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Research Paper

The effect of spinal cord injury on the expression of TGF-β and TNF-α in rat articular cartilage☆

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Abstract

Objective: To observe the expression of TGF-β and TNF-α in the spinal cord injured rat model and discuss the significance of the articular cartilage metabolism. **Methods:** 36 SD female rats were randomly divided into 2 groups: Rats models of spinal cord injury were implemented by Allen method. T_{10} laminectomy was performed in the control group. Both groups of rats were killed respectively in 1w, 3w and 6w. Hematoxylin-eosin stain was given to each slice in the model group and control group. Immunohistochemical stain was applied by using ABC method in the expression of TGF-β and TNF-α. Those expressed level were performed in image analysis and statistics process. **Results:** TGF-β and TNF-α were mainly distributed on the surface layer of the articular cartilage, with a weak expression in control group. The expression of TNF-α in the model group was more significant than that in the control group in the 1w, and still remained an evident difference with that in control group until the 6w($P < 0.05$). TGF-β expression of the model group had no remarkable difference with the control group in the 1w ($P > 0.05$) and prominently became stronger at 6w($P < 0.05$). **Conclusion:** The expression of TNF-α occurred early in the development of spinal cord injury, and the expression of TGF-β became stronger with the revival of spinal neural function. Both expressions were strengthened in articular cartilage in the 3rd week.

Keywords: spinal cord injury (SCI); articular cartilage; transforming growth factor(TGF-β); tumor necrosis factor (TNF-α)

INTRODUCTION

Spinal Cord Injury (SCI) is a kind of severe nervous system trauma. Bone mass of patient is lost after spinal cord injury with bone micro-mechanism cataplasised significantly[1]. Nowadays people believe that chondrocyte responds to both stimulation of biochemistry and physically, and that many kinds of cytokines exist in arthrodial cartilage. These are important to regulate damage and reparation of arthrodial cartilage [2]. The level of chondrocyte measured can be evaluated the state metabolism of cartilage. To observe the expressions of TGF-β and TNF-α in the rat model with spinal cord injured and discuss their significance in the articular cartilage metabolism, corresponding basic studies were carried on in our research. It may be valuable in the clinical application in the articular cartilage re-establishment and reparative process.

MATERIALS AND METHODS

Animals and reagents

Healthy female SD rats with a weight of 250 g~300 g were provided by Experimental Animal Center of Xi'an Jiaotong University (license of Shaanxi animal center, No: 08-005). They were bred at a constant temperature (23 ± 3)℃ in our institute before the experiments, with a humidity of 40%-50% and an indoors noise less than 60dB. 36 mature female SD rats were randomly divided into 2 groups: a model group and control group, with 18 rats in each group. Observing the variation of TGF-β and TNF-α expression in the rats' knee joint cartilage at 1w,

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3w, 6w after the injury. In both the model group and control group, 6 rats were operated once a time.

Rabbit antimouse TGF-β_1 polyclonal antibody, rabbit antimouse TNF-α polyclonal antibody and AEC condensed liquor were provided by Huamei biology company (Beijing, China). Biotinylationed goat antirabbit immunoglobulin G antibody and 1.25 g/L parenzyme were provided by Maixin biotechnology development company (Fuzhou, China).

Model of spinal cord injury

Rats models of spinal cord injury were built by Allen method [3], and T_{10} laminectomy was performed in the control group. Both groups of rats were killed respectively in 1w, 3w and 6w; hematoxylin-eosin stain(H.E stain) was given to each slice of the model group and control group; pathological changes should be observed under the microscope.

Hematoxylin-eosin stain and immunohistochemical stain

Rat knee articular cartilage was fixed in 40 g/L polyoxymethylene after 16 hours and decalcificationed in 0.01% ethylenediamine tetraacetic acid between the 10th day and 14th day. Articular cartilage was dehydrated by gradient alcohol and commonly imbeded using paraffin. Each sample was chipped with 5 μm thick intermittently. 1~2 slices were selected and stained with HE staining and observed under light microscope. Immunohistochemical stain should be applied using ABC method (produced in Huamei biology company). Primary antibody was replaced by phosphate buffering saline solution (PBS) as negative control.

Image analysis and statistics process

The collected immunohistochemical images were scanned in gray level by using a German Leica inverted optical microscope and imaging system (Attention: Slice measured in gray scale value can not be counterstained). The results of images were analyzed with the SPSS12.0 commonly. An average gray level is calculated by statistic positive signal in picture. It expresses pictorial transmittance in the image analyzed, which rank into 256 grades. The extreme dark image was recorded as grade 0, and the extreme bright image was recorded as grade 256. The less gray level means the higher positive staining and the stronger immunoreactivity and vice versa.

RESULTS

Analyzing results of hematoxylin-eosin stain after spinal injury

In the HE stain group the cell nucleolus were stained with blue color and the cellular plasm with different degrees of red. The calcium salt was stained with blue or indigo color. The surface layer in the knee articular cartilage of rat was exfoliated, and surface of cartilage was not smooth and glossy. The overloading surface of cartilage was thinner, ruined and defected, especially outstanding in the surface of tibia. The cartilage cell was reduced, disorder alignmented and clustered. The more serious ones appeared cataplasia and necrosis (coagulate, broken and caryolysis of cell nucleolus).

Immunohistochemical allocation of dyeing in the two cytokines (ABC method)

High positive rates in the two cytokines were expressed in the surface layer of the articular cartilage with many cell corps with red color; the cartilage cell condensation was caused by the reactive hyperplasia in the majority intercellular layer with a red granules positive expression; deep layer was also found a part of cells with positive expression (***Fig 1-a***,***Fig 2-a***). Positive signals were presented as red granules in the immunohistochemical stain group, mainly located at the cellular plasm, and the cellular nucleus were presented different degrees of blue(***Fig 1-b***, ***Fig 2-b***).

Data result of average gray level between model group and control group

TGF-β and TNF-α were mainly distributed in the surface layer of the articular cartilage, with a weak expression in the negative control. In model group the positive expression intensity of TNF-α was evidently higher than that in the control group in the 1w ($P < 0.05$) and still remained an evident difference with that in control group in the 6w. TGF-β expression of the model group had no remarkable difference with the control group in the 1w and prominently became stronger in the 6w($P < 0.05$)(***Tab 1***).

Tab 1 **Result of average gray level between TGF-β and TNF-α after spinal injury** ($\bar{x} \pm s, n = 6$)

Group		average gray level		
		1 w	3 w	6 w
Model	TGF-β	186.34 ± 3.56	182.25 ± 4.73*	176.85 ± 7.68*
	TNF-α	181.13 ± 7.34*	178.02 ± 5.05*	175.56 ± 6.05**
Control	TGF-β	192.97 ± 8.66	191.40 ± 6.87	191.41 ± 8.53
	TNF-α	193.19 ± 8.64	190.78 ± 8.59	192.98 ± 8.26

Compared with control group, $^*P < 0.05$, $^{**}P < 0.01$.

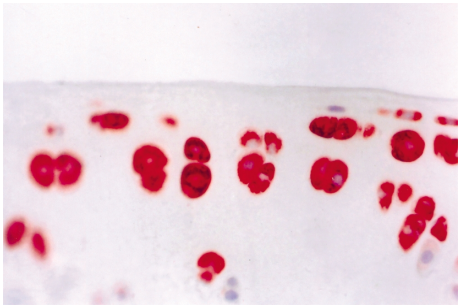

Fig 1-a Positive expressed signals in TGF-β after SCI. Positive rates were expressed in the surface layer of the articular cartilage, with cell corps in red. (ABC method,× 200)

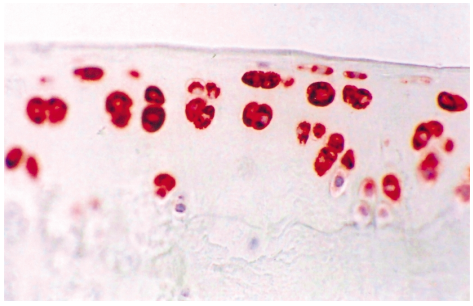

Fig 1-b Positive expressed signals in TGF-β after SCI. Positive signals were expressed as red granules, with the cellular nucleus in different degrees of blue. (ABC method,× 400)

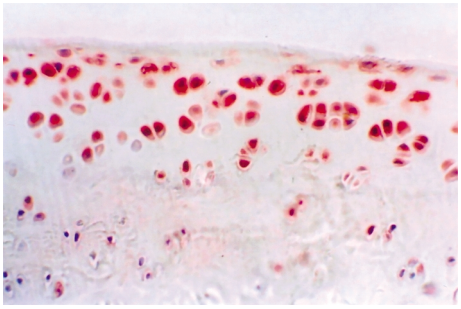

Fig 2-a Positive expressed signals in TNF-α after SCI. High positive rates were expressed in the surface layer of the articular cartilage, with more cell corps in red. (ABC method,× 200)

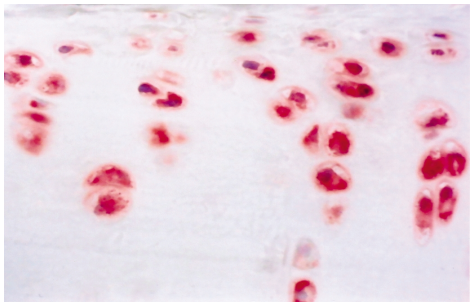

Fig 2-b Positive expressed signals in TNF-α after SCI. Positive signals were expressed as red granules, with the cellular nucleus in different degrees of blue.(ABC method,× 400)

DISCUSSION

Positive signals of transforming growth factor-beta (TGF-β) expressed as red granules mainly located at the cellular plasm. From normal rats' knee articular cartilage, positive expression was found in each layer, but positive signal of the surface layer were obviously higher than that of intercellular and deep layer, with a decrease trend progressively. Filvaroff E *et al* [4] reported that the expression of TGF-β was obvious in the surface layer, depletion layer and fairly low-grade of mature cell layer. TGF-β was participated in the cartilagineus reparative process by a series of effects on enhancing proliferate and inducing compositive activity of chondrocyte[5]. In addition, Assoian [6] showed that MES cell of cartilage osteotylus, nulli-mature and mature cartilage cell were also expressed as red granules in earlier chondrogenesis period,and it was confirmed in our study.

The result of average gray level showed that TGF-β expression of the model group had no remarkable difference with the control group in the 1w ($P > 0.05$) and progressively became stronger after the 3w ($P < 0.05$). Raab [7] reported that TGF-β has been taken part in the cartilage reparative process by a series of effects including reinforcing hyperplasy in cartilage cells and inducing synthetical cartilage cell activity. Heath *et al* [8] studied that the level of TGF-β ribonucleic acid was attained a peak value,15 days after bone fracture by detecting TGF-β ribonucleic acid with molecular hybridization. The peak value was related to activity of osteoblast in chondrosteosis. Generous augment in ribonucleic acid of TGF-β showed that some kind of autocrine growth regulatory mechanism existed in bone tissue and possibly concerned with bony remodeling processes [9]. In many investigations[10,11] the manifested level of gene expression of growth factor changed along with different periods in the union of fracture, and the effect of TGF-β also changed if cartilage has been conducted in the bone formation process. Some scholars [12] had detected a series of alteration of TGF-β in the process of fracture union by northern hybridization technique. The expression of TGF-β was detected at 6 d, emerged a hypo-valley at 10 d, but increased at 2 w post injury, and remained elevation up to 4 w.

Positive signals of TNF-α are similar to those of TGF-β, and TNF-α notability increased in the first week and entered slow-moving convalescence stage

after a week. It had a prominent increase in the 6 th week. Hayashi *et al* [13] observed in the model of spinal cord injury that the expression of immediate early genes and the ribonucleic acid of cytokine not only quickly presented but also up-regulated in acute stage after 1 hour. The study showed [14] that early-time expression of TNF-α was owed to some kind factors that were released by stimulating chondrocyte. The factors could be correlated to prostaglandin E2 and macrophage colony-stimulating factor that could stimulate cartilage resorption. TNF-α could stimulate chondrocyte to release prostaglandin E2 and macrophage colony-stimulating factor[15]. *In vitro*, those factors also inhibited cartilage matrix formation[16]. TNF-α still provoked the composition of collagenase and mediated that of degradation [17]. Streit *et al* [18] proved that a TNF-α mRNA level was obviously increased in the early stages with a peak level after being observed 24 hours post injury. Lee *et al* [19] observed that TNF-α message levels up-regulated as early as 1 hour post injury and returned to a baseline level within 3 days post injury. This coincided with the result of Bartholdi [20], who believed that TNF-α was expressed in a very narrow time window.

To sum up, the TNF-α expression increased in the first week, entered a slow-moving convalescence stage after 1 week, and had a prominent increase in the 6 th week; while the TGF-β expression lightly increased in the first week within the normal range, and maintained the increase until the 6th week. The expression of TNF-α occurred early in the developed of spinal cord injury and the expression of TGF-β became stronger with the revival of spinal neural function. Both expressions were strengthened in the articular cartilage at the third week.

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