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The importance of *IL28B* polymorphism in response to pegylated interferon α and ribavirin in chronic hepatitis caused by HCV genotype 1b

Znaczenie polimorfizmu *IL28B* w odpowiedzi na leczenie interferonem pegylowanym α i rybawiryną przewlekłego zapalenia wątroby wywołanego genotypem 1b HCV

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Słowa kluczowe: zapalenie wątroby, HCV, *IL28B*, leczenie.

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Abstract

Introduction: Treatment of chronic hepatitis C (CH-C) with peginterferon α (Peg-IFN) and ribavirin leads to a sustained virological response (SVR) in 50% of patients and depends on HCV and host factors. Polymorphism of the *IL28B* gene is associated with eradication of HCV and SVR.

Aim: The objective of this study was to examine patients from the region of southern Poland and determine whether CH-C therapy depends on the genetic variants of *IL28B* and whether this polymorphism may predict SVR.

Material and methods: One hundred forty-two patients with CH-C and the genotype 1b HCV were treated with Peg-IFN and ribavirin for 48 weeks. *IL28B* rs12979860 polymorphisms (C/T) were tested by PCR-RFLP. The HCV-RNA and alanine aminotransferase (ALT) levels were measured before treatment and after 12, 48 and 72 weeks.

Results: Viral load before and after 12 weeks of therapy was higher in the genotypes T/C and T/T than in C/C. 71.1% of patients with the genotype C/C achieved SVR vs. 41.4% with the genotype T/C and 23.5% with T/T. Patients with the genotype C/C responded better to treatment as compared to subjects with the genotypes T/C and T/T, and achieved better early response (12 weeks), at the end of treatment (48 weeks) and SVR. Alanine aminotransferase activity was similar among the groups. Tolerance of therapy was similar and independent of the genotypes.

Conclusions: The study confirmed the dependence of response to standard treatment of CH-C caused by the geno-

Streszczenie

Wstęp: Leczenie przewlekłego zapalenia wątroby typu C (PZW-C) pegylowanym interferonem α (Peg-IFN) z rybawiryną powoduje tzw. trwałą odpowiedź wirusologiczną (*sustained virological response* – SVR) u blisko 50% chorych. Odpowiedź ta ściśle zależy od HCV i czynników genetycznych chorego. Wykazano, że polimorfizm genu *IL28B* wiąże się z eradykacją wirusa i SVR.

Cel: Zbadanie, czy wyniki leczenia PZW-C u chorych z regionu Polski Południowej zależą od wariantów genetycznych *IL28B*, a polimorfizm może być czynnikiem predykcyjnym SVR.

Materiał i metody: Sto czterdzieści dwie osoby z PZW-C spowodowanym genotypem 1b HCV leczono standardowo Peg-IFN z rybawiryną przez 48 tygodni. Polimorfizmy rs12979860 *IL28B* (C/T) badano metodą PCR-RFLP. HCV-RNA i aktywność aminotransferazy alaninowej (ALT) w surowicy mierzono przed leczeniem i po 12, 48 i 72 tygodniach terapii.

Wyniki: Wiremia HCV-RNA przed terapią i po 12-tygodniowym leczeniu była wyższa u chorych z genotypem T/C i T/T. Trwałą odpowiedź wirusologiczną osiągnęło istotnie więcej chorych z genotypem C/C (71,1%) w porównaniu z 41,4% z genotypem T/C i 23,5% T/T. Chorzy z genotypem C/C lepiej reagowali na leczenie w porównaniu z osobami z genotypami T/C i T/T, osiągali wyższe wskaźniki odpowiedzi wczesnej (12 tygodni), na koniec leczenia (48 tygodni) i SVR. Aktywność ALT nie różniła się istotnie podczas terapii pomiędzy grupami. Tolerancja leczenia była podobna i nie zależała od genotypów C/C, T/C i T/T.

type 1b HCV on the patient's genetic predisposition. In contrast to some other reports, poor prognosis was observed in T/T homozygotes of *IL28B*.

Wnioski: Wyniki badania potwierdziły zależność odpowiedzi na standardowe leczenie PZW-C spowodowanego genotypem 1b HCV od predyspozycji genetycznej chorego, jednak w przeciwieństwie do niektórych doniesień złe rokowanie obserwowano u homozygot T/T *IL28B*.

Introduction

Hepatitis C virus (HCV) infection is a serious global problem, with the number of affected individuals worldwide approximating 170 million people and about 0.7 million individuals in Poland. The virus causes chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The standard antiviral therapy is pegylated interferon α (Peg-IFN) and ribavirin, but treatment response is not satisfactory. Sustained virological response (SVR), defined as undetectable HCV-RNA in blood 6 months after 48 weeks of treatment, is seen in 80% of subjects and is lower (about 50%) in the HCV genotype 1 infection [1]. The effectiveness of treatment depends on the HCV (genotype, viral load) and the genetic host factors [2-4]. In recent years, it has been shown that a single nucleotide polymorphism (SNP) in the region of the interleukin-28B (*IL-28B*) gene significantly correlates with spontaneous elimination of HCV and SVR [5]. The SNP rs12979860 was described in the *IL28B* region and the investigators demonstrated that the genetic profile is associated with favourable alleles C/C *IL28B* [2-7]. However, variants of the T/C or T/T *IL28B* gene are characterized by a lower percentage of SVR. The pharmacogenetics of chronic hepatitis is associated with a dependence of *IL28B* genotyping with the elimination of HCV and is important in the individualization of therapy, predicting the effectiveness and costs [3, 8].

The HCV genotype 1b occurs in more than 80% of patients in Poland [9], and the association of the response to treatment with the *IL28B* genetic determinant in this population has not been previously evaluated.

Aim

The aim of this study was to investigate whether in patients with chronic hepatitis caused by the HCV genotype 1b from the region of southern Poland, the HCV treatment outcomes depend on the rs12979860 variant in *IL28B*, and also whether this polymorphism can be considered a predictor of response to therapy.

Material and methods

The study included 142 consecutive adult patients of a Slavic ethnic origin, with chronic hepatitis induced by the HCV genotype 1b, who reported for treatment at University Hospital in Krakow and met the following criteria. Inclusion criteria: anti-HCV and HCV-RNA present in the serum and elevated alanine aminotransferase (ALT), evaluated at least 6 months before inclusion, histological examination confirming chronic hepatitis, body mass index (BMI) below 30 kg/m^2. Exclusion criteria: decompensated cirrhosis, liver cancer, HBV or HIV infection, autoimmune liver disease, alcohol abuse, diabetes, dyslipidaemia, severe chronic diseases, immunosuppressive therapy.

The patients were treated for 48 weeks: Peg-IFN α-2a (Roche), 180 µg/kg subcutaneously once per week with ribavirin (Roche) orally, 1-1.2 g daily or Peg-IFN α-2b (Schering-Plough, MSD) 1.5 µg/kg of body weight once a week with ribavirin (Schering-Plough, MSD), 1-1.2 g daily. After treatment, the patients were followed for 24 weeks.

The study was conducted in accordance with the Helsinki Declaration of 1975, and the patients were informed about the study and provided written consent to participate in the study.

Serum anti-HCV, HCV-RNA and ALT were determined. HCV-RNA was measured by reverse transcriptase – polymerase chain reaction (RT-PCR) (Cobas TaqMan HCV Test v. 2.0, Roche Molecular Systems, Inc., USA). HCV-RNA and ALT were determined before treatment and after 12, 48 and 72 weeks. Early virological response (EVR) was defined as undetectable HCV-RNA or at least a 2-log drop in HCV RNA from the baseline at week 12 of treatment; end treatment response (ETR) was defined as undetectable HCV-RNA at the end of the 48-week treatment.

Liver biopsies were performed within the 12 months prior to enrolment. Biopsies containing at least 6 portal spaces were stained with haematoxylin and eosin, the Gomori-Masson method and trichrome for the presence of connective tissue. In staging the fibrotic liver changes, the Batts and Ludwig scale was applied. The characteristics of patients are shown in Table I.

Genomic DNA was isolated from peripheral blood samples using proteinase K – a set of ion-exchange extraction column (A&A Biotechnology, Gdynia, Poland). An amplification product 190 bp in length was obtained using a standard PCR with primers 5'-GCC TCT TCC TCC TGC GGG ACA AG and 5'-GCG CGG GCA AGT ATT CAA CCC T Bsh1236I. After digestion with endonuclease, the resulting products were distributed in the agarose gel electrophoresis. Option C was digested with the enzyme. All the laboratory procedures were carried out on blinded samples.

Table I. Characteristics of patients with chronic hepatitis treated with Peg-IFN and ribavirin depending on the genotypes of *IL28B*
Tabela I. *Charakterystyka chorych na przewlekłe zapalenie wątroby leczonych Peg-IFN i rybawiryną w zależności od genotypu IL28B*

Parameter	C/C (*n* = 38)	T/C (*n* = 70)	T/T (*n* = 34)	Value of *p*
Age [years]	42.2 ±11.6	45.2 ±10.5	44.2 ±13.6	0.698
Women, *n* (%)	14 (36.8)	32 (45.7)	14 (41.2)	0.544
Men, *n* (%)	24 (63.2)	38 (54.3)	20 (58.8)	
Duration of infection [years]	9.7 ±2.7	11.4 ±3.1	8.5 ±1.9	0.420
Body mass index [kg/m^2]	28.1 ±4.5	25.3 ±3.2	26.6 ±4.5	0.106
Viral load, × 10^6 IU/ml	2.82 ±3.17	5.08 ±8.27	3.85 ±6.36	0.560
Liver fibrosis, *n* (%)				
F0-2	28 (73.7)	60 (85.7)	26 (76.5)	0.210
F3-4	10 (26.3)	10 (14.3)	8 (23.5)	0.125
ALT [U/l]	74.1 ±27.6	88.0 ±31.0	118.6 ±36.7	0.330
Peg-IFN α-2a, *n* (%)	20 (52.6)	34 (48.6)	14 (41.2)	0.954
Peg-IFN α-2b, *n* (%)	18 (47.4)	36 (51.4)	20 (58.8)	

Mean ± SD, n – number, p for Fisher's test, liver fibrosis according to Batts-Ludwig (F0-2 less advanced, F3-4 more advanced)

Table II. HCV-RNA viraemia during treatment and response of patients to therapy of chronic hepatitis depending on the genotypes of *IL28B*
Tabela II. *Wiremia HCV-RNA podczas leczenia i odpowiedź na terapię chorych na przewlekłe zapalenie wątroby w zależności od genotypu IL28B*

	C/C (*n* = 38)	T/C (*n* = 70)	T/T (*n* = 34)	Value of *p* CC vs. TC and TT
HCV-RNA viral load (value × 10^6 IU/ml)				
Before treatment	2.82 ±3.17	5.08 ±8.27	3.85 ±6.36	NS
After 12 weeks	0.01 ±0.02	0.09 ±0.13	0.35 ±0.75	0.01
Response to therapy, *n* (%)				
Early – EVR	24 (63.2)	24 (34.3)	6 (17.7)	0.025
End of therapy – ETR	35 (92.1)	47 (67.1)	18 (52.9)	0.043
Sustained – SVR	27 (71.1)	29 (41.4)	8 (23.5)	0.006

Mean ± SD, n – number, p for Fisher's test, NS – not significant (p > 0.05), vs. = one against another, virological response: EVR – 12 weeks, ETR – 48 weeks, SVR – 6 months after the 48-week treatment

Statistical analysis

Statistical analysis was performed using the chi-square test and Fisher's exact probability test to compare categorical variables. After analysis of results with the Shapiro-Wilk test, the results were compared by the Wilcoxon-Mann-Whitney U-test, Kruskal-Wallis test or Student's t-test. The analyses were performed using Statistica v. 9.0 (StatSoft, Inc., USA). The results are presented as mean values and standard deviations (SD), and $p < 0.05$ in the two-sided test was taken as statistically significant.

Results

The genotyping of *IL28B* showed the presence of the genotype C/C in 38 patients (26.8%), genotype T/C in 70 patients (49.2%) and genotype T/T in 34 patients (24%). HCV-RNA viral load in serum of patients before treatment and after 12 weeks, during the evaluation of early response to therapy in the groups of genotypes C/C, T/C and T/T, is shown in Table II. The baseline viral load was not significantly lower in patients with the genotype C/C (2.82 ±3.17 × 10^6 IU/ml) as compared to individuals with T/C and T/T (respectively: 5.08 ±8.27 and 3.85 ±6.36 × 10^6 IU/ml). HCV-RNA viral load after 12 weeks of treatment in patients with the genotype C/C was significantly ($p < 0.01$) lower (0.01 ±0.02 × 10^6 IU/ml) as compared with T/C and much lower than in the genotype T/T (Table II). Additionally, the Spearman's rank correlation performed between HCV-RNA viral load and ALT activity in patients before treatment and after 12 weeks of therapy did not confirm the presence of any significant relationships.

Among all patients treated with Peg-IFN and ribavirin, 38% achieved EVR, 69.7% achieved ETR, and 44.4% achieved SVR. Response to treatment depending on the genotypes C/C, T/C and T/T of *IL28B* is shown in Table II. The EVR, ETR and SVR in patients with the genotype C/C as compared with the genotypes T/C and T/T were significantly higher. The SVR, which is the criterion of the effectiveness of HCV infection therapy, was achieved in 71.1% of patients with the genotype C/C of *IL28B*, compared with 41.4% in patients with the genotype T/C, and 23.5% in the genotype T/T ($p = 0.006$). Tolerance of treatment was similar and independent of the genotypes C/C, T/C and T/T of *IL28B*.

The ALT activity assessed at the baseline and after 12, 24, 48 and 72 weeks did not differ significantly between the groups with the genotypes C/C, T/T and T/C.

Thus, the independent predictors of SVR in patients with the genotype C/C of *IL28B* and infected with the difficult to treat HCV genotype 1b were: viral load after 12 weeks of treatment, EVR, and ETR; and in patients

with elevated ALT at the baseline, they were enzyme activity after 4 and 12 weeks of therapy.

Discussion

Genetic studies have shown that in patients with chronic hepatitis caused by the HCV gene, polymorphism rs12979860 in *IL28B* is closely related to the effectiveness of treatment with Peg-IFN and ribavirin [4-6, 10]. Moreover, it has been shown that genetic alterations in *IL28B* may be predictors of response to this treatment [3, 4, 10-13]. The association of host genetic changes with the treatment outcome in patients with chronic hepatitis have not yet been tested in a population of Polish patients infected with the HCV genotype 1b. We have shown that HCV-RNA in serum of patients at the baseline did not differ significantly among patients with the genotypes C/C, T/C and T/T *IL28B*. However, after 12 weeks of treatment, HCV-RNA viral load was significantly lower in patients with the genotype C/C versus T/T and did not differ between the genotypes C/C and T/C. These results differ from previous studies of other authors who showed a higher initial viral load of the HCV genotype C/C in an American population [6, 11]. Early treatment response (EVR) reached 63.2% in patients with the genotype C/C, was significantly lower (34.3%) in individuals with the genotype T/C, and was only 17.7% in T/T. These results are poorer than those obtained by other authors [2, 4]. Thompson *et al.* described EVR in 87% of patients with the genotype C/C *IL28B* and in 38% and 28%, respectively, of patients with the genotype T/C and T/T [4]. These differences may be due to a different patient population and an initial viral load, which are prognostic factors of response [2]. Several authors have also confirmed a significantly better response to treatment in patients with the genotype C/C compared to the genotypes T/C and T/T [2, 4, 7].

Virological response at the end of treatment (ETR) reached 92.1% in patients with the genotype C/C, 67.1% with T/C, and only 52.9% with the genotype T/T. These results are similar to other studies [4-6]. The SVR is the most important indicator of treatment efficacy of patients with HCV. In this study, SVR was observed in 71.1% of patients with the genotype C/C and was significantly (p = 0.03) lower in subjects with the genotype T/C (41.4%) and with the genotype T/T (23.5%). The multivariate analysis showed that the genotype C/C of *IL28B* was associated with a significantly higher incidence of ETR and SVR achieved by the patients. The results of these studies are similar to those presented by other authors [4-6].

The mean ALT level exceeded the upper normal limit at the baseline in all the groups and during treatment was similar regardless of the genotype *IL28B*. Contrary results were obtained by other authors who observed higher ALT in patients with the genotype C/C as compared with T/C and T/T [11]. It should be noted that normal ALT activities are often recorded in patients with chronic hepatitis caused by HCV.

In conclusion, patients with chronic hepatitis caused by the difficult-to-treat HCV genotype 1b treated with Peg-IFN and ribavirin achieved a sustained virological response, which in patients with the genotype C/C *IL28B* was significantly better in comparison to the genotype T/C (71.1% vs. 41.4%) and the genotype T/T (71.1% vs. 23.5%). The study confirmed, in a patient population from the region of southern Poland, that the response to standard treatment of HCV genotype 1b chronic hepatitis depends on polymorphism of the *IL28B* gene, which may be a predictor of virus elimination in these patients.

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References

1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001; 358: 958-65.
2. Fattovich G, Covolo L, Bibert S, et al. IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C. Aliment Pharmacol Ther 2011; 33: 1162-72.
3. Ahlenstiel G, Booth DR, George J. IL28B in hepatitis C virus infection: translating pharmacogenomics into clinical practice. J Gastroenterol 2010; 45: 903-10.
4. Thompson AJ, Muir AJ, Sulkowski MS, et al. Interleukin 28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 2010; 139: 120-9.
5. Clark PJ, Thompson AJ, McHutchison JG. IL28B genomic-based treatment paradigms for patients with chronic hepatitis C infection: the future of personalized HCV therapies. Am J Gastroenterol 2011; 106: 38-45.
6. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009; 461: 399-401.
7. Balagopal A, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. Gastroenterology 2010; 139: 1865-76.
8. Afdhal NH, McHutchison JG, Zeuzem S, et al. Hepatitis C pharmacogenetics: state of the art in 2010. Hepatology 2011; 53: 336-45.
9. Juszczyk J, Beniowski M, Berak H, et al. Effectiveness of combined treatment with pegylated interferona-2a and ribavirin in chronic hepatitis C – study phase summary. Med Sci Monit 2004; 10 (S1): 5-11.

10. Montes-Cano MA, García-Lozano JR, Abad-Molina C, et al. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. Hepatology 2010; 52: 33-7.
11. Grebely J, Petoumenos K, Hellard M, et al.; ATAHC Study Group. Potential role for interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. Hepatology 2010; 52: 1216-24.
12. McCarthy JJ, Li JH, Thompson A, et al. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology 2010; 138: 2307-14.
13. Pearlman BL. The IL-28 genotype: how it will affect the care of patients with hepatitis C virus infection. Curr Gastroenterol Rep 2011; 13: 78-86.