多重因素影響干擾素-RIBAVIRIN 對慢性C型肝炎長期療效之評估

計畫類別：□ 個別型計畫  □ 整合型計畫
計畫編號：NSC89-2314-B-040-019-
執行期間：89年8月01日至90年7月31日

計畫主持人：邱慧玲
共同主持人：楊基澄

本成果報告包括以下應繳交之附件：
□ 赴國外出差或研習心得報告一份
□ 赴大陸地區出差或研習心得報告一份
□ 出席國際學術會議心得報告及發表之論文各一份
□ 國際合作研究計畫國外研究報告書一份

執行單位：中山醫學大學醫技系

中華民國90年10月30日
一、中文摘要

關鍵詞：C型肝炎病毒、干擾素、ribavirin、NS5A_2209-2248序列

C型肝炎病毒(hev)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。此年度計畫已完成30個病人的NS5A_2209-2248序列分析，經由臨床療效分析結果顯示病人的性別及年齡與長期療效無關，與NS5A_2209-2248部分序列是否突變亦無相關。30個分析病例中，27個病例在此區域中無胺基酸突變發生，其中14個具完全療效，6人無療效，7人有復發現象產生。具突變的3例中，分別為2218His→Arg（無療效），2219His→Arg（完全療效），227 Ile→Phe(復發)。這些突變種的功能性分析將是下一步之研究重點。

二、英文摘要

Keywords: hepatitis C virus, interferon, ribavirin, NS5A_2209-2248 region, mutation

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 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 to have no impact on the treatment efficiency, including sex and age of patient. The mutation in the NS5A_2209-2248 were also irrelevant to the treatment efficiency since in the total of 30 patients analyzed, 27 were without amino acid change in this region (14 responders, 6 non-responders, and 7 relapsers), and 1 with 2218His→Arg (non-responder), 1 with 2219His→Arg (responder), and another one with 2227 Ile→Phe (relapser). The functional analysis of these mutants will be our next objective.

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-α) 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. 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\textsuperscript{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 the impact of host factors, especially in the immune system such as TNF-\(\alpha\) and LT gene polymorphism and some of them did show notable differences. In this study, we will elucidate the impact of NS5A\textsubscript{2209-2248} (ISDR) sequences 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 PCR primers and sequencing primers were synthesized with a DNA synthesizer (model 391, Applied Biosystems Japan, Chiba, Japan). The nucleotides 6703 to 7320 (numbered on the basis of the sequence of HCV-J) of the HCV were amplified by a set of primers (5' TGGATGGAGTGCGGTTGCACAGGTA3') and (5' TCTTTCTCCGTGGAGGTGGTATTGG 3'). Both strands of the PCR product will be sequenced with Prism dye termination kit (Applied Biosystems Japan) and by an automated DNA sequencer (model 373S, Applied Biosystems Japan), according to the manufacturer’s instructions and the primers used are (5' CAGGTACGCTCCGGCGGTGCA3') - nucleotide 6722 to 6741 for the sense strand, and (5' GGGGCCTTGGTAGGTGGCAA3') - nucleotide 7275 to 7294 for the antisense strand. The resulting amino acid sequence will be compared with that identified in HCV-J.

The complete process for interferon therapy is time-consuming, expensive and may cause some side effects\textsuperscript{1}, therefore, it would be very useful to be able to predict the efficacy of interferon therapy to HCV infection. In a study conducted in Japan, Enomoto and his colleagues have firstly suggested that in patients with chronic HCV-1b infection, there is a significant correlation between response to interferon treatment and mutations in the NS5A\textsubscript{2209-2248} region, termed the interferon sensitivity determining region (ISDR), before interferon therapy\textsuperscript{2} and another following study supported this hypothesis\textsuperscript{3}. However, other studies done by groups in north America or Europe have controversial results showing that no correlation exists between response to interferon therapy and mutations in the NS5A\textsubscript{2209-2248} region\textsuperscript{4-7}. Interestingly, the NS5A protein, a potent transcriptional activator, has been suggested to play a role in the viral replication and hepatocarcinogenesis\textsuperscript{8-9}. This nonstructural protein, , has also been found to mediate the repression of the IFN-induced protein kinase, PKR, a mediator of IFN-induced antiviral resistance and a target of viral and cellular inhibitors\textsuperscript{10}. Thus, inactivation of PKR by NS5A protein may be one mechanism by which HCV avoids the IFN-induced antiviral effects. All these studies suggested NS5A protein play a role, which is mysterious and fairly unclear, in the process of interferon therapy.
Because of the large geographical differences observed for HCV, to assess whether any relationship existing between IFN-alpha sensitivity and mutation in the NS5A region in local isolates would be of great value in ensuring a long term success of HCV therapy in Taiwan. From our current data, not relatedness could be drawn between the sequence variation of NS5A ISDR region and treatment efficiency, however, 3 mutations (2218His→Arg, 2219His→Arg, and 2227 Ile→Phe) were identified in our study group. The impact of these mutations on the function of NS5A will be of interest for us to study further.

五、参考文献