行政院國家科學委員會補助專題研究計畫成果報告

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※**多重因素影響干擾素**-RIBAVIRIN **對慢性Ｃ型肝炎長期療效之評估**　※
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計畫類別：¦**個別型計畫**　　□**整合型計畫**
計畫編號：NSC89－2320－B－040－032－
執行期間：88 **年** 8 **月** 01 **日至** 89 **年** 7 **月** 31 **日**

計畫主持人：邱慧玲
共同主持人：楊基滐

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執行單位：中山醫學院醫技系

中　華　民　國 89 **年** 10 **月** 30 **日**

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共同主持人：楊基傑　彰化秀傳醫院胃腸科

一、　中文摘要

關鍵詞：C 型肝炎病毒、干擾素、ribavirin、合併療法、腫瘤壞死因子、
C型肝炎病毒(HCV)為輸血性肝炎的主要致病原之一，造成肝癌、肝硬化等嚴重致死疾病之重要因素，對人類健康影響甚巨。對於慢性 C 型肝炎病毒感染，目前最新的治療方法為利用干擾素及 ribavirin 的合併療法，在臨床實驗評估報告中，此方法對總體治療率有很大的助益。本研究計畫即旨在收集臨床病例，希望藉由結合臨床數據的分析及實驗室的基礎研究，來探討多重因素與此合併療法長期療效之間的關係。目前已完成之分析顯示病人的性別及年齡與長期療效無關，但具有同質性腫瘤壞死因子基因之病人之治癒率明顯高於異質性病人。但此相關性仍需更多病人及更多分析確定之。藉此希望對 C 型肝炎病毒臨床長期治療方面有所助益。

二、　英文摘要

Keywords: hepatitis C virus, interferon, ribavirin, tumor necrosis factor, gene polymorphism
Recently, ribavirin has been evaluated as a therapy of chronic hepatitis C alone and in combination with alpha interferon. Most of the results of interferon-ribavirin combination antiviral therapy were promising. For a more precise estimation of the efficacy and tolerability of interferon-ribavirin combination therapy for chronic hepatitis C, we hope to clarify the relationship between the treatment efficiency and multifactors through this study.　Several factors include age and sex of patients, were found no impact on the treatment efficiency. Meanwhile, the response rate of patients with homologous TNF1 allele is significantly higher than patients with heterologous allle. More patients are still needed for analysis to ensure this relationship.　These knowledges will be of great value in ensuring a long-term success of HCV therapy in Taiwan.

三、　緣由與目的

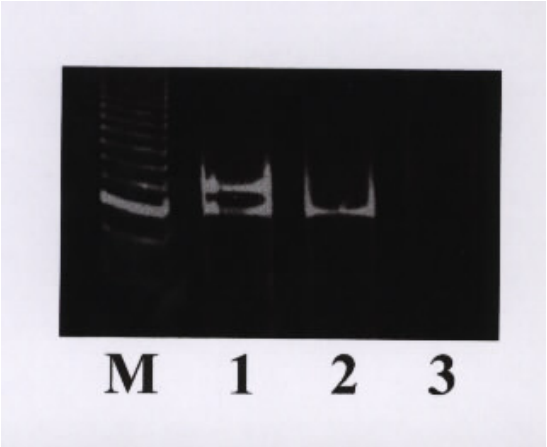
HCV is a major etiologic agent of transfusion-associated hepatitis and infects around 1% of the general population worldwide. In Taiwan, the prevalence rate of HCV in adult is 1-2%, which is believed to be underestimated. Interferon-α2b (IFN-alpha) used to be the main strategy for treatment, unfortunately, the most frequent genotype in Asia (including Taiwan), HCV-1b, is the most resistant to interferon treatment (the rate of complete response is only 10%) and this phenomenon makes the therapy of chronic HCV infection a difficult task[1]. In the current strategy for treatment of viral infection, antiviral agents aim at altering viral replication cycle and modifying the host immunity. For immunodulation, interferon can enhance NK activity, maturation of cytotoxic T cells and cell surface expression of HLA class I antigen, thereby promoting immune clearance of infected cells[2-3]. Since no definitive therapy has been approved for HCV chronic infection, there is still urgent need regarding optimal candidacy for HCV treatment. The adjuvant use of drugs, such as ribavirin, in combination with interferon may hold promise at enhancing viral eradication. Since the full course of treatment is time consuming and expensive, it will be of great

value if any factors can be used in precise prediction for the outcome of interferon - ribavirin combination treatment. The previous efforts were almost focused on the influence of viral factors; such as genotype, RNA load, and ISDR sequences on the treatment efficiency and some of them did show notable differences. In this study, we will elucidate the impact of host factors, especially in the immune system such as TNF-α and LT gene polymorphism during the treatment, on the treatment efficiency.

四、 結果與討論、

This study is cooperated with the department of Internal Medicine Gastroenterology of Show Chwan Memorial Hospital. Patients received recombinant interferon 3MU thrice weekly and daily oral ribavirin for 24 weeks. Blood samples were taken before entry, monthly during therapy, at the end of treatment, and 8 weeks after cessation of therapy.

The treatment efficiency was evaluated by the clinical physician based on the ALT value at the end of treatment. The TNF-α gene polymorphism was analyzed by a PCR - RFLP. First, the prepared genomic DNA is amplified by a PCR reaction. The sequences of primers used for TNF-α amplification is as follow: TNFα-5': AGG CAA TAG GTT TTG AGG GCC AT; TNFα-3': TTG GGG ACA CAA GCA TCA AGG '
After the amplification, the PCR product was cleaned, subjected to a NcoI enzyme digestion and then analyzed on a 2% agarose gel. One band (149 bp) will be seen on the gel if the PCR product remains intact which represents the TNF2 allele, while the present of three bands (149, 129 and 20 bps) represents heterologous allele and homologous TNF1 allele would be digested into two fragments (129 and 20 bps). The representative DNA profile for TNFα gene polymorphism analysis is shown in the figure below.



Lane 1 was loaded with herterologous TNF allele (showing 2 bands with 149 and 129 bp, respectively; the 20 bp band is too small to be seen in this gel); lane 2 represents a homologous TNF1 allele (with a 129 bp band and the 20 bp band is too small to be seen in this gel); a standard marker is located at lane M. Of the total 42 cases studied, 36 were determined as heterologous allele (13 responders, 4 relapsers and 19 non-responders), 6 cases were homologous TNF1 allele (4 responders, 1 relapser and 1 non-responder); no TNF2 allele has been detected so far.

Ribavirin is a nucleoside analogue and in vitro study showed that ribavirin inhibits RNA, protein synthesis and cell proliferation induced by mitogenic factors in PBMC and primary human hepatocytes[4-5]. Ribavirin also inhibits viral-induced macrophage production of TNF, IL-1, and preserves Th1 cytokine production but inhibits Th2 cytokine response[6]. Recently, it has been evaluated as a therapy of chronic hepatitis C in combination with alpha interferon and most of the results were promising [7-17] despite of a few studies found no difference between single interferon treatment and interferon - ribavirin combination therapy[18]. It was suggested that ribavirin may exert its effects by suppressing viral replication rather than by eradicating the virus[10, 17-18]. Clinically, the combination treatment was frequently associated with significant side effects[7]. The optimal use and regimen of combination therapy awaits further investigation. Human TNF-α is a cytokine consist of 157 amino

acid and mainly produced by activated macrophages. This 17 KDa polypeptide possesses pleiotropic properties including the induction of the immune response to infectious agents and exerting direct antiviral effects. Despite of that low levels of TNF-α can contribute to cell protection, excessive amounts of that may cause cell damage and TNF-α has been involved in the pathogenesis of a diversity of liver conditions including viral hepatitis. As regards to HCV infection, raised serum TNF-α levels have been detected in chronic HCV infection and high TNF-α values were associated with the resistance to interferon therapy, especially with genotype 1b. Furthermore, the expression of TNF-α is tightly regulated at the transcriptional and posttranstriptional level and mutations at position -308 and -238 of the promoter region have been shown to alter its expression[20]. Interestingly, a mutation at position 308 is associated with higher constitutive and inducible levels of TNF-α whereas the impact of that at position -238 is not clear yet. In the present data, we could not see any correlation between the TNFα promoter gene polymorphism and the treatment efficiency. We can see the response rate of patients with homologous TNF1 allele is significantly higher than patients with heterologous allele. This finding is indefinite since only less than half of our study cases have been analyzed, therefore, we will continue to analyze the remaining cases to obtain clearer conclusion.

五、 計畫成果自評

In this study, we collected more cases than planned. Since contamination problems in detection of replicative intermediate in PBMC can not be solved, instead, we analyzed the TNF-α gene polymorphism and serum level of TNF-α during the treatment. Although no definite conclusion is drawn from this study, we hope more information will be provided from the continuing project.

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