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Full Length Research Paper

The effect of TGF-β_1 on the Bax in rats with hypoxic brain damage after surgical treatment of gliomatosis cerebri (HBD/GC)

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The effect of transforming growth factor beta 1 (TGF-β_1) on Bax in rats with hypoxic brain damage after surgical treatment of gliomatosis cerebri (HBD/GC) were investigated. The SD rats were randomly divided into three groups: Sham control group, HBD/GC model group, HBD/GC + TGF-β_1 injection treatment (E) group. The SD rats were treated in hypoxic environment after a surgical treatment of gliomatosis cerebri to establishe HBD/GC models. The expressions relative rate of Bax in CA1 region of hippocampus were detected by Reverse transcription-Polymerase chain reaction (RT-PCR) and Immunohistochemistry (ICH). After 24 h of operation, there was a significant decrease in Bax expression in E groups, while an increase in HBD/GC model group. TGF-β_1 could comparatively increase the Bax mRNA and protein expressions in the hippocampal CA1 region of HBD/GC rats. These results suggested that TGF-β_1 might develop its improvement by increasing Bax mRNA and protein expression in the CA1 region of hippocampal of HBD/GC rats.

Key words: Transforming growth factor beta 1, HBD/GC, Bax.

INTRODUCTION

Gliomatosis cerebri (GC) is a rate tumor of the central nervous system. It is characterized by diffuse neoplastic infiltration of glial cells in varying stages of differentiation, with the preservation of anatomical architecture the sparing of neurones (Zhao et al., 2008). There has been considerable progress in the initiation of clinical trials to establish treatment regimes specifically designed for GC an optimal therapeutic strategy is still not well established. (Zhang et al., 2005) and (Zheng et al., 2003) propose that surgery is an optional therapeutic tool for GC, and the degree of operational incision is an important factor affecting the prognosis. However, the hypoxic brain damage (HBD) after surgical treatment significantly reduced the therapeutic effect of the GC surgery.

Up to now, there are no effective therapeutics tools for this case. Growing studies showed that transforming growth factor beta 1 (TGF-β_1) has several pharmacological activities such as anti-inflammation, adjusting differentiation and proliferation of immunologic cells and non-immunologic cells, and enhancing epithelial recovery (Spurgeon et al., 2005). Nevertheless, the effect of TGF-β_1 in rats with hypoxic brain damage after surgical treatment of gliomatosis cerebri (HBD/GC) and its mechanism are all still unclear. Bax, as a proapoptosis gene of the Bcl-2 family, has extensive amino acid homology with Bcl-2, may contribute to cell death, which is the key regulators of apoptosis (Zhang et al., 2000). Therefore, we assumed that TGF-β_1 might have some effects on the Bax expressions in rats with HBD/GC.

In the present work, we establish HBD/GC model by putting the SD rats to hypoxic environment after a surgical treatment of gliomatosis cerebri. We also detected the expressions relative rate of Bax in CA1 region of hippocampus by Reverse transcription-polymerase chain reaction (RT-PCR) and Immunohisto-

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Table 1. Primers for the various genes investigated in this study.

Gene	Direction	Primer sequence (5'-3')	Product size (bp)
β-actin	Forward	CCTCTATGCCAACACAGTGC	211
	Reverse	GTACTCCTGCTTGCTGATCC	
Bax	Forward	AAGCTGAGCGAGTGTCTCCGGCG	284
	Reverse	GCCACAAAGATGGTCACTGTCTGCC	

chemistry (ICH) methods.

MATERIALS AND METHODS

Reagents and apparatus

TGF-β_1 was purchased from Sunshine Pharmaceutical (Shenyang, China). Trizol and Bax (rabbit polyaclonal) were obtained from Wuhan Biostor (Wuhan, China); Y-maze (type MG -3) was obtained from Zhangjiagang Biomedical Instrument Factory (Zhangjiagang, China).

Laboratory animals

Fifty-four SD rats, aged >16 months, weighing (300 ± 41) g, of either gender, were provided by the Experimental Animal Center of the Second Military Medical University of Chinese PLA between July 2009 and December 2011. The rats were housed under controlled conditions (room temperature, 22 ± 2°C).

Establishment of HBD/GC rats models

Following the rats were intraperitoneally anesthetized by chloral hydrate, a median incision was made at the cervical part. Muscle was carefully bluntly dissected to expose bilateral common carotid artery. A No.7 surgical suture was embedded under common carotid artery. Vagus nerve was avoided to be stimulated.

Grouping and drug intervention

Fifty-four Wistar rats were randomly divided into three groups: Sham-operation group (n = 18): Surgical suture embedding, then surgical suture was drawn out and incision was sutured, while without hypoxic treatment. HBD/GC model group (n = 18): rat models of HBD/GC were developed according to the previous method. TGF-β_1 treatment group (n = 18): following a cannula was buried in lateral cerebral ventricle for one week, rat models of HBD/GC were developed and injected with 20 ng of TGF-β_1 via lateral cerebral ventricle. The experimental temperature was kept at 25℃. Rats in the Sham operation group and HBD/GC group were intraperitoneally injected with the same amount of normal saline.

Reverse transcription-polymerase chain reaction (RT-PCR)

Rats were anaesthetized with 10% of urethane and then sacrificed by transcardiac perfusion with PBS followed by separate their hippocampus immediately, cryoprotected by immersion in 30% sucrose for 24 h at 4 to 8℃ and frozen in a tissue-freezing medium. The total RNA was extracted with Trizol reagent according to the manufacturer's protocol. A_{260} readings were taken on the extracted samples to quantify the amount of RNA present, and A_{260}/A_{280} ratios were calculated to determine the purity. The reverse transcription reaction was carried out with 1 mM of dNTP, 40 U/μl of Rnase innhibitor, 5 U/μl of AMV-RT and 5 U/μl of AMV- Optimized Taq in the provided reaction buffer at 50℃ for 30 min. The primers were designed and synthesized based on the published gene sequence as shown in Table 1. The PCR reaction was done in a thermal cycler (Model 7500, Beckman) with an initial denaturation step at 94℃ for 5 min, followed by a variable number of cycles of denaturation 94℃ for 45 s, annealing for 45 s, elongation 72℃ for 1 min and a final elongation step at 72℃ for 7 min. The numbers of cycles was 30 for Bax, and were defined after titration between 20 and 45 cycles and were within the logarithmic phase of amplification. The amplified products were run on a 1.5% agarose gel with ethidium bromide. Images were documented with a digital CCD camera in a BX60, and the intensity of the product bands was measured with Flous-SmutiImage Spectrum software (Bio-Rad, USA).

Immunohistochemistry (ICH)

Rats were anaesthetized with 10% of urethane and then sacrificed by transcardiac perfusion with PBS followed by separate their hippocampus immediately, cryoprotected by immersion in 30% sucrose for 24 h at 4 to 8℃ and frozen in a tissue-freezing medium. The brains were cut on a freezing microtome at the level of the anterior hippocampus, into six adjacent series of 4-μm-thick coronal sections.

The sections were dehydrated through an alcohol series. Prior to immunohistochemical processing, sections were rinsed in 2% PBS-Triton X-100 and mounted onto gelatine-coated slides. Immunohistochemistry was performed on slide-mounted sections utilizing the following antibodies: Bax (dilution 1:100). The sections were incubated overnight at room temperature with the primary antibody diluted in PBS-bovine serum albumin (BSA). After rinsing, sections were incubated for 1 h at room temperature in biotinylated goat antimouse serum (1:500), sections were incubated for 1 h in avidin–biotin–horseradish peroxidase complex (1:200). Following rinses, sections were placed for 30 min in chromagen solution consisting of 0.05% diaminobenzidine and 0.01% H_2O_2. The reaction was monitored visually and stopped by rinses of 0.1 M PBS.

In order to minimize variability, sections from all animals were stained simultaneously. Cell counts were performed blindly in all sections using a Nikon Eclipse E800 microscope. Counts were made in six randomly selected optical fields under 400× magnification by individuals who were blinded to diagnosis. Bax immunoreactivity was assessed semi-quantitatively using Image Pro Plus software Version 4.5.129 (Media Cybernetics). The percentage area covered by immunoreactivity was measured and the mean value taken.

Table 2. Expression relative rate of Bax mRNA in the hippocampal CA1 region.

Group	24 h after operation
Sham	0.17 ± 0.007
HBD/GC	0.987 ± 0.047△
E	0.583 ± .017△*

(VS Sham: △P < 0.05; VS HBD/GC: *P < 0.05).

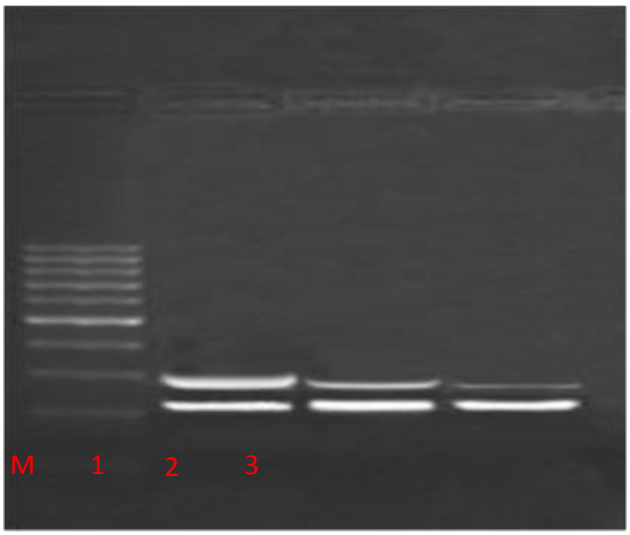

Figure 1. Comparison of the RT-PCR of Bax in different groups. (M:Mark; 1: Bax mRNA expression in CA1 region of hippocampus in HBD/GC model group at postoperative 24 h; 2: Bax mRNA expression in CA1 region of hippocampus in TGF-β_1 treatment group at postoperative 24 h; 3: Bax mRNA expression in CA1 region of hippocampus in Sham operation group at postoperative 24 h).

Table 3. The IOD of Bax immunoreactivity in the hippocampal CA1 region.

Group	24 h after operation
Sham	9.17 ± 1.73
HBD/GC	28.33 ± 5.17△
E	16.67 ± 3.47△*

(VS Sham: △P < 0.05; VS HBD/GC: *P < 0.05).

Statistical analysis

The database was set up with the SPSS 16.0 software package for analysis. Data were represented as mean ± S.D. The means of multiple groups were compared with one-way ANOVA, after the equal check of variance, and the two-two comparisons among the means were performed by Student's t-test. P < 0.05 was considered as statistically significant.

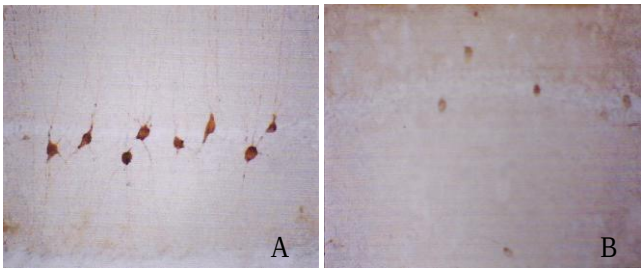

Figure 2. Bax positive cells of CA1 region of hippocampus in different groups (×400). (Figure 2A: Bax immunoreactivity in CA1 region of hippocampus in HBD/GC model group at postoperative 24 h; Figure 2B: Bax immunoreactivity in CA1 region of hippocampus in TGF-β_1 treatment group at postoperative 24 h).

RESULTS

Effects of TGF-β_1 on the expression of Bax mRNA in CA1 region of hippocampal of rats

After 24 h of administration, Bax mRNA in the HBD/GC group and TGF-β_1 treatment group were significantly more than those in the Sham-operation group (P < 0.05), while in TGF-β_1 treatment group was significantly less than those in the HBD/GC group (P < 0.05) (Table 2, Figure 1). These results suggested that TGF-β_1 could significantly down-regulating the decreased expressions of Bax mRNA of HBD/GC rats.

Effects of TGF-β_1 on the expressions of Bax protein in CA1 region of hippocampal of rats

After 24 h administration, Bax protein in the HBD/GC group and TGF-β_1 treatment group were significantly much more than those in the sham-operation group (P < 0.05), while in TGF-β_1 treatment group was significantly less than those in the HBD/GC group (P < 0.05) (Table 3, Figure 2). These results suggested that TGF-β_1 could significantly down-regulating the decreased expressions of Bax protein of HBD/GC rats.

DISCUSSION AND CONCLUSION

TGF-β_1 is a family of related proteins that regulate many cellular processes including growth, differentiation, extracellular matrix formation, and immunosuppression (Katabami et al., 2005 and TenDijke et al., 2002). Every cell in the body produces TGF-β and has receptors for it. TGF-β_1 is one of the isoforms (TGF-β1-5), and arrests the cell cycle in the G1 phase, thereby inhibiting cell proliferation and triggering apoptosis (Damdinsuren et al., 2006). Growing studies showed that TGF-β_1 plays important roles in growth, differentiation and repair of neuron cells. When neuron cells were treated with hypoxia, the expression of TGF-β_1 significantly increased,

indicating that TGF-β_1 could repair the injury induced by hypoxia (Lesen et al., 2002; Magy et al., 2002). Meanwhile, HenrichNoack et al. (1996) also found that TGF-β_1 could inhibit the apoptosis of neuron cell damage induced by hypoxia, however, its real mechanism are still unclear.

In our present work, we establish HBD/GC model by putting the SD rats to hypoxic environment after a surgical treatment of GC. We then detected the expressions relative rate of Bax in CA1 region of hippocampus by RT-PCR and ICH methods. These results suggested that TGF-β_1 could down-regulating the decreased expressions of Bax mRNA and protein of HBD/GC rats. However, the precise mechanism for the down-regulation of TGF-β_1 requires further investigation.

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