* 15: 6-10, 1990)

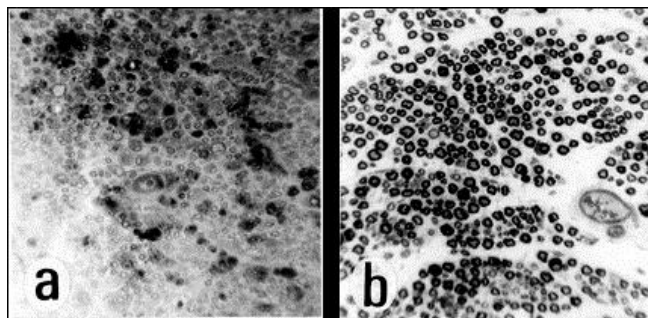


Fig.3. Photomicrographs of semithin sections stained with toluidine blue. Rat sciatic nerves were crushed. One group of rats were laser treated 20 consecutive days after injury. Twenty-one days after injury the nerves were excised and stained. a. Site of crush injury of nonirradiated nerve: Nerve fibers seem to be smaller and mostly nonmyelinated, and numerous macrophages and phagocytes are seen. b. Site of crush injury of laser treated nerve: Most axons are ensheathed with myelin and a very few infiltrating macrophages are observed. (Rochkind et al, *Neurosurgery* 20: 843-847, 1987)

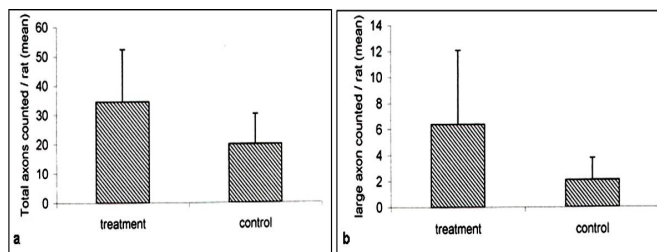


Fig. 4. Bar graphs illustrating the results of a double-blind randomized study evaluating regeneration of the transected rat sciatic nerve after suturing and postoperative low-power laser treatment. a: Graph showing a statistically significant increase in the total number of axons in the laser-treated group ($p = 0.026$), compared with the nontreated control group. b: Graph showing a statistically significant increase in large diameter axons in the laser-treated group ($p = 0.021$) compared with the nontreated control group. (Shamir, Rochkind, *Journal of Reconstructive Microsurgery* 17:133-138, 2001)

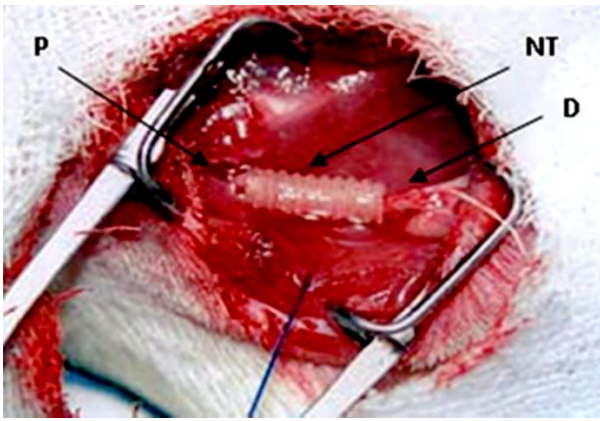


Fig. 5. Intraoperative photograph of the neurotube (NT) reconstruction procedure. A neurotube is placed between the proximal (P) and distal (D) parts of the rat sciatic nerve for the reconnection of a 0.5 cm nerve defect (arrows). (Rochkind et al, Photomedicine and Laser Surgery 25: 137–143, 2007)



Fig. 6. Photograph of the sciatic nerve of an adult rat 3 months after neurotube (NT) reconstruction. The neurotube recreated the anatomical connection of the previously transected and divided nerve, and a distance of 0.5 cm was recreated. (Rochkind et al, Photomedicine and Laser Surgery 25: 137–143, 2007)

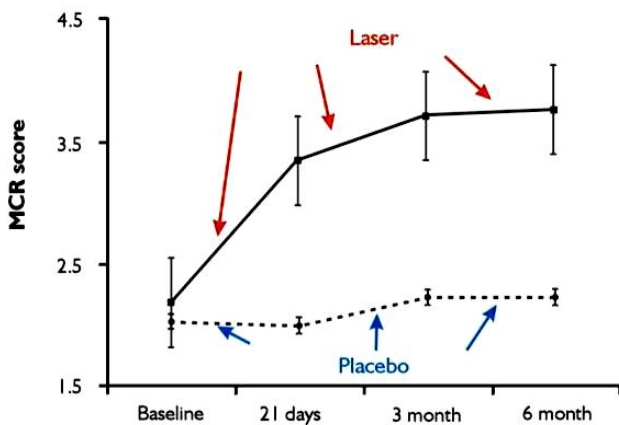


Fig. 7 Graph of the motor function follow-up in injured patients who underwent either 780-nm laser phototherapy or placebo treatment. Mean motor function (\pm SD) of all affected muscles was examined in injured patients using the Medical Research Council (MRC) Grading System. The analysis of the results showed that at baseline the 780-nm laser-treated and placebo groups were in clinically similar conditions ($p = 0.887$). The analysis of motor function during the 6-month follow-up period compared with baseline showed statistically significant improvement ($p = 0.0001$) in the laser-treated group compared with the placebo group. (Rochkind et al, Photomedicine and Laser Surgery 25: 436–442, 2007)

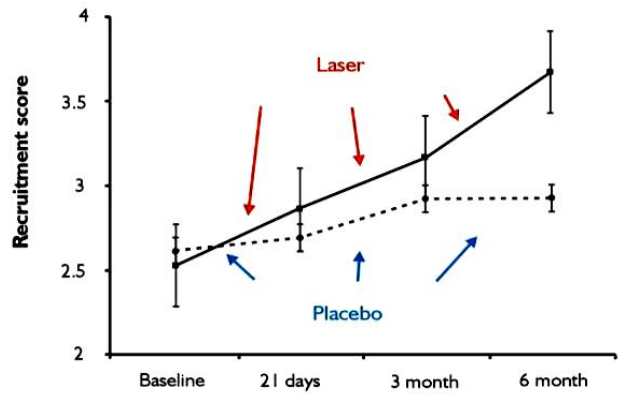


Fig. 8. Graph of the motor unit recruitment in injured patients who underwent either 780-nm laser phototherapy or placebo treatment. Motor unit recruitment, the mean of all examined muscles (\pm SD), was monitored in injured patients. The 780-nm laser-treated and placebo groups were in similar conditions at baseline ($p = 0.934$). In the laser-treated group, statistically significant improvement ($p = 0.0006$) was found in motor unit recruitment during the 6-month follow-up period, compared with the placebo group. (Rochkind et al, Photomedicine and Laser Surgery 25: 436–442, 2007)

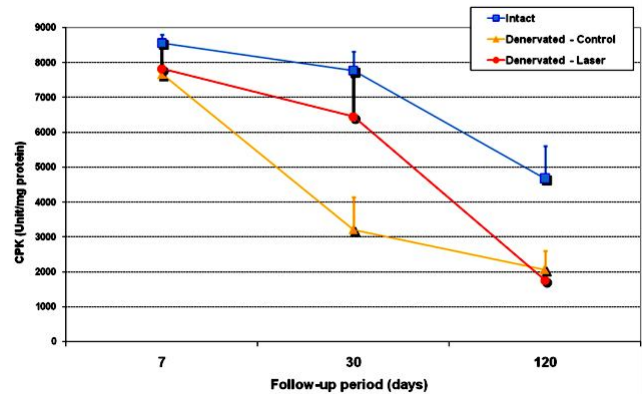


Fig. 9. Graph illustrating the results of study evaluating creatine kinase (CK) activity (unit/mg protein) in intact and denervated rat gastrocnemius muscle. Graph showing content CK (unit/mg protein) during 7, 30 and 120 days in intact and denervated muscles with and without laser treatment. In the control non-irradiated group, 30 days after denervation, the amount of CK decreased markedly to 41% of the normal value (intact muscle). In the same time delayed and attenuated decrease of the CK activity was observed in the laser treated group. The CK activity of the laser treated denervated muscle decreased only by 17% of the normal value. The analysis of CK activity in the denervated laser treated group, compared to the control denervated group showed statistically significant difference ($p = 0.008$)

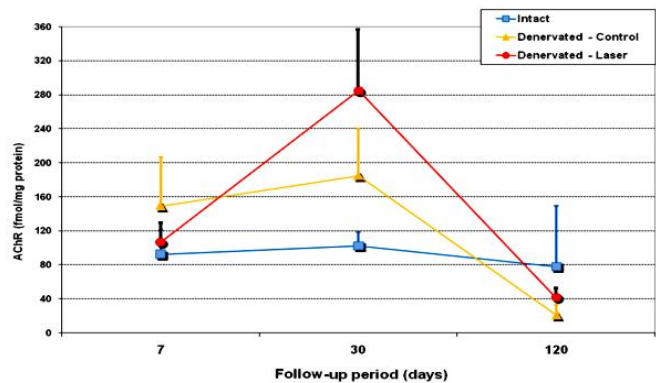


Fig. 10. Graph illustrating the results of study evaluating the level of acetylcholine receptors (AChR) in intact and denervated rat gastrocnemius muscle. Graph showing content AChR (fmol/mg protein) during 7, 30 and 120 days in intact and denervated muscles with and without laser treatment

In the control non-irradiated group, 7 days after muscle denervation, as expected, the amount of AChR increased to 161% of the normal value (intact muscle). In contrast, the amount of AChR of the laser irradiated denervated muscle remained near normal value. Thirty days after denervation in laser-treated group amount of AChR increased to 278% compared to 180% in non-laser group. The amount of AChR in laser-treated group remains higher until 4 month after the denervation, in comparison to non-irradiated group.

Conclusions: Laser phototherapy accelerates and enhances axonal growth and regeneration after injury or reconstructive peripheral nerve procedure. Animal and clinical studies show the promoting action of phototherapy on peripheral nerve regeneration, which makes it possible to suggest that the time for broader clinical trials has come.

(Rochkind et al., *International Review of Neurobiology*, In Press, 2013)

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Рохкінд Шімон

Лазеротерапія при ушкодженні периферійного нерва: від фундаментальної науки до клінічних досліджень

Резюме. У науковій роботі показано, що лазерне фотолікування пришвидшує і поліпшує ріст аксонів і регенерацію після ушкодження, а також сприяє реконструкції периферійного нерва. Пілотні клінічні та експериментальні дослідження на тваринах свідчать про стимуляційний вплив лазерної фототерапії на регенерацію периферійного нерва і це дозволяє стверджувати, що настав час для більш значних клінічних випробувань.

Ключові слова: периферійний нерв, лазерна фототерапія.

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Динамика азотистых метаболитов в ткани щитовидной железы при резекции печени

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Резюме. В опытах на 120 белых крысах (самках) массой исследовали влияние резекции печени (РП, 15-20% массы органа) на динамику азотистых метаболитов в ткани щитовидной железы. Установлено, что формирование при РП артериальной гипергаммониемии сопровождается отсроченным (на 14-е сутки после операции) увеличением концентрации аммиака в ткани щитовидной железы. Содержание глутамина в ткани щитовидной железы не изменяется в течение 14-и суток после РП. Даже в условиях артериальной гиперглутаминемии, отмеченной на 3-и и 14-е сутки после операции. РП вызывает стойкое накопление мочевины тканью щитовидной железы при отсутствии увеличения её концентрации в артериальной крови. В статье обсуждаются возможные механизмы выявленной изменений азотистого метаболизма в щитовидной железе после РП.

Ключевые слова: печень, резекция, азот, метаболизм, щитовидная железа.

Постановка проблемы и анализ последних исследований.

Исследованиями установлено, что в ответ на нарушение обезвреживания аммиака в печени после её резекции происходят компенсаторные изменения азотистого метаболизма в органах желудочно-кишечного тракта [1], лёгких [2] и почках [3,4]. Они проявляются активацией реакций нейтрализации аммиака, стимуляцией его выведения из организма и транзитной ретенционной задержкой в тканях. Известно, что в регуляции азотистого метаболизма принимают участие гормоны щитовидной железы [5]. Вместе с тем состояние самого азотистого метаболизма в ткани данного эндокринного органа при резекции печени остаётся не исследованным.

Целью настоящей работы явилось изучение динамики азотистых метаболитов в ткани щитовидной железы при резекции печени.

Материал и методы исследования

Опыт проведён на 120 белых беспородных крысах массой 180-220 г. Резекцию печени (РП) осуществляли под эфирным наркозом, удаляя 15-20% массы органа. Животные разделены на 7 серий опытов. 1 серия интактные животные (норма), 2,3 и 4 серии опытов – животные, исследованные, соответственно, на 3-и, 7-е и 14-е сутки после лапаротомии («ложнооперированные» животные), 5,6 и 7 серии – животные, соответственно, на 3-и, 7-е и 14-е сутки после РП.

Объектом исследования служили ткань щитовидной железы и артериальная кровь, которую брали из аорты, пунктируя её предварительно гепаринизированным инсулиновым шприцом. Животных забивали декапитацией на фоне этилового наркоза (40 мг этилового натрия / кг массы). Перед забоем у животных производили забор крови из бифуркационного отдела аорты. Для определения содержания азотистых метаболитов ткань щитовидной железы предварительно замораживали в жидком азоте и растирали в порошок, из которого готовили навеску тканей для получения 10% гомогената в 60% растворе ТХУ. Гомогенат центрифугировали на холоду в течении 10 мин при 3000 об/мин. Полученный супернатант использовали для изучения содержания аммиака, глутамина и мочевины. Аммиак в ткани щитовидной железы определяли микродиффузионным методом [6], в депротенизированной плазме крови – фенилтипохлоридным методом [7]. Глутамин в ткани щитовидной железы и депротенизированной