

## Original Article

# Soluble TRAIL levels decreased in chronic hepatitis C treatment with pegylated interferon $\alpha$ plus ribavirin: association with viral responses

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**Abstract:** The molecular mechanisms and pathogenesis of chronic hepatitis C (CHC) infection are unclear. Innate immune cells such as natural killer (NK) cells and dendritic cells are responsible from molecular mechanism of CHC. NK cell cytotoxicity such as TRAIL expression is important pathway for viral clearance. The aim of this study was to evaluate the relationship between HCV RNA and sTRAIL levels during the first 12 weeks of Peg-IFN $\alpha$  and ribavirin treatment. Twelve treatment naive patients with CHC treated with Peg-IFN $\alpha$  and ribavirin were included in this study. Circulating sTRAIL and HCV RNA levels were measured at baseline, 4th and 12th week of treatment and their correlation was investigated. sTRAIL and HCV RNA levels decreased gradually with Peg-IFN $\alpha$  plus ribavirin treatment. The differences were significant between day 0, 4th week and 12th week of treatment. The expression of sTRAIL was correlated with HCV RNA level at baseline, at 4th and 12th week of treatment ( $P = 0.021$ ,  $P = 0.012$ ,  $P = 0.001$  respectively). IFN binds to its receptor on the infected hepatocyte surface during Peg-IFN $\alpha$  and ribavirin treatment. So the polarized phenotype of NK cell is not displayed and NK cell cytotoxicity such as TRAIL expression is blocked. We suggest that the decreased level of circulating sTRAIL may reflect increased binding to its ligand expressed on hepatocyte and decreased TRAIL production under the influence of Peg-IFN $\alpha$  plus ribavirin treatment. Therefore TRAIL may be probably a immunologically predictive factor such as HCV RNA during treatment.

**Keywords:** sTRAIL, chronic hepatitis C, HCV RNA, interferon

## Introduction

The hepatitis C virus (HCV) is a significant pathogen that causes fibrosis, cirrhosis, hepatocellular cancer as a result of the damage of liver cells. Chronic hepatitis C (CHC) infection is a public health problem that affects more than 170 million people globally [1]. A majority of patients infected with HCV do not spontaneously clear the virus and become chronically infected. The molecular mechanisms and pathogenesis of HCV persistence are not yet well understood. Chronic hepatitis develops in more than 80% of all HCV infected patients and in 20% of these patients lead to liver cirrhosis [2, 3]. Most studies on the immunology of CHC have focused on the innate immune response

[4, 5]. Innate immune cells such as natural killer (NK) cells and dendritic cells are responsible from molecular mechanism of liver injury in CHC. Previous studies have demonstrated that apoptosis and hepatocyte necrosis can cause liver injury [6, 7]. Following activation of innate immune system, cellular immunity including NK cell activation and antigen-specific CD8 cell proliferation occurs [3, 8]. CD8+ T lymphocytes directly kill infected cells via direct cell-cell contact and release antiviral cytokines [e.g. interferon (IFN), tumour necrosis factor (TNF)] [9]. IFNs are produced in vivo and also IFN $\alpha$  is currently used as a therapeutic drug for therapy of chronic hepatitis B and C. The well known standard treatment of chronic HCV infection is pegylated IFN $\alpha$  (Peg-IFN $\alpha$ ) in combination with

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**Table 1.** Laboratory characteristics of the patients

Parameter	Pre-treatment (n = 12)	4th week of treatment (n = 12)	Pre-treatment vs. 4th week p value	12th week of treatment (n = 12)	4th vs. 12th week p value	Pre-treatment vs. 12th week p value
Age (years)	48.08 ± 3.55	48.08 ± 3.55		48.08 ± 3.55		
HCV-RNA (IU/mL)	1443370.83 ± 395745.68	3705.08 ± 1734.24	0.002	601.58 ± 520.90	0.012	0.002
TRAIL (pg/mL)	300.24 ± 13.11	274.23 ± 8.37	0.034	228.39 ± 8.76	0.011	0.002
WBC (mm <sup>3</sup> )	6833.33 ± 731.37	3708.33 ± 236.60	0.002	3216.67 ± 282.80	0.041	0.002
Hemoglobin (g/dL)	13.39 ± 0.49	11.59 ± 0.49	0.003	11.03 ± 0.36	0.064	0.002
Platelets (mm <sup>3</sup> )	185583.33 ± 13161.85	152416.67 ± 12010.39	0.024	134916.67 ± 11595.75	0.032	0.003
MPV (um <sup>3</sup> )	11.08 ± 0.36	11.23 ± 0.43	0.078	11.27 ± 0.32	0.08	0.078
ALT (U/L)	61.67 ± 11.22	34.67 ± 10.65	0.002	24.17 ± 3.89	0.057	0.004
AST (U/L)	57.33 ± 17.00	30.33 ± 6.42	0.005	27.42 ± 2.89	0.064	0.016
AFP (ng/mL)	4.65 ± 0.69	3.67 ± 0.39	0.017	2.93 ± 0.40	0.023	0.006

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**Table 2.** Distribution of HCV-RNA, TRAIL level and HCV genotype during antiviral treatment in patient with chronic hepatitis C

Patient number	HCV genotype	HCV RNA level pre-treatment	TRAIL level pre-treatment	HCV RNA 4th week of treatment	TRAIL 4th week of treatment	HCV RNA 12th week of treatment	TRAIL 12th week of treatment
1	1b	1791562	379.29	10156	301.43	0	223.57
2	1b	2909650	251.43	0	230.71	0	217.86
3	1b	132613	258.57	51	237.86	12	265
4	1b	2778120	252.86	8520	267.14	0	217.86
5	1b	1269606	285	0	269.29	0	245
6	1b	160769	254.29	18194	238.57	6267	217.14
7	1b	936424	364.29	75	301.43	0	276.43
8	1b	1766555	331.43	7414	319.29	940	225.71
9	1b	4467157	315	12	298.57	0	269.29
10	1b	36497	273.57	0	268.57	0	186.43
11	1b	317297	295.71	39	262.86	0	181.43
12	1b	754200	341.43	0	295	0	215

ribavirin [10, 11]. IFN shows direct antiviral and immunomodulatory effects by binding to own receptor on the infected hepatocyte surface.

Furthermore, hepatocytes infected with HCV are the most likely sources of IFN $\alpha$ . But IFN $\alpha$  level may not be sufficient to manage viral clearance. Chronic exposure to HCV-induced IFN $\alpha$  causes a polarized NK cell phenotype that contributes to liver injury due to increased NK cell cytotoxicity such as TRAIL expression and degranulation but no viral clearance occurs owing to decline in IFN $\gamma$  production [12]. Consequently, NK cells contribute to viral clearance with elimination of infected hepatocytes via TRAIL-dependent mechanism.

Another pathway of viral clearance occurs via the ligands and receptors of the TNF superfamily such as TNF $\alpha$ /TNF-receptor 1, Fas ligand (FasL) and TRAIL receptor 1 and 2. It is shown that lymphocytes, recognising the viral antigen on hepatocytes, express cytolytic Fas ligand, while hepatocytes in the vicinity of lymphocytes exhibit enhanced Fas expression and become susceptible to FasL-mediated death [13]. Ligand binding induces the formation of signal complex, resulting in the activation of caspase-8 which culminates apoptosis of hepatocyte [9]. Consequently, TNF and Fas have been well described. TRAIL/TRAIL receptor 1-2 are last receptor system. TRAIL, a type II transmembrane glycoprotein and also called Apo2L, selectively induce apoptosis in HCV infected cells but not normal cells. TRAIL is recognized as the key receptor of apoptosis in infected

hepatocytes. Previous studies have showed that the expression level of TRAIL is much higher in the HCV infected group than healthy controls [14, 15].

According to our knowledge, TRAIL expression in relation to HCV RNA level during antiviral treatment has not been described yet in the literature. In this present study, we assessed the association between the level of TRAIL expression and HCV RNA level during the first 12 weeks of treatment.

### Material and method

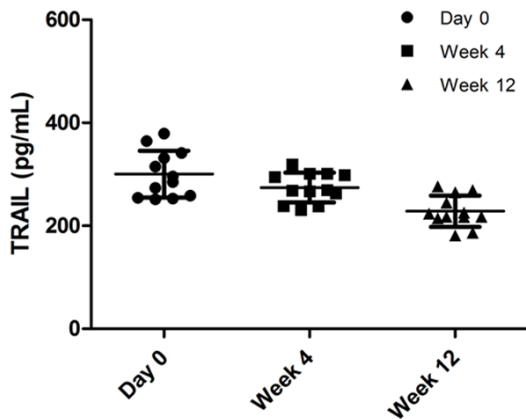
#### Patients

Twelve treatment naive patients with CHC were treated with Peg-IFN $\alpha$ -2a at a dose of 180  $\mu$ g/week or Peg-IFN $\alpha$ -2b at the standard dose, 1.5  $\mu$ g/kg of body weight/week, both in combination with oral ribavirin at a dose of 1000-1200 mg/day, according to body weight (75 kg, 1000 mg/day;  $\geq$  75 kg, 1200 mg/day). All patients gave written informed consent. The study was approved by our hospital local committee on ethics. Blood samples of patients were tested for complete blood counts, serum alanine aminotransferase (ALT), aspartat aminotransferase (AST), alpha fetoprotein (AFP), HCV genotype (only baseline) sTRAIL and HCV RNA levels at baseline, 4th and 12th week of treatment.

#### Laboratory tests

HCV RNA levels were measured using Abbott m2000sp real time system (Abbott Molecular

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**Figure 1.** sTRAIL levels. Pre-treatment:  $300.24 \pm 13.11$ , 4th week of treatment:  $274.23 \pm 8.37$ , 12th week of treatment:  $228.39 \pm 8.76$ . Data is expressed as Mean  $\pm$  SD.

Diagnostic, ABD), which has a lower limit of quantification of 15 IU/ml. Abbott m 2000 r (Abbott Molecular Diagnostic, ABD) was used for genotype determination

TRAIL concentrations in serum samples were analyzed using a TRAIL/APO2L ELISA kit (Diaclone, France) according to the manufacturer's instructions. The absorbance values were measured using a spectrophotometer set at 450 nm, and the concentrations of sTRAIL (pg/ml) calculated from OD readings of recombinant standards.

### Statistical analysis

Analysis of the data was performed with SPSS 18.0 (New York, USA). The data were presented as means  $\pm$  SEM. Comparison of parameters was performed using Wilcoxon signed rank test. To assess relationship between measured parameters, we used Spearman correlation. *P*-value less than 0.05 indicated significance for all analyses.

### Results

Laboratory characteristics of the patients was shown in **Table 1**. Mean baseline HCV-RNA level was  $1443370.83 \pm 395745.68$  IU/mL; TRAIL level was  $300.24 \pm 13.11$  pg/mL; ALT level was  $61.67 \pm 11.22$  U/L; AST level was  $57.33 \pm 17.00$  U/L and AFP level was  $4.65 \pm 0.69$  ng/mL.

Distribution of HCV-RNA, TRAIL level and HCV genotype during antiviral treatment in patient

with CHC was presented in **Table 2**. HCV-RNA levels decreased gradually with Peg-IFN $\alpha$  plus ribavirin treatment and the differences were significant between day 0 and 4th week ( $P = 0.002$ ,  $P < 0.005$ ); between day 0 and 12th week ( $P = 0.002$ ,  $P < 0.005$ ); between 4th week and 12th week ( $P = 0.012$ ,  $P < 0.05$ ).

sTRAIL levels decreased with Peg-IFN $\alpha$  plus ribavirin treatment and the differences were significant between day 0 and 4th week ( $P = 0.0034$ ,  $P < 0.05$ ); between day 0 and 12th week ( $P = 0.002$ ,  $P < 0.005$ ); between 4th week and 12th week ( $P = 0.011$ ,  $P < 0.05$ ) (**Figure 1**).

The expression of sTRAIL was correlated with HCV RNA level at baseline, at 4th and 12th week of treatment ( $P = 0.021$ ,  $P = 0.012$ ,  $P = 0.001$  respectively).

ALT and AST levels also decreased significantly between day 0 and 12th week ( $P = 0.004$ ,  $P < 0.005$  and  $P = 0.016$ ,  $P < 0.05$  respectively).

We also observed significant decrease in AFP level and the differences were significant between day 0 and 4th week ( $P = 0.017$ ,  $P < 0.05$ ); day 0 and 12th week ( $P = 0.006$ ,  $P < 0.01$ ); 4th week and 12th week ( $P = 0.023$ ,  $P < 0.05$ ).

### Discussion

CHC leads to liver injury. Disease progression and respond to treatment depend on host immune responses and viral replication. Information regarding pathogenetic mechanism of CHC infection is limited. When NK cells are activated in chronically HCV-infected patients, they exhibit a polarized phenotype with increased TRAIL expression and degranulation, but not IFN $\gamma$ . Consequently, IFN $\gamma$ -mediated viral clearance is more effective than elimination of virus-infected hepatocytes by cytotoxic mechanisms [12]. Stegmann et al. also showed that IFN $\alpha$  induced TRAIL has a highly functional role and contributed to elimination of infected hepatocytes in CHC infection [16]. These results explain decreases in HCV-RNA level during Peg-IFN $\alpha$  based therapy. Therefore, guidelines recommend the use of Peg-IFN $\alpha$  in combination with ribavirin for the treatment of CHC.

Apoptosis (programmed cell death) is an active physiological process that leads to the ordered

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destruction of cells without release of intracellular contents into the extracellular environment [17]. It is a fundamental process to maturation and homeostasis of the immune system. Apoptosis can be induced passively through lack of essential survival signals or actively through ligand induced trimerization of specific death receptors of the TNF receptor family such as Fas, the TNF receptor or the TRAIL receptor [18]. The responsibility of Fas/FasL system is recognized as contributing to increased apoptosis. Recently, interest has focused on TRAIL, several studies have indicated that TRAIL has a important role in the pathophysiology of different disease states including cancer, viral infections and autoimmune diseases. The apoptosis following interaction of TRAIL with its ligand may contribute to these diseases [19-22].

It is known that the TRAIL level is significantly higher in patients with CHC [23, 24]. Because TRAIL is a defense mechanism to eliminate infected hepatocytes. The purpose of CHC treatment is to achieve the sustained virological response (SVR) after combined Peg-IFN $\alpha$  and ribavirin treatment. HCV genotype and serum HCV RNA levels are the most important pretreatment predictive viral factors. The recognized predictive factors for SVR during treatment is rapid virological response [defined as an undetectable serum HCV RNA level at week 4 of treatment with Peg-IFN $\alpha$  plus ribavirin (RVR)] and early virological response (defined as serum HCV RNA being either undetectable or 2 log<sub>10</sub> lower at week12 than before treatment (EVR)). These predictive factors are important milestones in the treatment of patients with CHC. There is a positive correlation between the magnitude of the decrease in the HCV RNA level at week 4 and 12 and the probability of SVR [25]. But clinicians would like to establish probably other predictive factors relation to SVR. We asked to whether sTRAIL level may be a predictive factor such as HCV RNA during the first 12 weeks of antiviral therapy. In the present study, we analyzed HCV RNA level and the kinetics of sTRAIL at baseline, at 4th and 12th weeks of treatment in patients with CHC and investigated correlation between HCV RNA and sTRAIL level. We found that sTRAIL and HCV RNA level decreased significantly and the expression of sTRAIL correlated with the HCV RNA level at baseline, at 4th and 12th weeks of treatment. We suggest that the

decreased level of circulating sTRAIL may reflect increased binding to its ligand expressed on hepatocyte or lymphocyte under the influence of Peg-IFN $\alpha$  plus ribavirin treatment. Recently, TRAIL-receptor 2, 4 and caspase-8 were found to be up-regulated in patients with CHC infection, whereas TRAIL-receptor 3 to be down-regulated and SVR correlated with high expression of TRAIL and pro-apoptotic TRAIL-receptor 2 on HCV infected hepatocytes [26].

Several investigators also analysed whether TRAIL has the predictive feature on different issues of CHC. Ahlenstiel et al. reported that NK cell response may be accepted as a biomarker for virologic response to standart Peg-IFN $\alpha$  and ribavirin therapy in CHC [27]. Because Peg-IFN $\alpha$  activates NK cells during early phase of treatment. So cytotoxic function of NK cells is strongly stimulated, which measure by degranulation and TRAIL expression. Consequently, a higher frequency of TRAIL-expressing NK cell and increased degranulation and TRAIL expression correlate to virologic response. Brost et al. also found that TRAIL was highly expressed in patients achieving SVR compared to non-responders [26]. SVR correlated with high expression of TRAIL on HCV infected hepatocytes. Piekarska et al. showed a strong inverse association between the expression of TRAIL and stage of fibrosis, grade of inflammation. The highest expression of TRAIL was determined in patients with low grade and low stage of disease [28]. Therefore TRAIL may have a pathophysiological predictor role in CHC patients. Thus far, only one report analysed sTRAIL level during antiviral therapy [29]. In contrast with our results, they found that the sTRAIL levels increased within 24 hours in all patients and high sTRAIL levels continued at the 4th and 12th week of therapy compared with baseline. This difference may be depend on effects related to unknown other immune mechanisms during antiviral therapy.

TRAIL-related apoptosis is the essential mechanism for the elimination of virally infected cells. This is first study on the expression of sTRAIL in relation to HCV RNA level during the first 12 weeks of standard treatment. According to our results, TRAIL may be probably a immunologically predictive factor such as HCV RNA. But the role of TRAIL expression under antiviral therapy is also not completely understood so

far. Therefore, additional studies need to investigate the level of TRAIL expression in the different phases of antiviral therapy.

**Disclosure of conflict of interest**

None.

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