Study on the Expression Difference of IL-1β in Rabbits after Free Bone Flap Suspension

Miao Cheng[1], Xuejiao Li[2], Xin Chen[2], Lianrui Li[2], Zhiqiang Wang[1*]

[1] First Division Alar Hospital, Xinjiang Production and Construction Corps, Alar 843300, Xinjiang, China

[2] College of Animal Science and Technology, Tarim University, Alar 843300, Xinjiang, China

Abstract: Objective: The aim is to investigate the effect of free bone flap suspension technique on postoperative IL-1β expression in rabbits and to evaluate its regulatory effect on postoperative inflammatory response. Methods: Thirty New Zealand white rabbits were selected and divided into the suspension group, the non-suspension group and the control group. The levels of IL-1β in peripheral blood, CRP and immunohistochemical AOD were detected on the 1st, 3rd and 7th days after surgery, respectively. Results: On the first day after surgery, the concentration of IL-1β in the suspension group was 2.86±0.40 ng/L, significantly lower than 4.92±0.55 ng/L in the non-suspension group (P<0.001); On the 7th day, the suspension group decreased to 1.45±0.29 ng/L, approaching the control group. Immunohistochemical AOD values showed 0.34±0.06 in the suspension group, significantly lower than 0.58±0.05 in the non-suspension group (P<0.001). White blood cell and CRP levels recovered more quickly in the suspension group after surgery. Conclusion: Free bone flap suspension can effectively reduce postoperative IL-1β expression and inflammatory response, and has good biological and clinical application prospects.

***Keywords*:** free bone flap, IL-1β, inflammatory response, rabbit model

1. Introduction

Craniocerebral injury is a common acute and critical condition in neurosurgery. Postoperative complications include epidural hematoma, intracranial effusion, etc., which greatly affect the prognosis of patients. Although traditional craniotomy can effectively relieve intracranial pressure, the potential space formed between the bone flap and the dura mater after surgery can easily induce inflammatory responses, delay the repair of the surgical area, and increase the occurrence of complications. As an improved surgical approach, the dural suspension technique optimizes the attachment state of the bone flap by intervening in the spatial structure, which is expected to enhance the physiological stability after surgery. In recent years, IL-1β (interleukin-1 β), as a classic pro-inflammatory cytokine, has been highly correlated with the intensity of local inflammation after surgical trauma, making it an important objective indicator for assessing the degree of tissue damage in neurosurgery, predicting postoperative brain edema and neurological function recovery. Based on the animal experimental model, this paper evaluated the effect of free bone flap suspension in regulating postoperative inflammation by dynamically monitoring the differences in IL-1β expression in rabbits of each treatment group after craniotomy and combining it with immunohistochemical and blood routine indicators, with the aim of confirming the possible value of this technique in reducing postoperative inflammatory stress and improving tissue repair, And to provide theoretical basis and technical support for the standardization and improvement of neurosurgery in the future.

2. Data and methods

2.1 Specimens

The study selected 30 healthy adult New Zealand white rabbits, regardless of gender and weighing 2.5 to 4.1 kg, from the same regular laboratory animal supply base with the same living background and health status. The animals were randomly divided into three groups: Group A (free bone flap suspension group), Group B (non-suspension craniotomy group), and Group C (control group), with 10 animals in each group. Group A used the technique of suspending the dura dura with a free bone flap, while group B only underwent standard craniotomy, and group C did not receive any form of treatment as a basic reference. The tests were carried out under aseptic conditions and all animals were monitored for body temperature, food intake, activity status and consciousness[1] after the operation. Blood samples were collected on the 1st, 3rd, 7th, and 14th days after the operation for routine blood tests, biochemical tests, and ELISA tests. The animals were sacrificed on the 7th day after the operation, and brain tissue was taken for HE staining and immunohistochemical analysis. The entire study process was in line with animal experiment ethics and followed the 3R principle to reduce unnecessary animal sacrifice[2].

2.2 Methods and reagents

A model of traumatic brain injury was established using standard craniotomy in the trial. Fixed on the operating table after intravenous anesthesia before the operation, a osteogenic window was drilled at the top of the head, and the dura mater was illuminated with a microscope. Rabbits in Group B only underwent traditional surgery to restore the bone flap, while rabbits in group A chose free bone flap suspension after the dura mater was sutured: four holes were drilled in the bone flap, then the thread pre-sutured in the dura mater was threaded out of the bone eye, and a knot was tied when the bone flap was repositioned to ensure a tight fit between the bone flap and the dura mater and minimize[3] the potential cavity. Peripheral blood was taken at the set time points after the surgery, and changes in the concentrations of IL-1β,TNF-α, and IL-10 were determined by ELISA. The reagents used included: imported IL-1β, TNF-α, IL-10 standard kits (R&D Systems), tissue staining using HE staining solution and immunohistochemical kits (ZSBG-Bio), all reagents were operated according to the instructions and quality verification was completed. The entire test process was carried out on the molecular test platform of Tarim University.

2.3 Determination of immunohistochemical results

Paraffin-embedded sections of animal brain tissue were taken within 7 days after surgery, and HE staining and immunohistochemical staining were performed. Immunohistochemical analysis was performed using the SABC method to detect IL-1β expression, and positive cells were brownish[4] yellow after DAB staining. The sections were evaluated using the blind method of two senior pathologists who did not know the experimental grouping, and the five-field average method. Staining intensity (0 colorless, 1 pale yellow, 2 yellow, 3 brownish-yellow) and percentage of positive cells (0-100% on a 0-3 scale) were evaluated on each section, and the sum of the two scores was the final score. A score of more than 2 indicates positive expression. With the help of ImageJ image analysis software, values of average optical density (AOD) were measured to assess protein expression intensity[5]. After the data recording was completed, it was reviewed uniformly by the principal experimenters to ensure the accuracy and repeatability of the interpretation.

2.4 Statistical processing

All experimental data were entered into Microsoft Excel sheets and then imported into SPSS 26.0 statistical analysis software for processing. When dealing with continuous variables, normality tests were first conducted. For data that conformed to a normal distribution, Mean± standard deviation (Mean±SD) was used for comparisons between groups, and One-way ANOVA was used for comparisons of three or more groups. At different time points after the surgery, Repeated Measures ANOVA were used to observe the dynamic changes. Rank sum tests were performed on non-normal distribution or heteroscedasticity data. The significance level between groups was set as $P<0.05$, and significant results were further analyzed pairwise using LSD or Bonferroni. Chart production using the GraphPad Prism 8 software package to create bar charts and line charts for data visualization.

3. Results

3.1 Differences in IL-1β expression among different treatment groups

To clarify the regulatory effect of free bone flap suspension on postoperative inflammatory response, ELISA was used in this study to dynamically detect the expression levels of IL-1β in peripheral blood of rabbits in the three groups before surgery and on the 1st, 3rd, and 7th days after surgery. IL-1β, as a typical pro-inflammatory factor, can directly reflect tissue stress and repair. Group A received free bone flap suspension, group B received traditional craniotomy without suspension, and Group C was the control group that did not receive surgery. By analyzing the expression trends at different time points and the differences between groups, the intervention ability of the procedure on inflammatory factor levels was evaluated to provide experimental evidence for the optimization of postoperative rehabilitation pathways. The relevant data are shown in Table 1.

Table 1. Expression levels of IL-1β in rabbits at different time points (ng/L, x youdaoplaceholder3 ± s)

Time points	Group A (Suspension Group)	Group B (unsuspended group)	Group C (control group)	F value	P value
Preoperative	1.22 ± 0.35	1.25 ± 0.33	1.20 ± 0.30	0.15	0.865
Day 1	2.86 ± 0.40	4.92 ± 0.55	1.35 ± 0.28	75.62	<0.001
Day 3	2.01 ± 0.37	4.18 ± 0.48	1.28 ± 0.25	88.53	<0.001
Day 7	1.45 ± 0.29	3.52 ± 0.44	1.19 ± 0.31	102.77	<0.001

From the above table, it can be observed that the baseline levels of IL-1β in the three groups of rabbits before surgery were roughly the same (P=0.865), and there was no statistically significant difference, so they were well comparable. One day after the operation, IL-1β content in group B increased significantly to 4.92±0.55 ng·L-1, which was significantly higher than that before the operation, suggesting that the traditional craniotomy caused a severe inflammatory response; IL-1β in group A rose to 2.86±0.40 ng/L, a significantly lower increase than that in group B (P<0.001), suggesting that free bone flap suspension could effectively alleviate inflammatory activation. On day 3, although the value in Group B slightly decreased to 4.18±0.48 ng/L, it remained at A relatively high level, while in group A it rapidly dropped to 2.01±0.37 ng/L, with the inflammatory response significantly controlled; By day 7, IL-1β levels in Group A had largely returned to 1.45±0.29 ng/L, which was quite close to 1.19±0.31 ng/L in the control group, but those in group B remained within a relatively high range of 3.52±0.44 ng/L. F values were above 75 at all time points after the operation and P<0.001, indicating a significant difference between the two groups. This indicates that free bone flap suspension is more conducive to controlling IL-1β expression, significantly reducing inflammatory responses and promoting postoperative recovery, showing a good biological intervention effect.

3.2 Differences in immunohistochemical staining among the groups

By immunohistochemical detection of IL-1β expression in brain tissue, it was found that the number of staining positive cells in group A was smaller, the staining intensity was relatively weaker, and the average optical density (AOD value) was significantly lower than that in group B, suggesting a relatively mild inflammatory response; However, there was more inflammatory cell infiltration around the cortex in group B, and the positive expression of IL-1β was significantly enhanced. Group C had less positive expression and a more complete structure, making it a negative control.

Table 2. Immunohistochemical positive scores and AOD values of IL-1β in brain tissue of rabbits in each group (x youdaoplaceholder5 ± s)

Grouping	Positive cell score (0 to 6 points)	Percentage of positive cells (%)	Staining intensity score	AOD value	P value (compared with Group A)
Group A	2.25 ± 0.42	18.3 ± 3.7	1.8 ± 0.3	0.34 ± 0.06	-
Group B	4.80 ± 0.50	41.7 ± 4.5	2.7 ± 0.2	0.58 ± 0.05	<0.001
Group C	0.75 ± 0.22	6.2 ± 2.1	1.1 ± 0.1	0.16 ± 0.04	<0.001

Note: The scoring criteria are the cell positive rate plus staining intensity, and the sum of the two is the total score; The average optical density was analyzed using ImageJ software.

Analysis of immunohistochemical staining results in Table 2 shows that IL-1β expression in the brain tissue of the three groups of rabbits was significantly different at 7 days after surgery. The positive cell score in group A (free bone flap suspension group) was 2.25±0.42, the percentage of positive cells was 18.3±3.7%, and the AOD value was 0.34±0.06, indicating that the expression of IL-1β in brain tissue was moderate and the inflammatory response was relatively mild; In Group B (non-suspension group), all indicators showed significant improvement. The positive cell score reached 4.80±0.50, the positive percentage was 41.7±4.5%, and the AOD value was as high as 0.58±0.05, suggesting obvious local inflammatory activation and more IL-1β expression. There was extensive infiltration of inflammatory cells and tissue damage. The positive score of group C (control group) was only 0.75±0.22 points, and the AOD value was 0.16±0.04 points, both significantly lower than those of group A and group B (P<0.001), indicating that it was the basic state among the negative controls. By statistical test, it was found that the difference in AOD values between group A and group B was extremely significant (P<0.001), indicating that free bone flap suspension could effectively inhibit the overexpression of IL-1β in brain tissue after surgery, The relief of local inflammatory response and improvement of the tissue repair microenvironment further confirmed the biological superiority of the procedure at the pathological morphological level.

3.3 Changes in inflammatory markers such as white blood cells and CRP

Dynamic tests of white blood cell count, lymphocyte count, and CRP concentration on days 1, 3, and 7 after surgery indicated that in group B, there was a higher level of systemic inflammatory response in the early postoperative period, with significantly elevated white blood cells and CRP, and the duration was longer; In group A, the inflammatory response decreased significantly on the third day and basically returned to the normal range on the seventh day. All indicators in Group C remained stable with no significant fluctuations. Statistical analysis showed that the white blood cell count and CRP in group A were significantly better than those in group B on the 3rd and 7th days after surgery (P<0.05), indicating that the free bone flap suspension technique helped to accelerate the control of postoperative inflammation.

Table 3. Changes in postoperative inflammatory indicators of rabbits in each group (x youdaoplaceholder5 ± s)

Time points	Grouping	White blood cell count (×10⁹/L)	Lymphocyte count (×10⁹/L)	CRP concentration (mg/L)	P value (white blood cells)	P value (CRP)
Day 1	Group A	7.25 ± 0.78	2.20 ± 0.35	12.3 ± 1.8	-	-
	Group B	9.75 ± 1.05	1.80 ± 0.28	21.7 ± 2.5	<0.001	<0.001
	Group C	5.35 ± 0.42	3.10 ± 0.31	5.6 ± 0.9	<0.001	<0.001
Day 3	Group A	6.05 ± 0.63	2.65 ± 0.38	9.1 ± 1.2	-	-
	Group B	8.85 ± 0.82	1.95 ± 0.33	18.4 ± 2.0	<0.001	<0.001
Day 7	Group A	5.45 ± 0.50	3.00 ± 0.45	6.5 ± 1.1	-	-
	Group B	7.90 ± 0.69	2.25 ± 0.37	14.2 ± 1.7	<0.001	<0.001

Note: CRP is a C-reactive protein that reflects the level of inflammation in the system; P values were based on ANOVA analysis and LSD pairwise comparisons.

4. Discussion

4.1 Intervention mechanism of free bone flap suspension on inflammatory response

The free bone flap suspension technique physically improves the cranial cavity structure after surgery, effectively reducing the continuous mechanical stimulation and exudation response induced by the potential space between the bone flap and the dura mater after restoration. In traditional craniotomy, the bone flap is simply repositioned without achieving a tight fit between the dura mater and the bone surface, which can easily result in "dead space", providing a breeding ground for postoperative bleeding, effusion and retention of local inflammatory factors. The suspension technique fixes the bone flap at the central position of the sutured dura mater, significantly reducing the tissue tension in the surgical area and the chance of exudate accumulation, which is conducive to the reconstruction of dura mater blood supply and tissue healing. The free bone flap suspension technique can reshape the anatomical relationship of the cranial cavity immediately after surgery, eliminating the potential dead space between the dura mater and the bone surface caused by the traditional craniotomy of "simple bone flap reduction", thereby blocking the resulting continuous mechanical stimulation and exudation cascade reaction. By performing tension suturing in the center of the dura mater and fixing the bone flap, the dura mater is closely attached to the skull, significantly reducing the tension in the surgical area, minimizing bleeding, effusion and retention of local pro-inflammatory factors, and creating an ideal microenvironment for dura mater revascularization and tissue healing.

4.2 Analysis of the relationship between IL-1β expression and inflammatory response

IL-1β, a key preinflammatory factor produced by monocytes and macrophages, plays a broad role in tissue damage after surgery, cytokine amplification responses, and changes in blood-brain barrier permeability. Its dynamic changes have been recognized as a sensitive indicator for evaluating the degree of inflammation in brain tissue after surgery. ELISA and immunohistochemical techniques were used in this study to dynamically monitor the expression of IL-1β in the serum and brain tissue of rabbits after surgery. The results showed that IL-1β expression in the non-suspension group continued to rise from day 1 to day 7 after surgery, suggesting a very strong inflammatory response and a relatively slow recovery process; But the expression in the free bone flap suspension group began to decrease 3 days after surgery, and at 7 days it was similar to that of the control group, which was statistically significant.

4.3 Clinical significance and prospects of free bone flap suspension technique in neurosurgery

Free bone flap suspension is one of the structural remodeling procedures, which not only shows obvious advantages in inflammatory intervention, but also has a significant effect on the control of postoperative complications. A large number of literature and clinical data show that the incidence of epidural hematoma and intracranial effusion in patients after conventional craniotomy remains high, which directly affects postoperative recovery and prognosis. The suspension technique addresses "periosteal dead space" and significantly reduces the risk of postoperative bleeding and brain tissue space-occupying. The experimental model confirmed that the procedure could effectively reduce the levels of inflammatory factors and tissue damage in animals, suggesting that the procedure has significant clinical translational value. More importantly, this method is simple to operate, easy to obtain materials, does not add a significant economic burden, and has a good foundation for promotion.

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Author Bio

Corresponding author: Zhiqiang Wang (born 15 October 1970), male, Han, Lishui, Zhejiang Province; Chief Neurosurgeon; Master's degree. Research interests: Clinical management and surgical optimization of traumatic brain injury, comprehensive diagnosis and treatment of cerebrovascular disease, and development of hospital management systems in frontier regions.