行政院國家科學委員會專題研究計畫 期中進度報告

系統參與腦幹死亡之機制研究

計畫類別：個別型計畫
計畫編號：
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中華民國 年 月 日
A. Introduction

Previous studies from our laboratory revealed that reduction in the “life-and-death” signal during the progression towards death is associated with the progressive augmentation in both molecular synthesis and functional expression of the inducible nitric oxide synthase (iNOS) in the rostral ventrolateral medulla (RVLM), whose neuronal activity is intimately related to “life-and-death”. We further showed that NF-κB in the RVLM transcriptionally regulates this surge in iNOS gene expression, and activation of the “life-and-death” signal depends on stimulation of glutamate receptors on RVLM neurons. Similar to most proteins in the cytosol and nucleus of eukaryotic cells, iNOS, NF-κB and glutamate receptor expression are regulated by the ubiquitin-proteasome system, in a process that is energy-dependent. It follows that the ubiquitin-proteasome system may participate in brain stem death by regulating these chemical entities in the RVLM.

B. Guiding hypothesis

The proposed three-year study is an attempt to delineate the role of the ubiquitin-proteasome system in brain stem death. Based on an experimental endotoxemia model of brain stem death and employing a multidisciplinary study using the RVLM in Sprague-Dawley rats as the neural substrate, the guiding hypothesis for the proposed three-year study is that the ubiquitin-proteasome system participates actively in fatal endotoxemia by regulating expression of iNOS, NF-κB or glutamate receptor or apoptosis in RVLM.

C. Results obtained during the first 9 months of Project Year One

a. Members of the ubiquitin-proteasome system are present in the proteomic map of the RVLM

Based on 2-dimensional electrophoresis, and protein mass fingerprinting using tryptic peptide spectrum generated by MALDI-TOF mass spectrometry and complementary search results in conjunction with MS-Fit and MASCOT programs,
we have identified 16 protein spots that are associated with the ubiquitin-proteasome system or ubiquitin carboxyl-terminal hydrolase L-1 (UCH-L1) in the proteomic map of the RVLM (Fig. 1).

**Fig. 1.** Two-dimensional electrophoresis gel showing the location of components of the ubiquitin-proteasome system in the proteomic map of the RVLM. Spot 1, ubiquitin; Spot 2, ubiquitin-conjugating enzyme E2 variant 1; Spot 3, ubiquitin-conjugating enzyme E2N; Spot 4, ubiquitin-conjugating enzyme E2N; Spot 5, proteasome subunit alpha type 1; Spot 6, proteasome subunit alpha type 3; Spot 7, proteasome subunit alpha type 5; Spot 8, proteasome subunit beta type 3; Spot 9, proteasome subunit beta type 7 precursor; Spot 10, 26S protease regulatory subunit 7; Spot 11, 26S protease regulatory subunit 8; Spot 12, 26S proteasome non-ATPase regulatory subunit 9; Spot 13, UCH-L1; Spot 14, UCH-L1; Spot 15, UCH-L1; Spot 16, UCH-L3.
b. UCH-L1 and ubiquitin expression in the RVLM is down-regulated during experimental endotoxemia

Western blot analysis (Fig. 2) revealed that the protein expression level of UCH-L1 in the RVLM exhibited a progressive reduction over the three phases of experimental endotoxemia induced by *Escherichia coli* lipopolysaccharide (LPS; 15 mg/kg, i.v.). Whereas the free form of ubiquitin (monoubiquitin) level remained constant during Phase I, there was also a discernible decrease during Phases II and III.

![Western blot analysis](image)

**Fig. 2.** UCH-L1 or monoubiquitin expression in the RVLM under basal condition (B) or during the three phases (LI, LII, LIII) of experimental endotoxemia.

c. Polyubiquitination in the RVLM is reduced during experimental endotoxemia

The reduced amount of monoubiquitin and UCH-L1 implies that the process of protein degradation by the ubiquitin-proteasome system will be slowed down. Indeed, we found a progressive reduction of ubiquitinated products, as reflected by the decreased amount of polyubiquitin, alongside monoubiquitin, in the RVLM over the course of experimental endotoxemia (Fig. 3).

d. The ubiquitin-proteasome system in the RVLM is essential to survival during experimental endotoxemia

Pretreatment with microinjection of inhibitor of ubiquitin (ubiquitin aldehyde) or proteasome (lactacystin) bilaterally into the RVLM significantly augmented fatality induced by LPS (Table 1). Thus, a functional ubiquitin-proteasome system in the
RVLM is essential to survival during experimental endotoxemia.

**Fig. 3.** Polyubiquitin or monoubiquitin expression in the RVLM under basal condition (Lane 1) or during the three phases (Lanes 5-7) of experimental endotoxemia and corresponding period intervals after administration of saline (Lanes 2-4).
Table 1. Mortality rate within 240 min after administration of LPS (15 mg/kg, i.v.) in rats that received pretreatment of aCSF, ubiquitin aldehyde or lactacystin.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Died</th>
<th>Survived</th>
<th>Mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCSF + LPS</td>
<td>11</td>
<td>1</td>
<td>10</td>
<td>9.1%</td>
</tr>
<tr>
<td>Ubiquitin aldehyde (5 pmol) + LPS</td>
<td>2</td>
<td>2*</td>
<td>0</td>
<td>100.0%*</td>
</tr>
<tr>
<td>Ubiquitin aldehyde (5 fmol) + LPS</td>
<td>9</td>
<td>7*</td>
<td>2</td>
<td>77.7%*</td>
</tr>
<tr>
<td>Lactacystin (500 pmol) + LPS</td>
<td>10</td>
<td>5*</td>
<td>5</td>
<td>50.0%*</td>
</tr>
</tbody>
</table>

*P < 0.05 versus aCSF + LPS group in the Fisher exact test.

D. Conclusion

We conclude that the ubiquitin-proteasome system at the RVLM is essential to survival during fatal endotoxemia.