

Safety and efficacy of pegylated interferon and ribavirin in an aplastic anemia adolescent infected by hepatitis C virus

Xiaofei Li¹, Hua Li², Xianqiang Zhang², Quancheng Kan¹, Zujiang Yu²

¹Department of pharmacology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China

²Department of the Infectious disease, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China

johnyuem@zzu.edu.cn

Abstract: To analyze on the curative safety and efficacy, we dynamically observed the therapeutic and side-effects from a 43-year-old woman with aplastic anemia(AA) and infected by hepatitis C virus (HCV) after the combination treatment of PEG-IFN- α 2a plus ribavirin. During the combination treatment of PEG-IFN- α 2a (Peg-IFN- α ,135 μ g, weekly) plus ribavirin (900 mg/day) for 72 weeks, hepatitis C virus ribonucleic acid (HCV RNA), blood bio-chemistry and counts of blood cell(CBC) were detected every 4 or 8 weeks. At the end of the 24 weeks follow up, T leukocytes subsets, level of HCV RNA were assayed and bone marrow aspiration smeared again. From the above detected data, viral-load of HCV were declining at the first 12-weeks, disappeared at the 32th-week and lasted negatively till the end of the follow up; CBC including white blood cell(WBC), neutrophilicgranulocyte (Neu) and blood platelet were changed like “U ” model, that was, decreased at the first 3weeks, fluctuated in the next 44weeks, and elevated in the last 24 weeks; blood chemistry including serum alanine aminotransferase(ALT), aspartate aminotransferase (AST) were abnormal before antiviral therapy and recovered after injecting Peg-IFN alfa-2a for 8 weeks; T lymphocyte subsets and bone marrow aspiration smear diagnosis were similar as the pre-treatment in the follow up. These results suggested that chronic hepatitis C patient with AA could acquire sustained virologic response (SVR) and had no permanently affection to bone marrow hemopoiesis, although transient reduction in CBC after the therapy of Peg-IFN plus ribavirin. Otherwise, the delayed early virologic response (EVR) to Peg-IFN may be correlated with aplastic anemia.

[Xiaofei Li, Hua Li, Xianqiang Zhang, Quancheng Kan, Zujiang Yu. **Safety and efficacy of pegylated interferon and ribavirin in an aplastic anemia adolescent infected by hepatitis C virus.** *Life Sci J* 2013; 10(3): 67-71]. (ISSN: 1097-8135). <http://www.lifesciencesite.com> 12

Key words: Pegylated interferon; Hepatitis C; Aplastic anemia

Introduction:

Hepatitis C virus (HCV) remains a major medical problem. Antiviral treatment is only partially effective and a vaccine does not exist. Paradoxically, hepatitis C is the only human chronic viral disease that can be cured, as all other pathogenic viruses infecting humans either display self-limited courses or establish non-eradicable persistent infections. The combination treatment of peginterferon- α plus ribavirin, which provided limited rates of cure, was associated with frequent side effects[1]. Hepatitis-associated aplastic anemia (HAAA) is a not uncommon syndrome in patients with bone marrow failure, with hepatitis documented in 2 to 5% of cases of AA occurring in the West and 4 to 10% in the Far East. The proportion of HAAA in severe aplastic anemia (SAA) was 3.3% in China[2]. Till now, to the patient with HAAA, there are no reports about chronic hepatitis C consisted of the combination treatment because, we usually afraid, those patients may be developing to SAA again after treatment[3,4]. Herein, we reported the motive change in sera indexes including HCV-RNA, CBC, blood bio-chemistry, etc, from an AA patient infected by

HCV following Peg-IFN plus ribavirin treatment. At the last, the SVR and bone marrow hemopoiesis were analyzed in the follow up.

Case presentation:

A 43-year-old China woman was diagnosed with AA and HCV infection (genotype Ib), a combination treatment of PEG-IFN- α 2a (135 μ g, weekly, Roche) and ribavirin (900 mg/day) was commenced for a period of 96 weeks. Before starting the combination treatment, her blood tests were un-normal with a platelet count of 56,000 cells/mm³, hemoglobin(Hb) of 8.4 g/dl, and a white blood cell (WBC) count of 3100 cells/mm³. Her serum HCV ribonucleic acid (RNA) levels were more 5.916 \times 10⁶units/ml(Roche), On admission, serology for HIV, and hepatitis A and B viruses was negative. Blood bio-chemistry showed the following: urea 30 mg/dl (normal range 17 to 50 mg/dl), creatinine 1.0 mg/dl (normal range 0.7 to 1.4 mg/dl), sodium 139 mMol/L (normal range 136 to 145 mMol/L), potassium 3.8 mMol/L (normal range 3.5 to 5.0 mMol/L), glucose 99 mg/dl (normal range 74 to 115 mg/dl), calcium 8.8

mg/dl (normal range 8.6 to 10.2 mg/dl), amylase 156 U/L (normal range 0 to 40 U/L), creatine phosphokinase 200 U/L (normal range 20 to 190 U/L), lactate dehydrogenase 296 U/L (normal range 200 to 460 U/L), uric acid 4.6 mg/dl (normal range 3.5 to 7.2 mg/dl), A B ultrasonic examination of the patient's abdomen (liver, spleen and bladder) was unremarkable. Screening for several auto-antibodies was negative. Thyroid function tests and complement serum levels were normal.

In 1988, the patient's bone marrow biopsy was profoundly hypocellular with a decrease in all haematopoietic cells; the bone marrow space was composed mostly of fat cells and marrow stroma. The diagnosis of AA was made in the patient. A mono-treatment of Stanozolol (2mg, tid) was commenced for a phase of 24 weeks, had curative effects and stopped the treatment. In 2008, the patient's bone marrow biopsy, CBC, and counts of Ret all showed AA (Figure I and table2) again and

HCV-antibody was positive. Because of afraid of the side-effects of Peg-IFN- α to the bone marrow hemopoiesis, the patient refused to be given the combination treatment. But following the repeated flare of hepatitis, the patient was to be hospitalized and commenced a combination treatment of PEG-IFN- α 2a (135 μ g, weekly) and ribavirin (900 mg/day). To our surprised, the treatment was well tolerated by the patient. During the phase of treatment (total 96 weeks), HCV RNA, blood bio-chemistry, CBC and liver functions were dynamically observed every 4~8 weeks. Peripheral-blood mononuclear cells were examined by flow cytometry to quantitate the number and phenotype of T lymphocytes with directly conjugated monoclonal antibodies against CD3⁺, CD4⁺, CD8⁺ and CD19⁺ at the end of the 24 weeks follow up. Level of HCV RNA was assayed and bone marrow aspiration smeared. Further investigations showed the following during the course of treatment.

I HCV RNA: Following the combination treatment (0~96 weeks), level of HCV RNA was wavyly declined at the first 12 weeks (early virologic response, EVR), fluctuated in the next 20 weeks, and became negative since the 32th week to the end of treatment (EOT) (Figure 3). In the follow up of 24 weeks, HCV RNA was also negative.

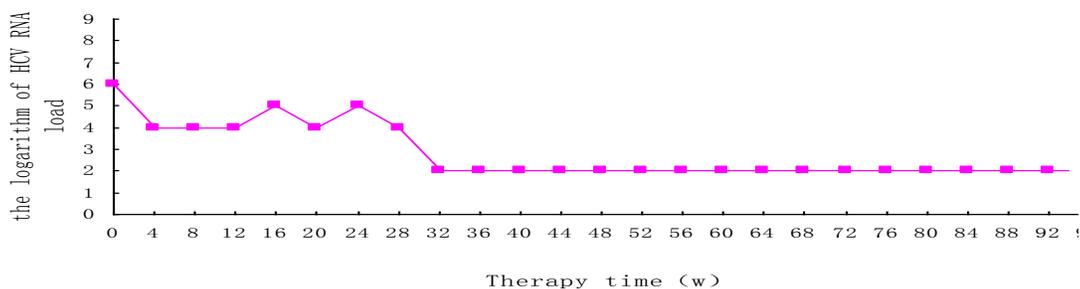


Fig. 1 the dynamic change of HCV RNA level

II Blood bio-chemistry: The serum ALT, AST and T-Bil (total bilirubin) (normal range for ALT, 6 to 40 U per liter; normal range for AST, 9 to 40 U and serum T-Bil, 0 to 25 μ mol per liter) of liver function were above normal in the pre-phase of combination treatment. ALT was 158 u/l and AST was 97 u/l. After the first month of combination therapy, the ALT and AST were descend, returned to normal at the 8th week and maintained to the EOT. Otherwise, T-BIL caused from ribavirin had no change during the treatment.

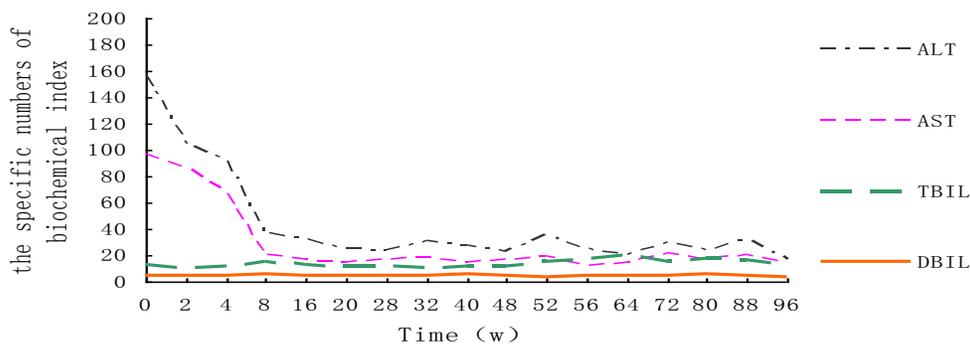


Fig 2 the change of biochemical index

III CBC: the patient's Hb was 8.4g/dl, platelet count was 2.3×10^5 cells/mm³ and WBC was 3100 cells/mm³ with an absolute neutrophil count of 2300 cells/mm³. At that time the patient was not still receiving any treatment. An examination of bone marrow confirmed the presence of aplastic anemia (figure4, table4). Following the combination treatment, the counts of WBC and neutrophil were declined and granulocyte colony-stimulating factor (G-CSF, 300µg im qod) was started at the 8th week. By 3 months the hematologic values had no improved (hemoglobin concentration, 10.1 g per deciliter; leukocyte count, 2100 per cubic millimeter; neutrophil count, 1100 per cubic millimeter; and platelet count, 6.7×10^4 per cubic millimeter) and we abandoned G-CSF injection. But to the 48th week, his bone marrow became slightly recovered. At the EOT, the Hb concentration was 14.2 g per deciliter, the leukocyte count was 3100 per cubic millimeter, the neutrophil count was 1700 per cubic millimeter, and the platelet count was 34,000 per cubic millimeter (figure5, table5)

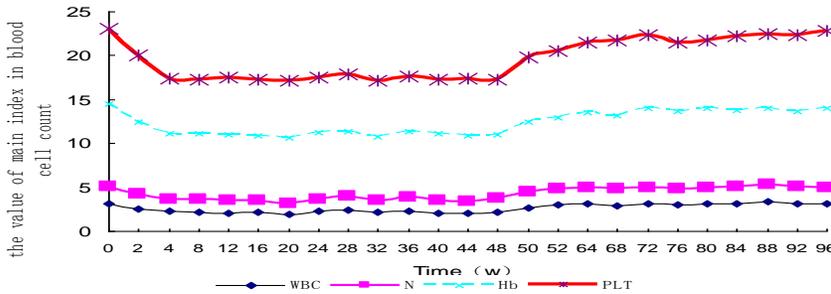
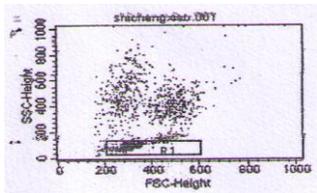
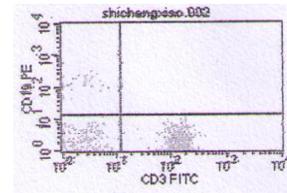


Fig 3 the change of main index in blood cell count

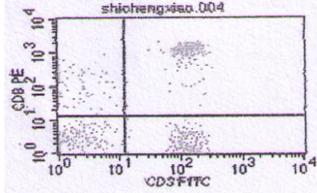
IV T lymphocyte subsets: T lymphocyte subsets were carried by cytometric analysis. At the EOT, The presence of mature of lymphocyte was testified by the use of anti-CD3⁺, anti-CD4⁺, CD19⁺ and anti-CD8⁺ antibodies. Flow-cytometric analysis showed normalities of the CD4⁺ and CD8⁺ counts in the patient. The percentage of CD3⁺ T cells was 71.2%, CD4⁺ T was 35.8%, CD8⁺ T was 33.5% and the ratio of CD4⁺/CD8⁺ was 1.1, as the follows.



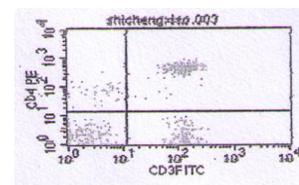
Scattering polar diagram of Peripheral-Blood cell R1 represented Peripheral-Blood Lymphocytes



lower limit on the right the Mature T cells of Peripheral-Blood (CD3⁺/CD19⁺)



Upper limit on the right CD8⁺ cells of Peripheral-Blood(CD3⁺/CD8⁺cell)



upper limit on the right CD4⁺ cells of Peripheral-Blood(CD3⁺/CD4⁺cell)

The percentage of CD3⁺, CD4⁺, CD8⁺ and the ratio of CD4⁺/CD8⁺T cells

Discussion

Hepatitis C virus(HCV), causative agent of chronic liver disease has affected approximately 175 million people worldwide which make up to almost 3% of the world's populations and 3 to 4 million new cases adds up to this figure annually[5]. Chronic HCV infection progresses towards more severe outcomes in the form of cirrhosis and hepatocellular carcinoma. Peg-IFN-α 2a or 2b plus ribavirin are currently the

standard regimen for patients with HCV infection[6]. A wide range of adverse reactions, including flu-like symptoms, nausea, anorexia, diarrhea, psychiatric symptoms, alopecia, leukopenia, thrombocytopenia, hemolytic anemia, dyspnea, rash, insomnia, and ataxia have been associated with Peg-IFN-α 2a plus ribavirin treatment[7]. Treatment with IFN-α has also been reported to trigger autoimmune phenomena in up to 3% of cases, with AIHA being the most prevalent and most

significant phenomena seen in clinical practice. Both IFNs and Peg-IFNs may induce neutropenia, but after the treatment, neutrophils became normal quickly. Furthermore, due to its inhibition of cellular growth, interference with oncogene expression and augmentation of lymphocyte cytotoxicity for target cells, IFN- α may cause bone marrow suppression, including potentially severe cytopenias and, very rarely, AA. So, it is a challenge that how to give the patient an appropriate therapy who infected by HCV associated AA.

In our case, at first, she refused the combination treatment because of its inhibitions of cellular growth of WBC, even development of severe AA. But from 2008, liver function continued above normal and made further progress in CBC, so she asked to be given combination treatment to control hepatitis. The data of examinations showed the early virologic response (EVR) continued to the twelfth week, but since the 13th week to the 32th, the level of HCV RNA was descended wavelly, and until to the 33th week, HCV RNA just became negative and lasted to the EOT. Meanwhile, ALT and AST of liver function recovered from the 8th week. In addition, CBC including WBC, neutrophil count, and platelet was changed like "U" model, which was, decreased at the first 3 weeks, fluctuated in the next 44 weeks, and elevated from the last 24 weeks in the phase of treatment. Her liver function tests, CBC, and HCV RNA remained normal during the follow up. Herein, the combination treatment may be inhibited of cellular growth of CBC, but it was transient and reversible. However, the delayed EVR may be associated with inhibition of cellular growth.

Numerous studies have shown that AA behaves as an immune-mediated disease[8,9]. AA is characterized by a diminished number of or absent bone marrow precursor cells and peripheral cytopenias. Meanwhile, many studies have also shown that lymphocytes from patients with aplastic anemia suppress the in vitro proliferation of hematopoietic progenitor cells from patients and normal donors[10]. Moreover, in aplastic anemia activated cytotoxic lymphocytes localize in the bone marrow, and there are increased levels of interferon gamma, a lymphokine product of activated CD8⁺ and CD4⁺ T cells, in the bone marrow. However, T-cell activation is common in viral infection, and cytotoxic T cells are thought to mediate the liver inflammation in hepatitis B and hepatitis C infection, but lymphocytes do not appear to be activated in uncomplicated sero-negative hepatitis[11,12]. The cellular immune response has been shown to be a major contributor to HCV clearance. Mohamed S. Abdel-Hakeem suggested that both HCV-specific CD4⁺ and CD8⁺ T cells become persistently defective with prolonged infection. Mohamed S et al have demonstrated that early

interferon therapy for HCV can rescue and select for long-lived poly-functional CD8⁺ memory T cells[13,14]. Treatment-induced memory T cells were similar in phenotype and function to natural memory T cells generated following spontaneously resolved infection. Kamal et al. demonstrated that SVR is associated with a recovery in HCV-specific CD4⁺ T-cell responses[15].

In our case, the delayed EVR was presented in the early phase of treatment, but the patient finally acquired the SVR. At the EOT, we detected the percentage and ratio in the CD4⁺/CD8⁺. To a certain extent, SVR associated the comparatively abundance counts of CD4⁺ and CD8⁺ T cells in her sera due to a relative increased in the number of CD4⁺T cells, decreased in the number of CD8⁺T cells and the ratio some liked in the healthy. However, the delayed EVR maybe also relatively lack of poly-functional CD8⁺ memory or CD4⁺T cells in AA although we had no directed data to prove the hypothesize in the early treatment phase(12 weeks). In other way, some data might be suggested the above theory that we gave the patient G-CSF injection, but she had no response.

Conclusions

Taking these data into account and considering our patient's clinical course (normal liver function tests at presentation, late onset of AA), the patient acquired the SVR of combination. Perhaps, both innate and adaptive immune responses of CD4⁺ and CD8⁺ T cells appeared to be active the virus clearance, although someone reported a patient to develop AA with chronic hepatitis C virus infection because of association with interferon alpha 2a injection. To the best of our knowledge, this is the first report of the special patient with AA being treated with Peg-IFN- α 2a for chronic HCV infection. But, as health care providers, physicians should be aware of complication of Peg-IFN- α 2a treatment. The present findings also need to be confirmed by a study of a larger number of samples and further follow-up.

Corresponding Author:

Zujiang Yu,
Department of the Infectious disease,
the First Affiliated Hospital of Zhengzhou University,
Zhengzhou, Henan 450052, China.
Email: johnyuem@zzu.edu.cn

References

- [1] Foster GR. Pegylated interferons for the treatment of chronic hepatitis C: pharmacological and clinical differences between peginterferon-alpha-2a and peginterferon-alpha-2b. *Drugs*. 2010; 70(2): 147-65
- [2] Feng YH, Yen CJ, Huang WT, Su WC, Chen

- TY, Tsao CJ. Clinical response of antilymphocyte globulin-based treatment in patients in taiwan with aplastic anemia: positive hepatitis C antibody may represent a response predictor. *Int J Hematol.* 2004; 79(2):133-7.
- [3] Rauff B, Idrees M, Shah SA, Butt S, Butt AM, Ali L, Hussain A, Irshad-Ur-Rehman, Ali M. Hepatitis associated aplastic anemia: a review. *Virology.* 2011; 28(8):87
- [4] Ramos-Casals M, García-Carrasco M, López-Medrano F, Trejo O, Forns X, López-Guillermo A, Muñoz C, Ingelmo M, Font J. Severe autoimmune cytopenias in treatment-naive hepatitis C virus infection: clinical description of 35 cases. *Medicine (Baltimore).* 2003; 82(2):87-96.
- [5] Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. *Antivir Ther.* 2005; 10(1):1-11.
- [6] Alexopoulou A, Papatheodoridis GV. Current progress in the treatment of chronic hepatitis C. *World J Gastroenterol.* 2012; 18(42):6060-9
- [7] Talwani R, Gilliam BL, Rizza SA, Nehra V, Temesgen Z. Current status of treatment for chronic hepatitis C virus infection. *Drugs Today (Barc).* 2012; 48(3):219-31
- [8] Li B, Liu S, Niu Y, Fang S, Wu X, Yu Z, Chen S, Yang L, Li Y. Altered expression of the TCR signaling related genes CD3 and FcεRIγ in patients with aplastic anemia. *J Hematol Oncol.* 2012; 5:6.
- [9] Ge M, Zheng Y, Li X, Shi J, Ge J, Li H, Feng S. The polymorphisms of T cell-specific TBX21 and STAT4 genes may contribute to the susceptibility of Chinese individuals to aplastic anemia. *Hum Immunol.* 2012; 73(1):118-21
- [10] Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. *Blood.* 2006; 108(8):2509-19
- [11] Nakao S, Feng X, Sugimori C. Immune pathophysiology of aplastic anemia. *Int J Hematol.* 2005; 82(3): 196-200.
- [12] Kondo Y, Ueno Y, Ninomiya M, Tamai K, Tanaka Y, Inoue J, Kakazu E, Kobayashi K, Kimura O, Miura M, Yamamoto T, Kobayashi T, Igarashi T, Shimosegawa T. Sequential immunological analysis of HBV/HCV co-infected patients during Peg-IFN/RBV therapy. *J Gastroenterol.* 2012; 47(12):1323-35.
- [13] Abdel-Hakeem MS, Bédard N, Badr G, Ostrowski M, S&kaly RP, Bruneau J, Willems B, Heathcote EJ, Shoukry NH. Comparison of immune restoration in early versus late alpha interferon therapy against hepatitis C virus. *J Virol.* 2010; 84(19):10429-35.
- [14] Badr G, Bédard N, Abdel-Hakeem MS, Trautmann L, Willems B, Villeneuve JP, Haddad EK, S&kaly RP, Bruneau J, Shoukry NH. Early interferon therapy for hepatitis C virus infection rescues polyfunctional, long-lived CD8+ memory T cells. *J Virol.* 2008; 82(20):10017-31.
- [15] Kamal SM, Amin A, Madwar M, Graham CS, He Q, Al Tawil A, Rasenack J, Nakano T, Robertson B, Ismail A, Koziel MJ. Cellular immune responses in seronegative sexual contacts of acute hepatitis C patients. *J Virol.* 2004; 78(22):12252-8.

7/11/2013