REVIEWS

Survey of Current Experimental Studies of Effects of Traditional Chinese Medicine on Peripheral Nerve Regeneration

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ABSTRACT The repairing and regeneration of peripheral nerves is a very complex biological and cytological process, its mechanism is unclear so far, and thus results in the lack of specific and effectual therapy and medicament. Chinese herbs and their effective components have their own inimitable predominance in promoting peripheral nerve regeneration, such as their multi-factorial, multi-target and multi-functional action, abundant source, inexpensive, etc. In this paper, the experimental studies reported in recent 5 years concerning the effects of Chinese herbs or their active components on peripheral nerve repairing and regeneration are reviewed in respects of the integral level, cellular level, molecular level and gene level.

KEY WORDS nerve regeneration, peripheral nerve, Chinese medicinal herbs

Peripheral nerve injuries could directly influence the patients' quality of life, and how to promote the repairing and regeneration of the injured peripheral nerve has all along been a hotspot in the field of basic medical and clinical research to which many researchers have devoted much effort and financial capability. Because the repairing and regeneration of peripheral nerve is a very complex biological and cytological process, its mechanism is unclear so far, and as a result, specific approaches or drugs aimed at this goal are absent. Chinese herbal medicine has certain effect in promoting the growth of peripheral nerves, and that is why it has been winning more and more attention in recent years. In this paper, the experimental studies concerning the effects of Chinese herbs or their active components on peripheral nerve regeneration in recent 5 years are reviewed.

ON INTEGRAL LEVEL

Wang BJ, et al . observed the effects of Compound Taizishen Granule (复方太子参颗粒) in rat models with peripheral nerve injury established by clamping their sciatic nerve[1] . The modified remedy originates from Liqi Buxue Decoction (理气补血汤), a time-honored recipe for treatment of wounds formulated by the famous expert of orthopaedica and traumatology, Dr. LIN Ru-gao, composed of heterophylla falsestarwort root, astragalus root, chuanxiong rhizome, asiabell root, prepared fleece flower tuber, Chinese angelica root, white peony root, teasel root, drynaria rhizome, grilled licorice root, etc., and given through gastrogavage. Result showed that the effect of Compound Taizishen Granule was significantly superior to that of placebo (normal saline) in improving sciatic nerve functional index (SFI), latency of the potential evoked by motor nerves (Lat), compound muscular action potential (MAP), the maximum contractility of triceps and the recovery rate of the axon count of myelinated nerves 2, 4 and 6 weeks after operation, indicating that the remedy can promote regeneration, repair and recover the function of the injured peripheral nerves.

The study by Zhang F et al. showed that 2, 4 or 6 weeks after rat's sciatic nerve was injured, SFI, Lat and MAP in the group treated with the extract of Ginkgo leaf, $EGb_{24/6}$, at the dose of 20 mg/d was better in recovery than those in the control group treated with normal saline[2] . They also found that in the number, mean diameter and section area of vessels and the myelinated nerve fiber as well as the thickness, and in the degree of maturation of the medullary sheath in the $EGb_{24/6}$ treated group the recovery was superior to those in the control group, and the number of degenerated nerve fibers was less in the former than in the latter. These results expounded that $EGb_{24/6}$ can promote nerve regeneration and the recovery of function indices .

Li LJ, et al. established peripheral nerve injured models by cutting off rats' sciatic nerve with one side of epineurium preserved, and 1 ml pilose antler polypeptides was injected into the target or-

* Supported by the Natural Science Foundation of China (No. 30572439)

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gan every other day after operation, the results showed that pilose antler polypeptides has effect similar to those described in the above-mentioned experiments[3].

ON CELLULAR LEVEL

Wang BJ, et al. demonstrated that the effects of Liqi Buxue Decoction (理气补血汤, LBD, composed of milkvetch root, asiabell root, teasel root, chuanxiong, etc.) on the recovery rate of myelinated nerve axon count, diameter of regenerated axon and thickness of myelin sheath of the injured peripheral nerves were superior to those of normal saline (used for control). Besides it was shown by transmission electron microscopic examination that abundant organelle and mature myelin sheath lay in the injured peripheral nerves in the LBD treated group, more than those in the control group, displaying the superior condition of nerve regeneration[4].

Ma YP, et al. reported the ultrastructure alterations of spinal neuropile in rats sufferring from low thoracic right hemi-transverse spinal cord injury, and the effect of Jisui Granule Ⅰ (脊髓Ⅰ号, composed of 13 Chinese herbs as chuanxiong, notoginseng, etc.) on the alterations were also investigated on the 1st, 3rd, 15th and 30th day after operation under transmission electron microscope to explore the relationship between Jisui Granule Ⅰ and regeneration of injured spinal neurons[5]. The results demonstrated that Jisui Granule Ⅰ has effect in protecting the neurons of injured spinal cord region, promoting regeneration of spinal neurons and perikaryon, axon and dendrite profile, and alleviating the response of glia cells and pericapillary cells to spinal injuries. So, it has been summarized that immediate administration of Jisui Granule Ⅰ after spinal cord injury could effectively protect the spinal tissues, inhibit the reactive proliferation of glia cells and pericapillary cells to create a microenvironment favourable to the regeneration of spinal neurons.

Zhou CJ, et al. studied the effect of Yiqi Huayu Recipe (益气化瘀方, YHR, composed of milkvetch root, chuanxiong, artificial moschus, artificial ox gallstone, stephania tetrandra, etc.) administered through gastrogavage at the dose of 5.03 g/kg of crude drug every day on neurons in neuromuscular junction in rat L5 nerve root compression models[6]. Results showed that under the condition of muscle miss innervation, the aggregation, sprouting and extension of nerve endings in the neuromuscular junction in the YHR treated model group were better than those in the control group, and in the process of neuro-repairing, the velocity and extent of nerve endings superposition with motor end plate as well as the re-building of neuro-muscular junction in that group was also better. The authors suggested that YHR can promote the proliferation of neurons, enhance their capacity of regeneration, quicken the neuromuscular junction reformation and significantly shorten the process of regeneration.

Zhang F, et al. studied the protection by $EGb_{24/6}$ at the dose of 2 ml/d of the impaired neurons. The results showed the number of neurons was significantly higher and the number of apoptotic neurons was significantly less in the $EGb_{24/6}$ group than those in the control group at different time points after surgery[7]. Furthermore, the ultra structure of L5 dorsal root ganglion (DRG) in the $EGb_{24/6}$ group was basically normal with abundant organelle, while the neurons in the control group had their nuclear gradually shrunk, nuclein becoming thin and sparse, satellite cells departing from the neuron, mitochondrial swelling, crest disappearing, and matrix lost. This study demonstrated that $EGb_{24/6}$ has certain effect in protecting neurons and can decrease neuronal apoptosis.

Pang SY, et al. observed the effect of 9 kinds of main ginsenoside monomers, Rb1, Rb3, Rd, Re, Rf, Rg1, Rg2, Rh1, Rh2, through MTT method on the activity of cultured spinal motor and sensory neurons from embryonic rats[8]. They found ginsenosides Rg1, Rb1, Re, Rf and Rh1 could increase the activity of the cultured cells, but ginsenosides Rb3, Rd, Rg2 and Rh2 had no influence on them. They also observed the effects of 9 ginsenoside monomers on the axon growth of spinal ganglionic axon and found that Rg1, Rb1, Re, Rf and Rh1 had effect in promoting growth of axon, with the effect corresponding to that in improving the activity of neurons[9].

Wang L, et al. assessed the effect of abdominally injected polysaccharides of lycii (PSL, 10mg/kg) on nerve regeneration after sciatic nerve injury[10]. Results showed both the number of nerve fibers on the far-end of anastomosis and diameter of myelinated nerve in the PSL treated group were inferior to those in the control group at the 4th, 8th week and the motor nerve conduction velocity (MNCV) at the 8th week in the PSL group was also lower than that in the control group, suggesting that PSL can inhibit the nerve regeneration of rats' broken sciatic nerves after anastomosis.

Schwann cells (SCs), an important glial cell of peripheral nervous system, not only has close cor-

relation with the genesis, development, configuration and function of peripheral nerves but also plays a key role in the repairing of peripheral nerve injuries. When Waaler's degeneration occurred in the distal end of the injured nerve, SCs columns would form Bungner band through division and proliferation to guide the growth of regenerated axons, and at the same time it secretes many kinds of neurotrophic factors and extracellular matrix and participates in constituting the microenvironment for peripheral nerve regeneration so as to induce, stimulate and regulate the regeneration of axon and development of myelin sheath[11].

Zhou CJ, et al. studied the effect of YHR on SCs in neuromuscular junction on the 10th, 20th 30th, and 60th day after L5 nerve root compression in rats, using immunohistochemistry, confocal laser scanning techniques and polyclonal antibody S-100 as SCs marker, with the motor endplates visualized by fluorescein-conjugated α-bungarotoxin (α-BTX)[12]. They found that after muscle lost innervation, the aggregation, sprouting rate and extension of the terminal SCs as well as the amount of overlap of terminal SCs with endplates and the reformation of neuromuscular junctions in the regeneration stage in the experimental group treated with YHR were superior to those in the control group, indicating that YHR could promote the proliferation and enhance the regeneration function of SCs, accelerate the reformation of neuromuscular junctions and shorten the process of regeneration. Furthermore, they observed the changes in distribution of S-100 protein and neurofilament (NF) at neuromuscular junction (NM) of soleus muscle in different phases and the overlapping area of S-100 and NF was measured with NIH image technique. They found the appearance and growth of SCs marked by S-100 protein were earlier than NF, and the aggregation, sprouting and extension of SCs and NF, as well as the overlapping area S-100 and NF in the group treated with YHR were better than those in the control, indicating that YHR can promote the growth of SCs and NF, and accelerate the nerve regeneration progress[13].

Hu XT, et al. investigated the effect of ginsenoside Rb1 on the proliferation of SCs *in vitro* by applying 10 μg/ml, 20 μg/ml, 200 μg/ml and 1 mg/ml ginsenoside Rb1 on the secondary generation of cultured cells of sciatic nerve from male SD rats on the 5th day of culture. The proliferation of SCs was determined at different times by MTT assay and thymidine incorporation assay[14]. It was found that 10 μg/ml of ginsenoside Rb1 could significantly induce SCs proliferation, which proved to be superior to DMEM medium, but higher concentrations of ginsenoside Rb1 (1 mg/ml) could significantly inhibit the proliferation of SCs, whereas 200 μg/ml of ginsenoside Rb1 showed an effect similar to that of DMEM cultured medium. Thus, it was held that ginsenoside Rb1 at its optimal concentration is effective in inducing the proliferation of SCs, but its higher concentration is cytotoxic for SCs. This result provides some basis for researching the routes of repairing nerve injury *in vivo*.

ON MOLECULAR LEVEL

Neurotrophic factors such as nerve growth factor (NGF), brain-derived neruotrophic factor and many others have been identified and are thought to be important in nerve repairing process, able to help nerve cells survive and maintain and promote axonal regeneration. Among them, NGF is the optimal representation[15]. Yin ZS, et al. reported an experiment on model rats with right sciatic nerve crush injuries, researched the effects of asiabell root, red sage root, milkvetch root, rehmannia root and Compound Shenji Zaisheng Granule (复方神肌再生冲剂, consisting of the above four Chinese single herbs and Chinese angelica root, safflower, etc.), using normal saline as the control (given through gastrogavege) to find the expression of NGF protein in the sciatic nerve tissues at the 2nd, 4th and 8th week after modeling by using immuno-histochemical method and image analysis[16]. Results showed that high level of NGF expression appeared in the group treated with red sage root and Compound Shenji Zaisheng Granule at the 4th and 8th week after surgery, suggesting the two remedies could prompt NGF expression in injured sciatic nerves of rats.

Protein tyrosine kinase (PTKs) is a common protein kinase existing widely in various cells, and a key substance for cell differentiation signal transmission. Extracellular signal is transmitted into nucleus by activating PTKs and stimulates cells to differentiate toward maturity. Jiang BG et al[17]. studied the effect and mechanism of Composite Hedysari Extract (CHE, composed of Radix Hedysari, Radix Paeoniae Rubra, Lumbricus, Angelica Sinensis, Rhizoma Chuanxiong, Epimedium, Peach Kernel, Flos Carthami, etc.) in different concentrations on PTKs in cultured sciatic nerve got from SD rats and cultured in PRMI-1 640 medium with 15% embryo bovine serum, taking NGF as the positive control and normal medium as the negative one. Results showed after 48, 72 and 96 h of culture, the activity of PTKs in the cultured sciatic nerve determined by [γ－^{32}P]ATP incorporation test with protein tyrosine ki-

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nase assay system was, in the negative control group, in inactive condition but, in the NGF group, got increased slightly, lasting, with little increasing, to the 96th hr, with the peak value as 0.1548; But in the CHE group, it got significantly increased, but decreased quickly, with the peak value appearing at the 48th h, which in the groups treated with different concentrations (1 : 1 600, 1 : 3 200 and 1 : 6 400), was 0.4460, 0.3326 and 0.4169 respectively, showing no significant change along with the changing of drug's concentration. By the way, the activity had no change at the concentration of 1 : 12 800. By rank-sum test, it was shown that significant difference appeared between the negative control with NGF and CHE at concentration over 1 : 5 400 at the 48th h, and the changes after 48 h was of no statistical meaning. Analysis also showed that the effect of CHE on the activity of PTKs was negatively correlated with the concentration and the acting time of the extracts. Besides, using the same method, the authors found in the NGF and CHE groups, the activity of protein kinase A (PKA) was notably increased, which in the CHE group was negatively correlated to the acting time and positively correlated with the concentrations used, showing significant difference . The summary was that the action of CHE in promoting nerve regeneration is realized through receptor-cAMP-PKA signal pathway to accelerate the proliferation of Schwann cells[18] .

ON GENE LEVEL

Liu M, et al. explored the molecular mechanism of Shenjing Zaishengsu (神经再生素, SZS, a Chinese herbal preparation with the effective ingredients extracted from milkvetch root, Chinese angelica root, safflower, prepared fleeceflower tuber, achyranthes root, spiny jujube seed, etc., Chinese patent number 01108209·7) by observing the changes of gene expression level when it was applied to promote nerve growth[19] . The experiment was conducted using differential display PCR (dd-PCR), with the cDNA fragments acquired from cultured cells of dorsal root ganglia (DRG), treated or untreated with SZS, which was further confirmed by Northern blot after hybridization, screening, clonal sequencing and DNA sequence retrieval analysis. Comparison of these sequence with data from GenBank showed that in the 8 differential fragments obtained, one could down-regulate gene and the others up-regulate it. Among them, one was found to be completely homologous to RRAJ5161 (the proliferating correlative gene) and another to F196315 (the small zinc finger-like protein DDP2). Three were found to be partially homologous to AK001757 gene and STA5SRR gene (lysyl-tRNA synthetase) respectively. This study indicated SZS plays an important role in selectively regulating gene expression during the process of stimulating nerve growth. In addition, they detected and compared the changes of gene expression of growth related protein 43 (6AP-43), low molecular neurofilament protein (NF-L), translation elongation factor 2A3-2 (EF-Ts and PRAJ5161) and F196315 in DRG cells cultured respectively with SZS, NGF and blank culture (for control) by using RT-PCR.[20] It was found that as compared with the control, in the SZS and the NGF groups, the gene expressions of GAP-43, NF-L, 2A3-2 and DDP2 were significantly up-regulated or changed to different extent in a time-dependent manner. This study indicated that, similar to NGF, SZS may act on the protein translation level so as to promote growth of nerve cells; on the skeletal level so as to maintain the shape and normal physiological activity of nerve cells; on the specific protein level of neurons so as to maintain the survival of cells and promote the growth of neuro-synapse; and on the trans-factor regulating level so as to advance the neuronal survival and protuberance extending

Gao XL, et al. studied the effect of Spinal Cord Granule Ⅰ (SCG Ⅰ) on calcitonin gene related peptide (DGRP) expression in DRG after spinal cord injury to investigate into its mechanism of promoting spinal cord regeneration in rats by using immunohistochemical method and quantitive image analysis[21] . The results they gained are as follows: (1) Spinal cord hemisection would cause CGRP expression to be down-regulated significantly and sustainably in ipsilateral 3 DRGs above and below the injury level. (2) SCG Ⅰ could evidently suppress but not check completely the injury caused down-regulation of CGRP expression. (3) The down-regulation of CGRP expression can not be relieved with administration of Buyang huanwu Decoction (补阳还五汤) or large dosage of hydrocortisone. (4) Marked evidence of repairing and regeneration could be seen in the injured spinal tissues after administration of SCG Ⅰ. So, the results not only suggests that the level of CGRP expression is correlated with the condition of repairing and regeneration of injured spinal cord tissues, but also indicates that SCG Ⅰ may evoke the repair and regeneration of injured spinal cord by regulating CGRP-mRNA expression. Moreover, GAO XL, et al. also found through the fluorescence immunohistochemical method in combination with laser scanning confocal microscopy that in the SCG Ⅰ treated group, c-Jun of DRG neuron was up-regulated, and the number of nerve cells with intact nuclei was significantly increased, suggesting that SCG Ⅰ had effect in improving repair and regeneration of

injured spinal cord by regulating c-Jun expression[22]. In addition, further study on the effect of SCG Ⅰ on nitric oxide synthase (NOS) expression in DRG cells in Wistar rats after spinal cord hemi-cross section, through NADPH-d histochemical method and image analyses, indicated that NOS in the 2 DRGs above and below the injured level was up-regulated after administering SCG Ⅰ with the strong positive rate reaching over 40%, significantly higher than that in the control group treated with normal saline, suggesting that NOS level is correlated with the condition of spinal regeneration and repairing, and SCG Ⅰ can promote regeneration of the spinal cord probably by regulating NOS expression level[23].

To sum up, a great deal of factors and elements are involved in the repair and regeneration process of peripheral nerve injury, including aspects such as blood vessel, metabolism and neuron nutrition, etc. Though some profound advance has been made in the research on the level of cellular, molecular and gene characters, the key mechanism is still uncertain and unresolved. There has been made insignificant progress, and effective pertinence therapy are still lacking because most experiments are focused on only one facet of the whole courses of peripheral nerve regeneration. Chinese herbs and their effective components have their own inimitable predominance in promoting peripheral nerve regeneration, such as their multi-factorial, multi-target and multi-functional action, abundant source and apness in price, etc. Introducing Chinese herbs and their effective components into the field of promoting nerve regeneration is meaningful to clinical practice and has gradually come into the highlight in researches, showing a favourable prospect. But recent researches on this topic are superficial, mostly limited to the observation or study of the effect of single recipe or drug to certain indexes, with the research of the substantial basis and action mechanism wait to be deepened, especially the action mechanism of compound recipe in participating in metabolism *in vivo* is scarcely studied, and improvement in aspects of drug screening, dose-form, etc. is urgently in need.

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(Received July 5, 2006)