行政院國家科學委員會專題研究計畫　成果報告

慢性 C 型肝炎併肝硬化病人對抗病毒治療反應低之機轉

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ABSTRACT

Chronic hepatitis C virus (HCV) infection can lead to liver cirrhosis in 20-30% of patients, which predisposes to hepatic decompensation and hepatocellular carcinoma (HCC). These hepatitis C patients with cirrhosis are associated with lower response rates to either interferon alfa monotherapy or combination therapy with interferon and ribavirin, about 50% or less of that in non-cirrhotic patients. The mechanism for the difference in response rates between non-cirrhotic and cirrhotic HCV-infected patients, however, is unclear. It is most likely that both host and viral factors are important.The hypothesis was : The poor response to interferon and interferon/ribavirin therapy in patients with chronic hepatitis C and cirrhosis is related to the impaired interferon alfa signaling pathways, which are interfered by structural changes in hepatocytes induced by prolonged necro-inflammation and/or factors associated with the cirrhotic process. The specific aims were :

1. To compare the gene expressions of JAK-STAT signaling pathways of interferon alfa in liver tissues obtained from chronic hepatitis C patients with or without cirrhosis.
2. To examine whether the expression of inhibitory factors for the JAK-STAT signaling pathway are increased in HCV-infected cirrhotic liver, including suppressor of cytokine signaling (SOCS) family.
3. To compare the expression of interferon-induced PKR (double stranded RNA-activated protein kinase) and OAS (2’-5’ oligoadenylate synthetase) between HCV-related cirrhotic or non-cirrhotic liver.

We found that in cirrhotic liver with chronic hepatitis C, STAT 1 phosphorylated forms (phosphorylation at Tyr-701) and STAT 3 phosphorylated forms (phosphorylation at Tyr-705) were increased compared with liver tissue from non-cirrhotic liver with chronic hepatitis C. Expression of both SOCS1 and SOCS 3 increased in cirrhotic liver. Unexpctedly, IL6 expression did not increase in cirrhotic liver tissues. The expressions of phosphorylated forms of PKR was increased in the cirrhotic liver with chronic hepatitis C. Our study support that SOCS 1 and SOCS 3 may be related to the low sustained response to interferon-base therapy in chronic hepatitis C patients with cirrhosis. To decrease or inhibit the expressions of SOCS-1 and SOCS-3 may increase the sustained viral response to interferon-base therapy for patients with chronic hepatitis C.

摘要

慢性 C 型肝炎病毒(HCV)感染會使 20-30%的病人在 20-30 年間產生肝硬化，並可能導致肝衰竭與肝細胞癌。C 型肝炎併肝硬化的病人對干擾素治療的反應較低，不論是干擾素單方治療或干擾素合併 Ribavirin 治療均比非肝硬化的病人差，其分子機轉並不清楚。本研究的假說是：C 型肝炎併肝硬化病人對干擾素為根基的治療反應差與干擾素信息傳遞路線受阻有關。本研究的特定目的包括探討：1. JAK STAT 信息途徑在 C 型肝炎肝硬化與非肝硬化組織是否表現不同；2. 比較細胞素訊息壓抑分子(suppressor of cytokine signaling, SOCS)家族在肝硬化與非肝硬化組織的表現差異；3. 比較干擾素誘導之抗病毒 PKR 蛋白在肝硬化與非硬化肝組織的差異。結果發現，C 型肝炎併肝硬化肝組織較多磷酸化 STAT 1 之分子(Tyr-701 位置)及磷酸化 STAT 3(Tyr-705)；SOCS 1 及 SOCS 3 的表現在肝硬化組織表現較強。尤其是後者，但肝硬化相關之 TGF-β 並未增加 SOCS 3 之表現。本研究支持 SOCS 1 及 SOCS 3 之增強表現可能影響干擾素α之訊息傳遞而使 C 型肝炎併肝硬化病人對干擾素或干擾素合併 Ribavirin 之療效變差。

BACKGROUND AND SIGNIFICANCE

The outcomes of the antiviral therapy for chronic hepatitis C are influenced by viral, host and interferon-related factors. Liver cirrhosis is one of the most important negative factor for successful treatment with interferon and interferon/ribavirin therapy. The SVR to interferon in HCV patients with cirrhosis is only about 50% of that in non-cirrhotic patients. Although pegylated interferon plus ribavirin can induce 30% of SVR in cirrhotic patients, the results is also half of that in non-cirrhotic patients. The mechanism underlying lower response to interferon in HCV-induced cirrhotic patients as compared to non-cirrhotic patients is unknown. Several studies have demonstrated a higher degree of HCV heterogeneity in the cirrhotic patients which might evade immune surveillance and resist antiviral treatment. However, it is not unanimous. The host factors associated with cirrhosis may play an important role.

The possible mechanisms of undesirable response to interferon and interferon/ribavirin in patients with chronic hepatitis C and cirrhosis include block of interferon alfa signaling and block of interferon-induced protein function by factors accompanied with cirrhotic process. In recent years, the action of IFNα has been identified to be through JAK-STAT signaling pathway. Although the JAK-STAT pathway was initially found to be activated by interferon, it is now known that a large number of cytokines, growth factors, and hormonal factors activate JAK and/or STAT proteins. Several inhibitory factors which can down-regulate the interferon –stimulated JAK-STAT pathway have recently been identified (17). These factors belong to 3 main families : suppressor of cytokine signaling (SOCS) family, protein tyrosine phosphatases (PTP), and p42/44 MAP kinase. The SOCS family includes SOCS 1,2,3, and CIS. SOCS 1-3 blocks Jak2. Whether these inhibitory factors are activated and play an important role in reducing the interferon alfa effectiveness in HCV-induced liver cirrhosis is unknown.

Fibrosis and cirrhosis is a complex process, involving the activation of extracellular matrix synthesis and the release of cytokines. Tranforming growth factor (TGF) beta play a major role in activating hepatic stellate cell and the fibrotic process (25). Increase intrahepatic mRNA expression of IL-2, 6 and 8 in human cirrhosis have also been observed. We are particularly interested in cytokines increase or activated in cirrhosis which might influence interferon action.

SPECIFIC AIMS

1. To compare the gene expressions of JAK-STAT signaling pathways of interferon alfa in liver tissues obtained from chronic hepatitis C patients with or without cirrhosis.
2. To examine whether the expression of inhibitory factors for the JAK-STAT signaling pathway are increased in HCV-infected cirrhotic liver, including suppressor of cytokine signaling (SOCS) family.
3. To compare the expression of interferon-induced PKR (double stranded RNA-activated protein kinase) and OAS (2'-5' oligoadenylate synthetase) between HCV-related cirrhotic or non-cirrhotic liver.

METHODS

We examined the expression the STATs, SOCS-1 & SOCS-3, TGF-β & IL-6 in liver samples obtained from HCV-related cirrhosis and non-cirrhotic patients . RT-PCR and western blotting were used to study the difference between the cirrhotic and noncirrhotic samples. Western blot analysis was performed according to Nguyen et al. Liver tissues were homogenized in lysis buffer, and subjected to SDS-PAGE gel electrophoresis. After electrophoresis, proteins were transferred to nitrocellulose membranes and blotted against primary antibodies and second antibodies.

RESULTS & DISSCUSSION

We found that in cirrhotic liver with chronic hepatitis C, STAT 1 phosphorylated forms (phosphorylation at Tyr-701) and STAT 3 phosphorylated forms (phosphorylation at Tyr-705) were increased compared with liver tissue from non-cirrhotic liver with chronic hepatitis C (Fig.1 and Fig.2). Expression of both SOCS1 (Fig.3) and SOCS 3 (Fig.4)increased in cirrhotic liver. Unexpctedly, IL6 expression did not increase in cirrhotic liver tissues (Fig.5). The expressions of phosphorylated forms of PKR was increased in the cirrhotic liver with chronic hepatitis C (Fig.6). Our study support that SOCS 1 and SOCS 3 may be related to the low sustained response to interferon-base therapy in chronic hepatitis C patients with cirrhosis. To decrease or inhibit the expressions of SOCS-1 and SOCS-3 may increase the sustained viral response to interferon-base therapy for patients with chronic hepatitis C.

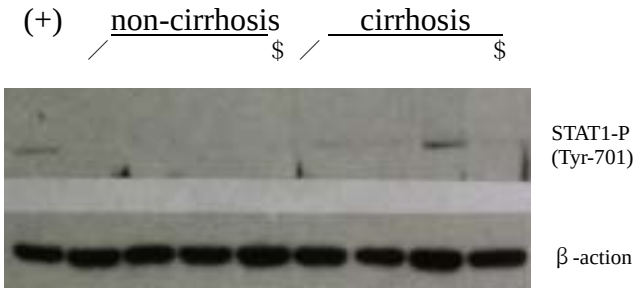

Fig. 1

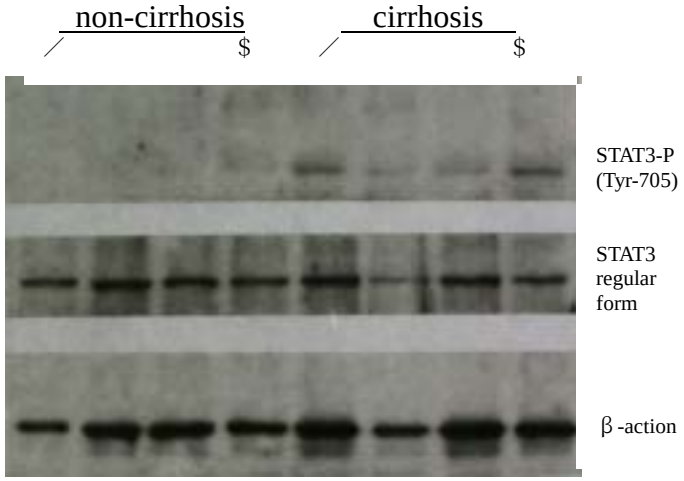

Fig. 2

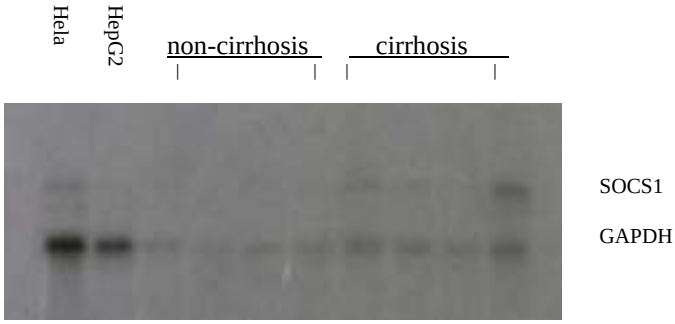

Fig.3

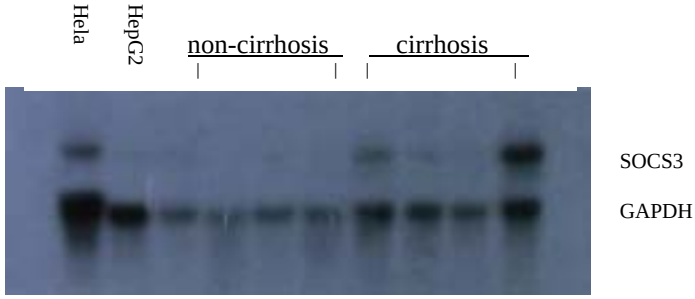

Fig.4

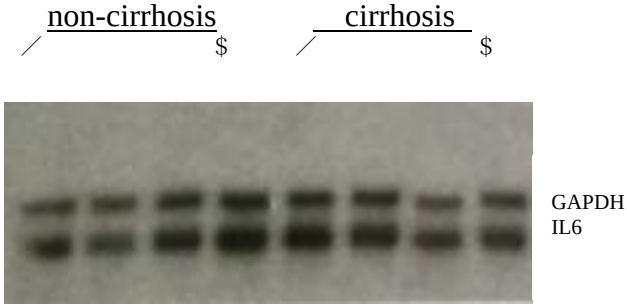

Fig.5

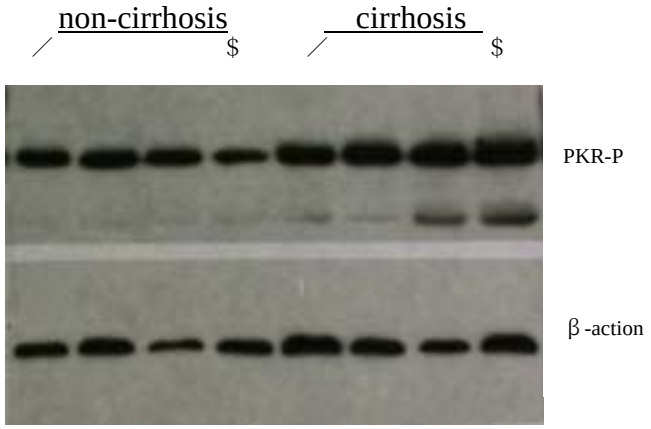

Fig.6