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行政院國家科學委員會專題研究計畫成果報告

中文計畫名稱: 利用癌症冬眠期小鼠探討中藥抗癌的免疫機制

英文計畫名稱: Study of the anti-tumor effect of Chinese herbs or anti-fungal drugs by using tumor dormant animal model

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中文摘要

本實驗使用由T型淋巴癌(L-5178Y)產生的腫瘤冬眠期小鼠(DBA/2)模式檢測中藥補中益氣湯對小鼠腹腔中淋巴癌細胞生長之影響及其抗癌免疫機制。二十隻冬眠期之小鼠分為給藥組及控制組(各十隻)，給藥組每日給予口服 0.45mg/kg 補中益氣湯蒸餾萃取粉劑以生理實驗水稀釋，連續給藥 7 日。控制組則灌食等量之生理食鹽水。灌食前及灌食後以連續稀釋法(Serial end point dilution)計算各小鼠腹腔液中癌細胞的數量。結果顯示在控制組 8 隻小鼠(兩隻在實驗過程中死亡)其腹腔癌細胞數量沒有顯著的差異($t=0.2831$，$P>0.05$)。而給藥組(10 隻小鼠)則發現腹腔癌細胞數量有顯著的減少並具有統計學上的差異($t=2.7178$，$P<0.05$)。兩組之腹腔液同時也用 flow cytometry 作 $CD4^+$ $CD8^+$, NK cells 及 macrophage 細胞的分析，其結果顯示給藥組小鼠腹腔液中 $CD4^+$及 $CD8^+$的細胞數量均有顯著的增加($P=0.008$，$P=0.007$)，但 NK 細胞及 macrophage 的數量並沒有顯著的影響。此一結果顯示補中益氣湯對癌細胞生長抑制作用可能主要是刺激 T 細胞的活化產生 lymphokines 進而產生 cytotoxic T cell 以消滅癌細胞。

關鍵詞 ：腫瘤冬眠期，補中益氣湯，T 型淋巴癌(L-5178Y)，抗癌免疫機制

英文摘要

In this study, the anti-tumor effect of Bu-Zhong-Yi-Qi-Tan was evaluated by using L5178Y lymphoma tumor dormant mice model. Twenty dormant mice were divided into two groups (10 for each group), the number of tumor cells in its peritoneal cavity was enumerated by serial end-point dilution (SEPD). Bu-Zhong-Yi-Qi-Tan extract powder was dissolved in saline and gave to the treatment group orally (0.45 mg/kg) every day for 7 consecutive days. The same volume of saline was given to control group orally daily for 7 days. After treatment, the number of tumor cells in peritoneal cavity was enumerated by SEPD again to compare it with before treatment. The results indicated that the mice after Bu-Zhong-Yi-Qi-Tan treatment, the number of tumor cells harbor in peritoneal cavity were decreased significantly ($t = 0.2831$, $p > 0.05$). In contrast, the control group without significant difference ($t = 0.2831$, $p > 0.05$). The change of $CD4^+$, $CD8^+$, NK and Macrophage cells in peritoneal fluid were analyzed by flow cytometry. The results indicated that the $CD4^+$, $CD8^+$ were increased significantly ($p = 0.008$, $p = 0.007$ respectively) in treatment group of mice. However, there were no any change number in NK or macrophage in either treatment or control group of mice. We conclude that the anti-tumor effect of Bu-Zhong-Yi-Qi-Tan is mediated by activation of T lymphocytes to produce lymphokines and cytotoxic T cells to eradicate the tumor cells.

Keywords: **Tumor dormant mice, Bu-Zhong-Yi-Qi-Tan, T-cell lymphoma, Anti-tumor immunological mechanisms**

計畫緣由與目的:

Bu-Zhong-Yi-Qi-Tan has been used widely as an immunostimulatant for cancer therapy. However, the mechanism of the action has not been elucidated. Tumor dormant state mouse is a sensitive animal model to study the immunological mechanism of anti-tumor effect. Since the tumor cell line was used in this study is a low metastatic lymphoma (L5178Y) which growth is restricted in the mouse peritoneal cavity. In peritoneal cavity, there are many immune cells which can easily be enumerated or monitored by either using SEPD or flow cytometry. The purpose of the study is to use this animal model to evaluation of the effect and mechanism of anti-cancer of Chinese herbs.

結果與討論:

The animal model of tumor dormancy has been established in our laboratory successively. We have overcome some technical hurdles, such as source of animals, animal facilities, animal surgery and finally the efficiency to production of tumor dormant mice has reached 70% which is better than what we did before in the United States. The *in vivo* experiment results showed that Bu-Zhong-Yi-Qi-Tan oral administration exerted its anti-tumor effect in the dose of 0.45mg/kg daily for 7 consecutive days. The average tumor burden per mouse was decreased from 1625.6 +/- 435.0 to 62.4 +/- 12.4, however, in control group, the tumor burden did not change significantly (average from 912 +/- 451 to 864 +/- 402 per mouse). Both $CD4^+$ and $CD8^+$cells were increased after Bu-Zhong-Yi-Qi-Tan treatment; the $CD4^+$ cells from baseline 15.67 % increased to 23.41% and $CD8^+$cells also was increased from 5.15% to 8.38% ($p = 0.008$ and $p = 0.007$ respectively). However, the rest of immune cells such as NK cells or macrophage did not find any change after drug treatment. The results implied that the tumor killing effect of Bu-Zhong-Yi-Qi-Tan is mediated through the effect of cytotoxic T lymphocytes by T lymphocyte activation. No any cured animal after drug treatment in this study, it may be due to the drug dosage and/or the length of treatment. Multiple dosage and duration of treatment experiments have been conducted try to answer the question. However due to power outage of animal facility during Nari typhoon ruin the whole experiments. This experiment has been restarted now and wish can get some further experimental results soon.

計畫成果自評:

There are several difficulties to establish the tumor dormant model in Taiwan, such as; 1) the source of inbred strain of DBA/2 mice, 2) inoculation of tumor cells subcutaneously, 3) surgical excision of tumor nodule, and 4) partial peritoneal lavage. We have lucky to overcome all problem and found a very effective way to produce tumor dormant mice. We are planning to use this model to screen all anti-tumor or immunostimulating herb or any synthetic potential anti-cancer compounds and study its mechanism of action.

參考文獻:

1. Weinhold K.J., Goldstain, L.T., and Wheelock, E.F. Tumor-dormant states established with L5178Y lymphoma cells in immunized syngeneic murine hosts. *Nature* 270 (3): 59-61,1977.

2. Liu, C.M., Y. Suzuki, L. Chen, T. Okayasu, C.E. Calkins, and E.F. Wheelock. Maintenance and Cure of the L5178Y Murine Tumor-dormant State by Interleukin-2: *In vivo* and *in vitro* Effects of Interleukin-2. *Cancer Res.* 50(5): 1361-1367, 1990.

3. Liu, C.M., T. Okayasu, P. Goldman, Y. Suzuke, K.Suzuke, and E.F. Wheelock. Immune Regulation of the L5178Y Murine Tumor-dormant State. *J. Exp. Med.* 164(4): 1259-1273,1986.

4. Chen. L., Y. Suzuki, C.M. Liu, and E.F. Wheelock. Maintenance and Cure of L5178Y Murine Tumor-dormant State by Interleukin-2: Dependence of Interleukin-2 on Induced Interferon-gamma and on Tumor Necrosis Factor for its Anti-tumor effects. *Cancer Res.* 50(5):1368-1374, 1990.

5. Suzuki, Y., L. Chen, C.M. Liu., T. Morita and E.F. Wheelock. Immune Regulation of the L5178Y Murine Tumor-dormant State: Induction of Cell and Soluble Factor and gamma Interferon. *Cancer Res.* 49(8):2028-2033, 1989.

6. Suzuki, Y., L. Chen, C.M. Liu, and E.F. Wheelock. Immune Regulation of the L5178Y Murine Tumor-dormant State: III. Endogenous Interferon-gamma Regulates Macrophage-Mediated Restraint on Tumor Cell Growth in Peritoneal Cell Culture by Prostaglandin E2. *Cancer Immunol. Immunother.* in press.

7. Suzuki, Y., C.M. Liu, D. Ben-Nathan, L. Chen, and E.F. Wheelock. Immune Regulation of the L5178Y Murine Tumor-dormant State: II. Interferon-gamma Requires Tumor Necrosis Factor to Restrain Tumor Cell Growth in Peritoneal Cell Culture from Tumor-dormant Mice. *J. Immunol.* 139(9): 3146-3152, 1987.