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ORIGINAL ARTICLE

Combined Therapy for Chronic HCV With Interferon-α Plus Ribavirin Improved Insulin Resistance but did not Affect the Adiponectin Levels

Shih-Ting Tseng [1], Wen-Ta Chiu [2], Hui-Er Wang [3], Kuan-Chou Chen [2,4*], Robert Y. Peng [5]

[1] Division of Endocrinology and Metabolism, Department of Internal Medicine, Kuang-Tien General Hospital, Taichung Hsien, Taiwan
[2] Taipei Medical University, Xin-Yi District, Taipei, Taiwan
[3] Department of Food Biotechnology, Hungkuang University, Shalu County, Taichung Hsien, Taiwan
[4] Department of Urology, Taipei Medical University–Shuang Ho Hospital, Taipei, Taiwan
[5] Research Institute of Biotechnology, Hungkuang University, Shalu County, Taichung Hsien, Taiwan

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Background/Purpose: Insulin resistance may play an important role in the pathogenesis of Type 2 diabetes in subjects infected with chronic hepatitis C virus (HCV). A combination therapy of interferon-α (IFN-α) plus ribavirin has been the mainstay of treatment for chronic HCV infection. Changes in insulin resistance and adiponectin levels after such a combination therapy, especially in Asian populations, are rarely reported. Hence, we performed this study to assess these changes before, during, and after the combination therapy.
Methods: The degree of homeostasis model assessment of insulin resistance and adiponectin levels were evaluated in nondiabetic subjects affiliated with chronic HCV before treatment and at 3 and 6 months, respectively, after the cessation of the combined therapy of IFN-α plus ribavirin.
Results: The parameters obtained before, during, and after cessation of the therapy showed that the degree of homeostasis model assessment of insulin resistance significantly changed over time with age-, sex-, and body mass index–adjusted conditions. In contrast, adiponectin levels were totally unaffected.
Conclusion: The extent of insulin resistance can be improved by the IFN-α plus ribavirin combination therapy. Despite a significant improvement in insulin resistance, the adiponectin levels remained unaffected.
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1. Introduction

Hepatitis C virus (HCV) infection is an important cause of liver cirrhosis in Taiwan. The association of chronic HCV infection with diabetes mellitus (DM) was first reported by Allison et al in 1994.[1] There is now emerging epidemiological data suggesting that HCV infection contributes to the development of DM. Latter, larger controlled studies have supported this conclusion.[2] Based on those studies,[3] 21–50% of subjects with chronic HCV infection were also affiliated with DM; the incidence rates were significantly higher than those found in the general population and/or among subjects with chronic hepatitis B infection. More importantly, the prevalence of anti-HCV antibody was significantly higher in subjects with DM than that in the general population.[4,5]

The cause of a higher DM prevalence in subjects with chronic HCV infection still remains unclear. Altered glucose metabolism has been well documented in subjects with liver cirrhosis[6] and HCV.[7,8] However, in Italy, Mangia et al[9] found a negative association between chronic HCV infection and DM in noncirrhotic hospitalized subjects. In another study, HCV genotype 1b has been shown to be associated with DM, and the prevalence of chronic HCV genotype 1b is 70–75% in the Taiwanese population.[10]

The HCV-associated diabetes was initially identified as Type 2 diabetes by the Third National Health and Nutrition Examination Survey III.[11] Interferon-α (IFN-α) has been proved to be an effective therapy for chronic HCV infection.[12] IFN therapy may increase insulin resistance in subjects with chronic HCV infection. IFN-α and ribavirin combination therapy is a mainstay of treatment for chronic HCV infection in recent years, with a higher restoration rate of liver function than IFN-α monotherapy.[13]

A recent report showed that the adiponectin levels were elevated in liver cirrhosis subjects.[14] One study revealed no significant change of adiponetin levels after INF-α therapy in chronic HCV-infected subjects, but the case number was small ($n = 11$ in the HCV group), and subjects received only monotherapy.[15] The adiponectin levels in subjects with chronic HCV and their response to IFN-α and ribavirin combination therapy remain unclear.

In this study, we measured insulin resistance in subjects with HCV infection by a simple and convenient method called homeostasis model assessment of insulin resistance (HOMA-IR).[16] It correlated with the gold standard of clamp-measured total glucose disposal well.[16] The changes in insulin resistance before and after

* Corresponding author. Department of Urology, Taipei Medical University–Shuang Ho Hospital, Taipei Medical University, 252, Wu-Xin Street, Xin-Yi District, Taipei 116, Taiwan.
E-mail: kc.chen416@msa.hinet.net (K.-C. Chen).

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treatment were measured; the changes in adiponectin levels were also evaluated.

2. Materials and Methods

2.1. Subject population

Thirty-six subjects (mean age ± standard deviation = 50.38 ± 11.96 years) with chronic HCV were consecutively followed at the outpatient clinics of Kuang Tien General Hospital, located in central Taiwan, from March 2004 to 2005. This study protocol is in agreement with the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the Kuang Tien General Hospital ethics committee. Informed consent was obtained. None of them took any medications or substances that could significantly affect glucose metabolism before entering the study. Subjects with a history of alcoholism were excluded. Abdominal sonography of all subjects was performed before entrance into the study, and those found to have liver cirrhosis were excluded. All subjects met the following inclusion criteria: elevated serum alanine aminotransferase (ALT) levels at least two times the upper limit of normal range before enrollment; positive serum HCV mRNA; plasma blood sugar levels less than 126 mg/dL. All subjects were negative for the hepatitis B surface antigen.

2.2. Laboratory investigations

Serum ALT and aspartate aminotransferase (AST), glucose, lipid profiles, ferritin, insulin, adiponectin, and HCV mRNA measurements were performed immediately after the blood specimens were obtained. The data collected involved samples before therapy, 3 and 6 months post-therapy, and 6 months after the cessation of treatment. Serum ALT and AST were measured with the ultraviolet spectrophotometric methods using a Hitachi Analyzer (Roche Diagnostics, Taipei, Taiwan). Fasting plasma glucose was determined by a Beckman Coulter LX20, LX20PRO Autoanalyzer (Beckman Coulter Inc., Taipei, Taiwan). Lipid profiles, such as total cholesterol, high-density lipoprotein, and triglyceride, were measured using Beckman Coulter LX20, LX20PRO (Beckman Coulter Inc.). The ferritin levels were measured using the BNP Plasma Protein Autoanalyzer (BN ProSpec, Dade Behring Inc., Eschborn, Germany). Serum insulin was determined by the method of radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). The serum adiponectin levels were determined with an adiponectin radioimmunoassay kit (LINCO Research Inc., St Charles, MO, USA), in which ^{125}I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum were used. HCV-RNA was examined before treatment, during treatment (at 12 and 24 weeks), and 6 months after the cessation of therapy by reverse transcription polymerase chain reaction (RT-PCR) with primers located in the 5V noncoding region of the HCV genome using a commercial kit (Amplicor HCV; Roche Diagnostics, Basel, Switzerland). In case the difference between the duplicate results was greater than 10% CV, the samples were tested twice. Insulin resistance was calculated by the HOMA-IR method as shown in Eq. (1).[16]

$$\mathrm{HOMA-IR} = (I_{FS} \times G_s)/22.5 \quad (1)$$

Where I_{FS} is the fasting serum insulin in μU/mL and G_s is the serum glucose concentration in mmol/L.

2.3. Treatment protocol

All subjects received subcutaneous injections of α-IFN-2a (Roferon-A; Hoffmann-La Roche, Basel, Switzerland) three times per week, each time with 3×10^6 units. Additionally, ribavirin (Robatrol; Hoffmann-La Roche) was supplied daily at a dosage of 1000 mg divided as *bid per os* for subjects with body weight less than 75 kg or 1200 mg divided as *bid per os* for those with body weight greater than 75 kg. The whole treatment continued for 6 months.

2.4. Statistical analysis

The Statistical Package for the Social Sciences 13.0 (SPSS Inc., Chicago, IL, USA) statistical software package was used for the analysis. First, descriptive statistics were computed. The Wilcoxon signed-rank test was used to compare the differences in glutamic pyruvic transaminase (GPT), fasting insulin, glucose, and ferritin levels. In answering questions concerning the change in insulin resistance before and after the treatment, a mixed model was used to evaluate the impact of treatment.

3. Results

The demographic data for all 36 subjects are shown in Table 1. During the treatment period, two subjects dropped out from the study because of the negative side effects of the IFN treatment, such as anemia or flu-like symptoms. After the 6-month treatment course was completed, data were collected every 3 months. However, four additional subjects dropped further, eventually resulting in only 30 volunteers having gone through the whole study course with us.

Simultaneously, the body mass index (BMI) and waist circumference values were compared. Significant deductions in BMI and waist circumference were observed at the third and the sixth months after treatment ($p < 0.001$); nonetheless, they did not show any significant differences at the 12th month (Table 2).

3.1. Biochemical evaluation

The transaminase levels significantly decreased after treatment. Fasting insulin levels declined during the treatment course and then slightly elevated after cessation of treatment for 6 months. Fasting glucose levels also diminished and continued to decrease even after the cessation of treatment. The ferritin levels rose to a maximum after 3 months and then continued to decrease even after the cessation of treatment until lower than the baseline at 12 months. No significant changes were found in serum cholesterol and triglyceride levels during the follow-up period. In contrast, high-density lipoprotein levels remarkably decreased at the third and sixth month and then increased considerably at the 12th month (Table 2).

3.2. Insulin resistance

HOMA-IR was applied to measure the insulin resistance. The mixed model was used, and the measurements were analyzed repeatedly,

Table 1 The demographic data of the volunteer patients

Variable	Status	*n*	%
Age (yr)	50.38 ± 11.96 (range: 26–75)	36	100.00
Gender, *n*	Female	20	55.56
	Male	16	44.44
Exposition time (yr)	>10	16	44.44
	<10	14	38.89
	Undefined	6	16.67
Previous medication history (%)	Never experienced before	29	80.56
	Experienced use of interferon	7	19.44
Hypertension history	None	28	77.78
	Yes	8	22.22
Family history of diabetes	Yes	8	22.22
	None	25	69.44
	Undefined	3	8.33

Table 2 Changes in values of BMI, waist circumference, some related biochemical data, and adiponectin levels before and after treatment*

Mean ± SD	Baseline	Treatment period (mo)		
		3	6	12
BMI (kg/m^2)	24.91 ± 3.34	23.44 ± 3.15*	22.81 ± 2.81*	23.13 ± 2.08
Waist circumference (cm)	81.3 ± 8.5	77.46 ± 8.6*	76.7 ± 7.6*	77.1 ± 8.1
GPT	167.1 ± 104.7	45.9 ± 33.0*	44.4 ± 42.0*	30.77 ± 42.0*
Insulin (μIU/mL)	23.39 ± 29.13	20.90 ± 21.86	17.08 ± 11.99	21.54 ± 23.45
Fasting glucose level (mg/dL)	101.6 ± 11.8	98.3 ± 13.8	98.7 ± 14.0	90.3 ± 21.4
Ferritin (ng/mL)	254.1 ± 203.5	661.8 ± 427.9*	477.5 ± 431.7†	107.8 ± 123.5‡
Total cholesterol (mg/dL)	153 ± 23	147 ± 28	152 ± 39	170 ± 28*
HDL (mg/dL)	45 ± 11	39 ± 8†	39 ± 9‡	52 ± 15‡
Triglyceride (mg/dL)	86 ± 47	96 ± 46	96 ± 47	103 ± 56
Adiponectin	23.6 ± 8.8	22.4 ± 9.7	23.1 ± 8.3	26.2 ± 12.7

BMI = body mass index; HDL = high-density lipoprotein; SD = standard deviation.
After the 6-month treatment course was completed, data were collected every 3 months. Thirty patients joined the whole course study.
* Wilcoxon signed-rank test: *$p < 0.001$; †$p < 0.05$; ‡$p < 0.01$.

in which time was chosen as the independent variable and the degree of HOMA-IR as the dependent variable, whereas the parameters age, sex, and BMI were chosen as the covariates.

As can be seen in Table 3, time revealed significantly an effect on HOMA-IR ($p = 0.021$). BMI also cross-interacted with time ($p = 0.010$). Apparently, the degree of HOMA-IR seemed to change with time (Figure 1). However, after adjusting for age, sex, and BMI (Table 4), it, in reality, did not show any significant difference versus time in the *post hoc* analysis (data not shown).

3.3. Adiponectin

A mixed model was applied for repeated-measurement analysis. The time course of treatment was chosen as the independent variable and the levels of adiponectin as the dependent variables, whereas the parameters age, sex, and BMI were treated as the covariates. Adiponectin levels did not significantly change after therapy (Tables 2 and 3 and Figure 2).

4. Discussion

In July 2007, a population-based study confirmed that people when infected with HCV could encounter an elevated risk of developing Type 2 diabetes.[17] As insulin resistance is a key feature of obesity; it may play an important role in the pathogenesis and chronicity of HCV. However, the mechanism still remains to be elucidated.

Results reveal that insulin resistance increased after 3 months of treatment with α-IFN-2a/ribavirin. The HOMA-IR values then gradually decreased until the ninth month after the cessation of treatment. This combined therapy did not affect the insulin sensitivity in these subjects; on the contrary, it even improved after treatment. By following up, changes in the values of HOMA-IR were found on age, sex, and BMI adjustments (Table 4). As can be seen, the *post hoc* analysis did not reveal any significant change (data not shown), a problem remains to further investigation. A previous study showed that a 2-week treatment with IFN did not induce any apparent changes in plasma glucose and insulin profiles. IFN therapy reduced insulin-mediated glucose uptake in the peripheral tissues by 17% (from 44.4 ± 3.2 to 37.3 ± 3.0 μmol/kg min) ($p < 0.05$), more significantly by 38% with splanchnic glucose uptake (from 47 ± 2% to 29 ± 3%) ($p < 0.01$).

Long-term INF-α treatment enhances glucose tolerance in diabetic and nondiabetic subjects with HCV infection.[18] INF-α stimulates counter-regulatory hormone secretion and causes elevation of blood sugar levels and hepatic insulin resistance. The effects may disappear when INF-α is administrated over longer periods.[19] A recent study pointed out that no significant change in insulin resistance occurred after a 6-month INF-α and ribavirin combination therapy,[20] being rather consistent with our results.

Thus, in alignment with the aforementioned concept, any parameters derived from insulin, such as HOMA-IR, may not properly represent insulin resistance in subjects with chronic hepatitis. However, most of the studies often recruited subjects with liver cirrhosis, whereas our study excluded liver cirrhotic subjects, which, accordingly, would exclude some potential hyperinsulinemia cases. INF-α therapy usually induces progressive weight loss, and decreased waist circumference was noted (Table 1). Ferritin levels also were elevated after 3 months of treatment and gradually decreased until reaching the lowest levels at the 12th month. Type 2 DM has been proved to be associated with elevated ferritin levels.[21] Iron deposition in the liver may induce insulin resistance.[22] Adiponectin is a recently discovered plasma protein that is closely related

Table 3 The fixed-effect test of HOMA-IR and adiponectin

Effect	HOMA-IR			Adiponectin		
	df	*F*	*p*	df	*F*	*p*
Intercept	1	2.244	0.149	1	1.524	0.237
Time	3	4.214	0.021*	3	0.665	0.588
Age	1	0.633	0.436	1	0.163	0.691
Sex	1	2.138	0.158	1	0.106	0.748
BMI	1	2.350	0.140	1	2.575	0.132
Age × time	3	1.786	0.194	3	1.195	0.351
Sex × time	3	1.091	0.386	3	1.176	0.358
BMI × time	3	5.144	0.010*	3	0.952	0.444

*$p < 0.05$.
BMI = body mass index; HOMA-IR = homeostasis model assessment of insulin resistance.

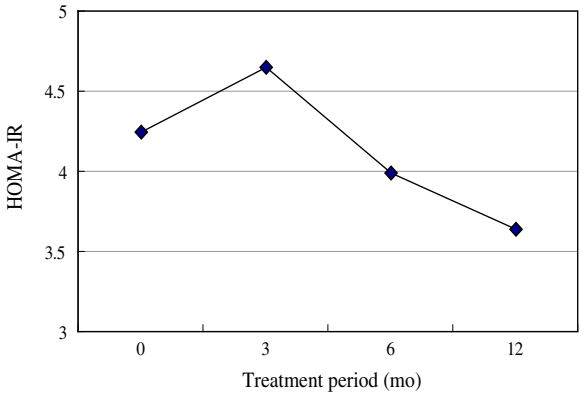

Figure 1 Time course change of nonadjusted degree of HOMA-IR values versus treatment period (mo). Degree of HOMA-IR increased in the first 3 months of combined therapy when compared with the baseline and then declined until the 12th month posttreatment. HOMA-IR = homeostasis model assessment of insulin resistance.

Table 4 Age-, sex-, and body mass index–adjusted degree of homeostasis model assessment of insulin resistance versus treatment period

Treatment period (mo)	Mean	Standard error
Baseline (0)	2.608	0.881
3	4.427	1.012
6	3.624	1.283
12	3.342	1.789

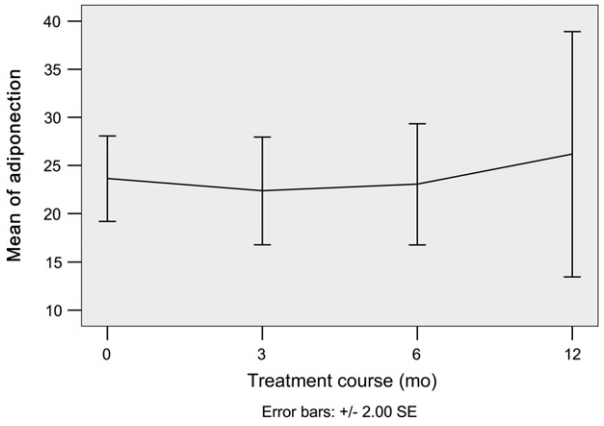

Figure 2 Change in adiponectin levels during the treatment course (mo). Error bars: ±2.00 standard error.

to glucose and lipid metabolism.[23] Its concentration has been reported to be significantly lower in the obese groups than that in the nonobese groups.[24] Adiponectin may play an important role in the pathogenesis of chronic HCV infection and even serve as an indicator of intrahepatic necroinflammation, steatosis, or fibrosis. Lu et al[15] indicated that treatment with IFN-α had resulted in a decrease in serum adiponectin levels but an improvement in insulin resistance in responders to the treatment. This study has the drawback of having too small number of subjects. Whether a larger population will behave differently is worth further tracking. In reality, the interaction mechanism between the liver adiponectin receptors and the combined treatment of INF-α plus ribavirin is rather complicated; a more systemic investigation might reveal clearer prognosis.

In conclusion, the present study reveals that the insulin resistance (presented as HOMA-IR) can be improved in parallel with unaffected adiponectin levels when treated with the combination therapy of IFN-α plus ribavirin. The reason for this remains to be further investigated.

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