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Effect of transplantation of neural stem cells on neuropathic pain in rats

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ABSTRACT

Objective : To observe the effects of different number of neural stem cells (NSCs) of intrathecal injection on partial sciatic nerve transaction (PST) in a rat model of neuropathic pain and the dorsal horn of the spinal cord, dorsal root ganglion (DRG) of glial cell line derived neurotrophic factor (GDNF) expression of. **Methods** : 84 adult male SD rats were randomly divided into seven groups: group sham (group I), group PST (group II), group PST + NSC_s 1×10^3(group III), group PST + NSC_s 1×10^4(group IV), group PST + NSC_s 1×10^5 (group V), group PST+NSC_s 1×10^6(group VI), group PST+ NSC_s 1×10^7(group VII). Three days after intrathecal injection NSCs. Record 1 days before operation, after 1, 3, 7, 14, 21 days of mechanical pain and heat pain threshold;and 7 to 21 days, immunohistochemistry and RT-PCR detection of the ipsilateral spinal dorsal horn and DRG GDNF expression. **Results** : Compared with I group, the pain threshold decreased 1 days after operation in group II ~VII, after 7 days, 14 days of minimum (P<0 05), IV, V, VI, VII group with the increase of NSCs content compared with group II,the pain threshold with increased gradually, seven days after the operation,The dorsal horn of the spinal cord and DRG GDNF expression was gradually upregulated (P < 005),21 days after operation, V, W, VII group with preoperative pain threshold were not statistically significant, but the expression of GDNF in V group was the highest (P < 0.05). **Conclusion** : Int rathecal $NSC_s1\times10^5$ are the most effective in alleviating PST-induced neuropathic pain in rats.

KEYWORDS

Neural stem cells; Glial derived neurotrophic factor; Cell transplantation; Partial sciatic nerve transaction.

INTRODUCTION

Neural stem cells (NSCs) in the experimental study of injury, neurodegenerative diseases, shock, multiple sclerosis, spinal cord and peripheral nerve injury of hypoxic ischemic brain, with reversal of neuronal damage, repair damaged nerve, promote the function recovery[1]. Research indicates that mouse branches of the sciatic nerve injury, stem cells intraventricular injection, generation and development can modulate pain, reduce the pain threshold[2]. NSCs in the regulation of the microenvironment in vivo, can differentiate into neuron and glial cells, The pain and injury to the slow release of anti nociceptive factor, Such as glial cell line derived neurotrophic factor (GDNF), In the promotion of tissue regeneration, neuronal survival, at the same time, prevent the initiation and development of chronic pain[3]. This study through the PST on rats, intrathecal injection of different amounts of NSCs, observe the pain threshold and the dorsal horn of the spinal cord, dorsal root ganglion (DRG) expression in GDNF, and to explore its possible mechanism.

MATERIALS AND METHODS

Clinical data

84 male SD rats of clean grade, body weight 150 ~ 180 g, free water, maintain 12 hours with the alternation of day and night, were divided into seven groups: group sham (group I), group PST (group II), group PST + NSC_s 1×10^3(group III), group PST + NSC_s 1×10^4(group IV), group PST + NSC_s 1×10^5 (group V), group PST+NSC_s 1×10^6(group VI), group PST+ NSC_s 1×10^7(group VII). After 2% pentobarbital sodium 50mg/ kg anesthesia, II and VII group according to the method of Lindenlaub T making right PST model, I group only exposed the sciatic nerve, don't cut off.

Measurement methods

The method according to the literature[4]. Rats were anesthetized, coupled to a PE-10 catheter slightly blunt needle lumbar L5 and L6 intervertebral vertebral canal puncture to animal, sudden lateral drift motion, as the sign of successful puncture, intrathecal injection with microsyringe was. Selection of the third generation of suspension culture of NSCs suspension, adjust the cell concentration, 3 days after operation, III to VI rats were intrathecally injected into 30 μ L cell suspension, density were 103/30 μ L, 104/30 μ L, 105/30 μ L, 106/30 μ L, 107/30 μ L. I, II group were injected with 30 μ L cell culture. Randomly selected six rats, on the right foot mechanical threshold determination of I, 3, 7, 14, 21 days and 1 days before and after operation (MWT) and heat pain threshold (TWL). After 7, 21 days after the determination of MWT and TWL, other 3 anesthetized, 4% paraformaldehyde perfusion fixation, take on the right side of the lumbar enlargement of the spinal cord dorsal horn and L5DRG, routinely fixed paraffin embedded. GDNF immunohistochemical staining by ABC method, 400 times were observed under light microscope.7, 21 days after operation, 3 rats were anesthetized, take on the right side of the dorsal horn of the spinal cord and L5 DRG. The total RNA was extracted from the spinal cord and DRG, using the spectrophotometer for identification of RNA quality and quantity. Using MMLV reverse transcription kit for synthesis of cDNA, using cDNA as template for polymerase chain reaction amplification, primers designed by Primer 5 software, electrophoresis confirmed the specificity of amplified PCR, the analysis software with Ct values of the samples and the corresponding copy number.

Statistical treatment

The experimental data were expressed asmean±standard deviation (mean±SD), and analyzed using analysis of variance, paired t-test and linear regression by the SPSS13.0 software package, P <0.05 was statistically significant for difference or correlation coefficient.

RESULTS

Compared with the I group, after 1, 3, 7, 14 days II ~ VI group and 21 days after operation, II, III, IV in group MWT and TWL were decreased ($P < 0.05$), of which 7, 14 day low threshold; compared with II, after 7, 14, 21 days IV, V VI, VII, and MWT and TWL value increased gradually ($P < 0.05$); 7 days after operation, I ~ VI group, after 14, 21 day of I to IV in group MWT and TWL were lower than group VI ($P < 0.05$) (TABLE 1).

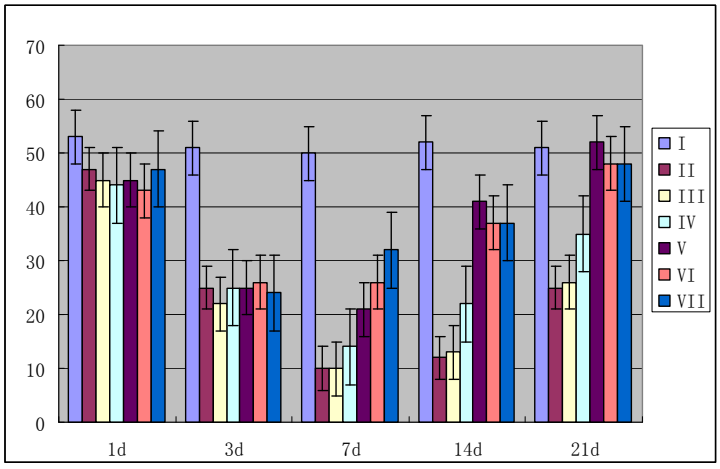
The cytoplasm or nucleus appear brown granules for GDNF expression positive cells in the spinal cord, 7 days after operation, seven rats of DRG GDNF expression have a similar change, positive cells in the I group than II group, positive cells slightly more, group III~VI with increasing graft volume, positive cells increased gradually, dyeing deepen; and after 21 days, 7 days in I group, no significant changes, II ~ VI group were decreased, but the higher positive expression cells in group V, stained with RT-PCR, the trend is similar.

DISCUSSION

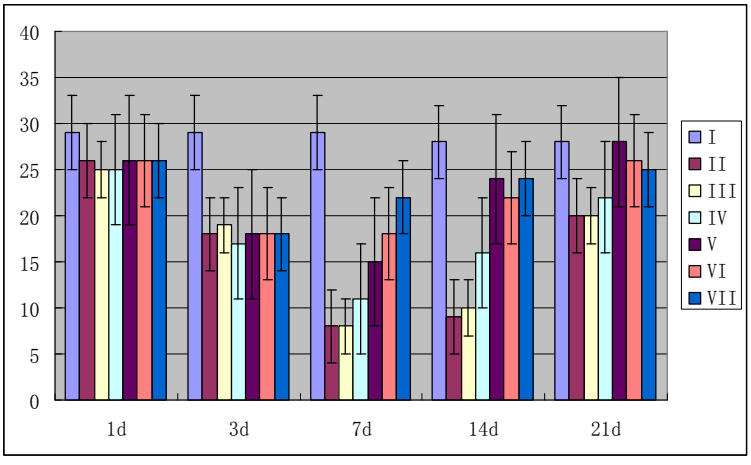
Neuropathic pain (NP) is a chronic intractable pain of nerve system injury caused by, its potential mechanism of complex, poor treatment results of traditional research confirms, sciatic nerve injury animal model, stem cell transplantation therapy can effectively alleviate the mechanical allodynia and thermal hyperalgesia in[5], make it become a feasible research direction the treatment of NP.. NSCs can migrate to the injury site, in the control of micro environment, differentiate into neurons and glial cells, establish synaptic connection with the host cells, synaptic reconstruction loop, restoration of synaptic transmitter release, inhibit the activation of astrocytes and microglia[2], restore the glial cells cytokine interactions between

neurons, expression due to the change of the primary sensory neurons of the neuropeptide, neuronal activity in normal senior center part of the prefrontal cortex, and possible biological micro pump form sustained release anti-inflammatory cytokines and neurotrophic factors, reverse further tissue damage[7], interrupt the process of the formation of NP. Stem cell transplantation is not to determine the number of NP, this study observed the effect of newborn rats from different number of intrathecal injection of NSCs on pain behavior in rats with Gao Min PST. This study observed 7 days after operation with increasing amount of MWT, transplantation, TWL gradually increased, the expression of GDNF increased; 21 days GDNF expression of V group was significantly higher than the other groups after operation. Although VI, VI group also reached the analgesic effect, quickly relieve V group of NP may be associated with the high expression of GDNF is closely related to it. Transplantation of 10^3 apparently number is too low, performance is weak, while the 10^6, the 10^7 transplanted amount, although the short term rapid increase pain threshold and GDNF secretion, long-term effect is not ideal. Generally, stem cells do not express the leukocyte differentiation antigen, does not cause immune host obvious rejection. But recently some scholars confirmed, allogeneic transplantation, advanced to stimulate the host immune rejection[10]. Some scholars study found, in acute brain injury, the higher amount of transplantation, the percentage of CD4+T cells increased significantly, possibly due to highly concentrate the antigen, immune host T cell mediated rejection, the experiment may exist the same mechanism;Mechanisms of stem cell treatment of NP, it is mainly the secretion of antinociceptive molecules play a role, such as GDNF. This study also observed that intrathecal injection of NSCs, expression of DRG and the ipsilateral spinal dorsal horn of GDNF obviously increased, and 7 days after operation, with the increased number of cells, GDNF expression was significantly increased. After 21 days of 10^5 cell array GDNF expression was the highest, which may partly explain the differences in animal behavior, 10^6, 10^7 cell array, the expression of GDNF may be related to the transplanted cells less and less residual. GDNF through its specific high affinity receptor GFRa-1 and Ret dependent pathway and Ret independent pathway mediated transmembrane signal transduction play a biological effect, play an important role in signal transduction and modulation of pain, promote the survival of injured neurons at the same time, reduce the ectopic discharge rate, block NP[8].

TABLE 1 : Comparison of seven groups of rats with MWT and TWL (mean±SD)



MWT



TWL

In summary, intrathecal injection of newborn rat derived NSCs, neuralgia behavior can reduce PST rats, the secretion of GDNF may be one of the mechanism of analgesia, while the number of cells in 10^5, can be most effectively relieve PST rats PN.

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