行政院國家科學委員會專題研究計劃成果報告

腦部 C-Fos 蛋白質在敗血症之整合型研究

Studies of c-fos Protein in Brain by Sepsis

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一、中文摘要

敗血症(Sepsis)目前雖然有很進步的藥物及儀器之診斷，但敗血症的死亡率仍高居 30-60%,目前有關敗血症之研究絕少提到敗血症與中樞神經(CNS)間的關係。但腦是神經、內分泌、免疫等相關部份的中樞，當受到侵襲下對維持身體平衡性恆常性的腦活動將會產生很大的變化。例如隨著敗血症而引起的敗血性腦症，被認為引起恆常性的制御不全，對身體帶來危機的狀態。目前有關敗血症的中樞神經研究了解非常稀少，因此為了觀察了解敗血症之中樞神經病變關聯性，本研究，使用老鼠(Wistar)做上腸間動脈閉塞症模型(occulsion of superior mensenteric artery)，一組為 sham，一組為上腸間動脈閉塞組控制組，一組為使用 corticosteroid 治療組之三組實驗組。

研究因敗血症而引起之腦病變之變化，用 immunochemistry 測量 c-fos protein 活性在腦部分布情形，測量血中之內毒素(endotoxin) 及細胞介質(interleukin)(IL-I, IL-6, IL-8)的變化相互之間的關係相聯性。期望此研究能對神經學與敗血症能夠明確了解且能對治療有助益，貢獻於臨床醫學，希望能劃開創造新的研究領域。

關鍵詞：.

①c-fos　②內毒素　③細胞激素

④敗血症　⑤上腸間動脈閉塞症

英文摘要

ABSTRACT

Objective: to determine the c-fos protein in brain , endotoxin levels , cytokine, in following occulsion of superior mesenteric artery of rat.

Design: prospective , randomized controlled.

Setting : surgical research department of the China Medical College .

Subject: 150 male wistar rats.

Interventions : used occulsion of superior mesennteric artery to obtain a model of septic shock in rats in vivo study the corticosteroid is applied in the treatment group.

Measurements :

Endotoxin measured the blood sample is treated by new perchloric acid method endotoxin concentration were determined by an endotoxin-specific-test . IL-8, IL-6, Il-I and TNF conncentration were determined by enzyme-linked immunosorbant assay(ELISA) . c-fos protein is studied by immunohistochemistry .Data is analyzed to identify and relationship of c-fos , cytokine and endotoxin

Conclusion

c-FOS immunoreactivity was observed in the limbic system in addition to that mentioned above in rats with SMA occlusion. This indicates that limbic function such as control of emotion and behavior is stimulated in endotoxemia and sepsis following SMA occlusion ,and suggests that these changes are linked to the etiology of mental abnormalities in sepsis.

Keywords:

①c-fos ②Endotoxin ③ Sepsis
④Superior meseteric artery occulsion
⑤Interleukin-1, Interleukin-6,Interleukin-8

二、緣由及目的

Introduction

In humans, the occlusion of the superior mesenteric artery (SMA occlusion) is a progressive and lethal disease accompanied initially by hypovolemic shock and acute renal failure, subsequently by endotoxemia and sepsis, and finally by multiple organ failure[1]. In serious cases of sepsis, neurologic dysfunctions including mental status abnormailities such as alteration of consciousness, seizures and convulsion are often seen [2,7,14]. Therefor, there should be some functional alterations and damages in the central nervous system, about which little is yet known. The present study aimed to clarify how the central nervous system is affected under sepsis following SMA occlusion in a rat model. We employed c-FOS expression as a marker of brain activation, and possible changes in c-FOS expression in brain were assessed in relation to levels of plasma endotoxin, which is thought to be an important factor in the production of various symptoms in sepsis and multiple organ failure.

Materials and Methods

c-FOS Immunohistochemistry

SMA Occlusion and Tissue Preparations. 150 male Wistar rates weighing 200-250 g were used. Under general anesthesia with sodium pentobarbital (50mg/kg), clipping of the SMA was performed in 24 rates. 50 animals had laparotomy and abdominal closure as a sham operation. Two, 4, 6 and 8 h after surgery, 50 animals at each time were perfused under deep anesthesia through the left ventricle with 80 ml 0.01 *M* phosphate-buffered saline (pH 7.4) and then with 300 ml of a fixative containing 4% paraformaldehyde, 0.35% glutaraldehyde and 0.2% picric acid in 0.1*M* phosphate buffer(pH 7.4). The coronal blocks of the brain were postfixed for 2 days in a fixative containing 4% paraformaldehyde and 2% picric acid in 0.1*M* phosphate buffer at 4C and placed for at least 48 h in 0.1*M* phosphate buffer (pH 7.4) containing 15% sucrose and 0.1% sodium azide at 4C.

c-FOS immunostaining. The sections were stored in free floating state for 4 days in 0.1*M* phosphate-buffered saline(pH 7.4) containing 0.3% Triton X-100(PBST). Serial sections at 200-μm intervals were processed for avidin-biotin-peroxidase complex immunohistochemistry. The sections were incubated for 4 days with c-FOS antibody(Cambridge Research Biochemical, Cambridge, UK, Diluted 1:10000) at 4C, for 2 h at room temperature with biotinylated anti-sheep IgG(Vector Lab, USA, diluted 1:1000). The peroxidase activity was demonstrated by incubating sections with 0.02% 3,3'-diaminobenzidine, 0.05%H_2O_2 and 0.3% nickel ammonium sulfate.

Measurement of the Plasma Endotoxin Level

In 50 male Wistar rates weighing 200-250g SMA clipping was performed under general anesthesia. The blood was drawn before the operation and 2,4,6, and 8 h after SMA clipping. Plasma endotoxin was measured with the chomogenic limulus method after preparation by the new PCA method.

三、RESULTS AND CONCLUSIONS

Macroscopic Findings

The ischemic change was observed in the rat intestine 2 h after SMA clipping.

After8 h, the intestines were dilated with a change to dark color, and infiltration of dark-red-color and badly-smelling ascites. In summary, intestinal necrosis, panperitonitis, a massive sympathetic response and peripheral circulatory disturbance were observed in rats 8 h after SMA clipping.

c-FOS Immunohistochemistry

In sham-operated rats, weak c-FOS immunoreactivity was detected in neuronal nuclei. Positive nuclei were scattered in the Pa, So and LHb(see legend to Fig. for key to abbreviations). c-FOS immunoreactivity was significantly increased in neuronal nuclei of certain regions 2, 4, 6, and 8 h after SMA clipping. Immuno-reactivity was detected in the cell nuclear region. Positive neurons were recognized as being scattered in rat nuclei after 2 and 4 h, after 6 h they were abundant, and the most predominant after 8 h. The neuronal cell bodies containing immunoreactivity were localized in specific areas of the brain, including the SFO, Pa, So, PVA, Pe, LHb, VMH, Ce, LC, NTS, and X from rostrally (Fig.1). The most predominant immunoreactivity was observed in the Pa, So, and LHb. However, no immunoreactivity was detected in the neocortex, hippocampus and striatum.

Plasma Endotoxin Level

The mean endotoxin level in plasma 0, 2, 4, 6, and 8 h after treatment were 6.48±5.57, 15.0±4.37, 10.0±4.18, 14.4±3.52 and 58.4±28.6 pg/ml respectively. Endotoxin levels were low and stable after 2, 4,and 6 h, but with a drastic change to an increase after 8 h (Fig 2, 3)

四、**DISCUSSION**

The present study demonstrated that c-FOS expression was increased in specific nuclei of rat brain in SMA occlusion of rat, and that the initial expression precedes the elevation of plasma endotoxin levels. This indicates that specific neuronal function of the central nervous system may be activated as a pathophysiologic response to occlusion of SMA in a phase earlier than the endotoxemia stage.

c-FOS is one of the immediate early genes whose expression is low or undetectable in quiescent cells but is activated transiently by extracellular stimulation[10]. Therefore ,these genes have been observed to be a marker of the activated neuron [5,9]. According to previous reports, stimulation of c-FOS expression in the central nervous system can be classified into three types: (1)growthe factors[9], electrical stimulation[11]and seizures[8];(2)brain injury such as ischemia[6,13],brain destruction[3]and amputation in the neuronal tract[12];and (3)nociceptive stress including peritoneal stimulation on injection of hyperosmolar NaCl, restriction and nociception of skin[20].

Firstly, brain ischemia during hypovolemia is the most likely cause of the increment of c-FOS expression in the present experiment. However, Jorgensen reported that c-FOS expression after brain ischemia was mainly detected in the hippocampus (CA, dentate gyrus),and Taniguchi also reported c-FOS expression in the hippocampus of rat brain with hypoxia. In our experiment, there was no c-FOS expression in the hyppocampus . therefore ,c-FOS expression in SMA occlusion cannot be explained by

brain ischemia or hypoxia. Secondly, peritoneal stimulation and nociceptive stimulation is the next explanation for the present c-FOS expression. Ceccatelli reported that c-FOS expression was observed in Pa of the hypothalamus, locus caeruleus, ventral nucleus of the medulla oblongata and NTS associated with the nociceptive stimulation. Although these areas are included in our c-FOS expression sites, c-FOS was more widely expressed in our experiment than in their report. This difference in c-FOS expression may account for the difference in the pathophysiologic response to peritoneal nociceptive activation and SMA occlusion.

To clarify how the central nervous system is involved in endotoxemia and sepsis, it is worthwhile to analyze the c-FOS expression sites physioanatomically. The expression sites in this study are categorized as follows: (1) brain stem; NTS, X, LC;(2)hypo-thalamus: Pa, So,Pe ,VMH;(3) circumventricular organs: SFO; and (4)limbic system: Ce, LHb and PV thalamic nucleus. The NTS and X are involved in autonomic regulation of the cardiovascular, respiratory and gastrointestinal function. Pa and So in the hypothalamus have a neuroendo-crine function including the so called hypothalamo hypophyseal tract, which is involved in the HPA(Hypothalamus-pituitary gland-adrenal cortex)axis. For example,through this axis antidiuretic hormone, synthesized in Pa and So,is secreted into the general circulation.

The stimulation of VMH produces an excitation of the sympathetic nervous system through so called HSA (hypothalamus-sympathetic nervous system-adrenal medulla) axis. The excitation of the HSA axis causes hypersecretion of glucagon and suppression of insulin secretion in the pancreas to induce gluconeogenesis, deamination and an increase in DNA synthesis in the liver(Fig.3).Circum-ventricular organs have fenestrated capillaries and because of their permeability they are said to be out side the blood-brain barrier. The SFO has a receptor for angiotensin II which induces antidiuretic hormone (ADH) secretion from the hypothalamus to increases body water. These physiological theories regarding c-FOS expression sites in the treated rat brain provide a good explanation for the pathophysiological response pbserved in patients with SMA occlusion.

五、計劃成果自評

1. 建立研究敗血症之腦性病變和其他如出血性，中風等等在腦部之 c-fos protein 之表現是否有關聯性，或有所差異。
2. 比較 cytokine、c-fos protein 及 endotoxin 之關聯性及差異性。
3. 解明 c-fos protein, Apotosis 及 sepsis 之病理及生理之病變機轉。
4. 解明 corticoidsteroid 治療組與 c-fos protein 是否有關聯性。

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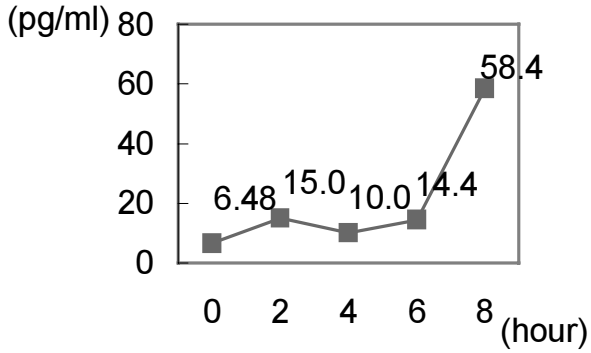

Fig. 3. The plasma endotoxin levels were low and stable after 2, 4, 6 but drastically changed to an increase after 6 h