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行政院國家科學委員會補助專題研究計畫成果報告

雙極性情感疾病患者之自體抗體之偵測及細胞激素之調節

計畫類別：■個別型計畫　□整合型計畫

計畫編號：NSC 90-2314-B-038-019

執行期間： 90 年 8 月 1 日至91 年 7 月 31 日

計畫主持人：呂思潔

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一、中文摘要

中樞神經系統與免疫系統間有密切的交互作用，在許多文獻中多獲得證實，其中有關精神壓力與精神疾病患者免疫異常情形的研究，引起我們的重視。比較特別的是，自體免疫機制被證實是精神分裂症可能的發病原因之一。本實驗的目的主要是檢測躁症患者對於抗熱休克蛋白抗體的表現。本實驗的血液樣本是取自躁期病患。患者的血清以西方點墨法檢測與 U-373MG 神經母細胞瘤細胞株細胞萃取物結合的抗體的存在，並進一步以 ELISA 測定抗熱休克蛋白的濃度。熱休克蛋白（Hsps）存在各種細胞中，且具有對抗感染及壓力的保護功能。

在抗 Hsp90 自體抗體部分，我們以 ELISA 檢測的結果發現，未用藥急性期患者抗 Hsp90 抗體比緩解期有意義的增加。已用藥急性期抗 Hsp90 抗體比未用藥急性期患者有意義的降低。已用藥急性期抗 Hsp70 及抗 Hsp90 自體抗體比正常控制組有意義的降低。而血清中抗 Hsp60 自體抗體在患者及正常控制組間沒有顯著意義的差別。我們推測急性躁期患者在 Hsp90 有免疫異常的反應，這種現象可能與躁症急性期的發病有關聯。

關鍵字: 躁症, 自體抗體, 熱休克蛋白, 自體免疫

Abstract

A close interaction between the central nervous system and the immune system have evidenced in many documents, in which we have paid attention to the study of immunological abnormalities both in psychological stress and patients with psychotic disorder. More specifically, an autoimmune mechanism was suggested as one of the possible factors in the pathogenesis of schizophrenia. The aim of this study was to investigate the presence of antibodies that react with heat shock proteins (Hsps) in sera from patient with mania. The sera were tested for antibodies binding to cell extracts of U-373MG glioblastoma cell line using western blot analysis. Hsps are intracellular proteins in most cells which are thought to serve protective functions against infection and cellular stress. In some instances, Hsps may be aberrantly expressed on target tissues interaction with the immune system might potentially result in autoimmune disease, as

suggested in juvenile diabetes mellitus or rheumatoid arthritis.

In the part of autoantibodies against Hsp90, we observed that un-medicated acute patients were significantly higher compared with remission patients. Sera anti-Hsp90 autoantibodies in medicated acute patients were significantly lower compared with un-medication acute patients. Sera anti-Hsp 70 and 90 autoantibodies in medicated acute patients were significantly lower compared with normal controls. In addition, there was no significant difference on autoantibodies against Hsp60 between patients and normal controls . We suggested that a presence of abnormal immune reactivity involving Hsp90 in patients with manic acute episode, and it may implicate in pathogenesis in acute episode with this disease.

Key word: mania, autoantibody, heat shock protein, autoimmunity

INTRODUCTION

The pathogenesis of psychiatry disorders is multifactorial with genetic, endocrine components with environmental. An autoimmune mechanism was suggested as a possible factor in the pathogenesis of psychiatry disorders. In several studies, high titers of antibodies for heat shock proteins of different molecular weights were found. Heat shock proteins (HSPs), also called stress proteins, are family of proteins present in almost all prokaryotic and eukaryotic cells. HSPs are required for a variety of critical functions ranging from protein synthesis to protein transport to protein degradation.

Immune responses to HSPs are implicated in the development of a number of human autoimmune diseases, including several inflammatory arthritis and type I diabetes mellitus. An increased antibodies to HSP 60 kDa and HSP 80-85 kDa in patients with schizophrenia was reported. Antibody to HSP 70 were found in never-medicated schizophrenic patient. The expression of HSP and formation of antibodies indicate that autoimmunity might play a role in the pathogenesis of the psychiatry disorder

MATERIALS AND METHODS

Subjects

Acute in-patients diagnosed as bipolar disorder, manic (DSM-IV) with YMRS scores $\geq$ 30, aged $\leq$ 45 years and free from acute infections or chronic diseases were recruited. During the index hospitalization, all the patients were treated with lithium and typical antipsychotics (haloperidol or chlorpromazine). Follow-up blood samples of patients were collected while in consequent remission (YMRS scores $\leq$ 12) free from any sign of depression. Age- and gender-matched healthy control subjects without any Axis I psychiatric disorder were recruited.

Western blot analysis of anti-HSP 60, 70, 90

auto-antibodies in patients with bipolar disorder

Heat shock protein (HSP-60, -70, -90, from StressGen, Victoria) were apply to 10% SDS-PAGE. Electrophoretic transfer were done from the gel to nitrocellulose sheets for overnight. The nitrocellulose sheet were cut into strips and incubated at room temperature with blocking buffer. The stripes were washed and incubated with patients serum as well as controls. Again the strips were washed 3 times, and secondary antibody conjugated with enzyme were added. After 1h incubation, the strips were washed and band detection were done by ECL according to Amersham using autoradiography as a visualization system.

ELISA analysis of auto-antibodies HSP-60, -70, and –90 in patients with bipolar disorder

In order to further determine the quantity of anti HSP-60, -70, -90 antibodies in patients serum, the ELISA with biotin-advidin amplify system were performed. The HSP –60, 70, -90, 1 μg/ml (StressGen, Victoria) were coated to the 96-well plates at 4°C overnight. Then the plates were blocked by 1% BSA in PBS at room temperature for 1-2 hour. After blocking the plates will be washed for 3 times, and the patients serum with proper dilution were added to the plates and incubated at 37°C for 2 hours. The secondary antibody conjugated with enzyme HRP were added to the plates and incubated for another 2 hours. After 3 times washing, the substrate (TMB) were added and the intensity of the plate were stopped (1N HCl). The absorbances of plates were measured by ELISA plate reader (Microplate Reader Emax, Molecular Devices) at 450 nm. The concentration of the anti-HSP were calculated according to the standard curve. The serum from normal controls were performed side by side.

RESULTS

The characteristic of study group were summarized on Table 1. As demonstrated on Fig 1, serum from patients as well as controls react with HSP 60, 70 and 90.

As demonstrated on Table 2. for anti-HSP 70 and 90, the medicated group were significantly lower than controls for anti-HSP 70 and 90, respectively. For anti-HSP 90, the non-medicated patients is significantly higher than the medicated ones. Moreover, the anti-HSP 90 Ab for un-medicated patients is significantly higher than patients with remission.

CONCLUSION

Since HSP are involved in diverse neuroprotective mechanisms, antibody against

HSP may inhibit neuroprotection. Our data demonstrated the interaction between autoimmune mechanism and HSP system in the pathogenesis of manic disorder. Therefore, the immunological influence on this system could result in a functional dysregulation thereby triggering the psychopathological phenotype of manic disorder and other psychiatric disorders.

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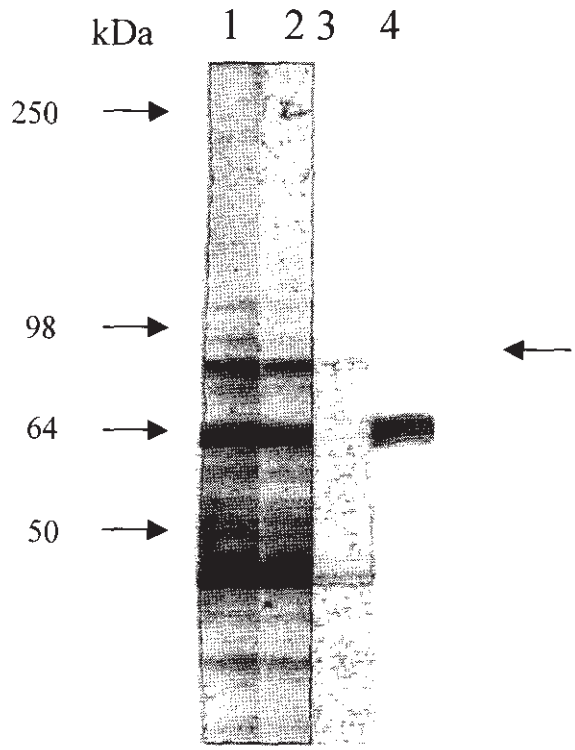

Figure 1. Binding of human srum to the U-373MG glioblastoma extract as analyzed by Western blot. Serum from a patient with mania : dilution 1:100 (lane 1) , 1:200 (lane 2) , 1:600 (lane 3) . Binding of rabbit polyclonal Hsp90 antibody to glioblastoma extract (lane 4).

Table 1. Percentage of autoantibodies against Hsp60, 70 and 90 analysed by Western blot.

Category	Acute manic episode (n=34) mediation	Acute manic episode (n=60) non-mediation	Acute manic episode (n=94) total	(n=42) remission	Normal Control (n=57)
Anti-Hsp60 Ab	17.65 % (6/34)	20.0 % (12/60)	19.15(18/94)	50.0 % (21/42)	15.79 % (9/57)
Anti-Hsp70 Ab	100 % (34/34)	100 % (60/60)	100 % (94/94)	100 % (42/42)	100 % (57/57)
Anti-Hsp90 Ab	50.0 %(17/34)	55.0 %(33/60)	53.19 % (50/94)	26.19 %(11/42)	47.37 % (27/57)

Table 2. Anti-Hsp60 ,-70 and -90 autoantibodies level in patients with mania (with or without medication).

Category	Bipolar disorder A^{m} mean (SD)	Bipolar disorder A^{un} mean (SD)	Bipolar disorder Remission (B) mean (SD)	Control subjects (C) mean (SD)	Comparison (p value) A^{m} vs. B #	A^{un} vs. B #	A^{m} vs. C #	A^{un} vs. C #	A^{m} vs A^{un} #
Anti-Hsp60 Ab [ng/ml]	19.60 (16.70) n=30	15.78 (6.21) n=39	19.10 (10.19) n=42	19.30 (9.77) n=40	0.9820	0.7014	0.9262	0.0606	0.1929
Anti-Hsp70 Ab [ng/ml]	29.10 (10.47) n=29	37.87 (36.19) n=41	36.35 (20.11) n=44	36.11 (14.92) n=35	0.0787	0.8095	0.0372 *	0.7888	0.2102
Anti-Hsp90 Ab [ng/ml]	19.03 (9.75) n=28	33.16 (21.46) n=37	23.22 (18.33) n=44	25.28 (15.56) n=43	0.2701	0.0274 *	0.0428 *	0.0614	0.0019 **

Unpaired t test

* $p<0.05$

** $p<0.01$

A^{m}: medicated acute mania

A^{un}: un-medicated acute mania