RESEARCH ARTICLE

Interferon Apha 2b for Treating Patients with JAK2V617F Positive Polycythemia Vera and Essential Thrombocytosis

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Abstract

Objective: To investigate interferon (IFN) alpha 2 b for treating patients with JAK2V617F positive polycythemia vera (PV) and essential thrombocytosis (ET). Methods: Interferon alpha 2 b was used to treat patients with JAK2V617F positive PV and ET. In control group, hydroxyurea was used. Endpoint of study was to compare rates of hematological and molecular remission. Results: Patients in the interferon alpha 2 b group achieved higher rates of hematologic and molecular remission than patients in the hydroxyurea group, with a lower incidence of thrombosis. Conclusion: Compared with hydroxyurea, interferon alpha 2 b could reduce JAK2V617F load for patients with PV and ET, and achieve higher molecular remission, improve treatment efficacy and reduce complications.

Keywords: Polycythemia vera - essential thrombocytosis - JAK2V617F mutation - Interferon - alpha 2 b

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Introduction

Polycythemia vera (PV) and essential thrombocythemia (ET) belong to common myeloproliferative neoplasms (MPN), characterized by unexplained blood cells increment, clinical thromboembolism, bleeding, and occasionally could convert into myelofibrosis or leukemia. It is suggested from biologic investigation that around 90% of patients with PV and 50% with ET could find kinase JAK2V617F point mutation, and this mutation is one of the important indicators in the diagnosis of PV and ET. In recent years, some studies demonstrated that mutation of copy number could be used as a surrogate for clinical response and mild residual disease (MRD) (Larsen et al., 2007; Kiladjian et al., 2008; Barosi et al., 2009). However, this is not reported in China (Bai et al., 2011). Currently, interferon (IFN) plays an important role in treating patients with MPN (Barbui et al., 2012), and IFN α is used in our department as a first-line regimen. By follow-up, we suggested that IFN αcould achieve high hematologic response rate, and also could be associated with high molecular response in patients with positive JAK2V617F.

Materials and Methods

All patients were recruited from out- and inpatient department of our hospital from January 2009 to June 2013 . Diagnostic criteria was adopted from WHO 2001 standard for PV and ET. Seventy-one patients were JAK2V617F positive. Forty seven patients (male 26, female 21; PV 27, ET 20) were treated with interferons alpha for more than 6 month, with a median age of 55 (41 to 78) years. Twenty four patients were treated with hydroxyurea due to intolerability or decline to use of interferon, including PV 15 (8 male, 7 female, with a median age of 53 years), ET 9 (4 male, 5 female, with a median age of 51 years).

Premedication: WBC > 50 * 10^9/L, platelet > 1000 x 10^9/L, hydroxyurea at a low dose of 0.5 ~ 1 g/d, combined with aspirin 100 mg/d. For patients with hemoglobin > 200 g/L, phlebotomy therapy was considered, 200 ~ 400 ml each time, 2 times a week. For patients peripheral blood leukocyte < 50 * 10^9/L < 180 g/L, hemoglobin < 180 g/L and platelet< 800 x 10^9/L, treatment with IFN α or hydroxyurea was initialed.

Interferon treatment group: IFN α2 b (from Harbin pharmaceutical company) 300 units, subcutaneously injected, three times a week; after reaching hematological complete remission, interferons alpha 2 b was maintained at 1 to 2 times a week. Hydroxyurea treatment group: hydroxyurea 0.5 ~ 1.5 g/d, adjust dose to 0.25 ~ 0.5/d when normal blood cells was achieved.

Response criteria was in line with standard of WHO 2001. Complete hematological remission (CHR) is defined as: no splenomegaly; normal blood count, white blood cells < 10.0 x 10^9/L, and platelet < 400 x 10^9/L, and hematokrit less than 45% (male), 42% (female) (and maintained for more than 3 months). Partial hematological remission (PHR) is defined as: blood count decreased to 50% of the original level, could be accompanied by splenomegaly. No response is defined as: do not reach

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Table 1. Comparision Between Two Groups of MPN Patients on Hematological Total Response Rate

	PV		ET	
	CHR%	PHR%	CHR%	PHR%
IFNα-2b	66.7 (18/27)	22.2 (6/27)	40 (8/20)	50 (10/20)
HU	6.7 (1/15)	40 (6/15)	11.1 (1/9)	33.3 (3/9)

PV, polycythemia vera; ET, essential thrombocythemia; MPN, myeloproliferative neoplasms; CHR, complete hematological response; PHR, partial hematological response; IFNα-2b , Interferon alpha 2 b; HU, hydroxyurea

Table 3. Comparision Between Two Groups of MPN Patients on Complications

	PV		ET	
	thrombus(%)	haemorrhage(%)	thrombus(%)	haemorrhage(%)
IFNα-2b	18.5 (5/27)*	11.1 (3/27)	20 (4/20)*	10 (2/20)
HU	26.6 (4/15)*	13.3 (2/15)	33.3 (3/9)*	11.1 (1/9)

*Comparision between IFNα-2b and HU, p<0.05; PV, polycythemia vera; ET, essential thrombocythemia; MPN, myeloproliferative neoplasms; IFNα-2b , interferon alpha 2 b; HU, hydroxyurea

Table 2. Comparision Between Two Groups of MPN Patients on Molecular Remission

	PV			ET		
	CMR%	PMR%	MMR%	CMR%	PMR%	MMR%
IFNα-2b	33.3 (9/27)	44.4 (12/27)	22.2(6/27)	26.7% (4/20)	33.3 (5/20)	4 (6/20)
HU	0 (0/15)	5 (1/15)	5(1/15)	0 (0/9)	0 (0/9)	11.1 (1/9)

PV, polycythemia vera; ET, essential thrombocythemia; MPN, myeloproliferative neoplasms; CMR, complete molecular response; PMR, partial molecular response; IFNα-2b , Interferon alpha 2 b; HU, hydroxyurea

partial remission. Molecular complete remission (CMR) is defined as: JAK2V617F mutation is not detected. Partial molecular remission (PMR) was defined as: load of JAK2V617F mutation is less than 50%. Mild molecular remission (MMR) is defined as load of JAK2V617F mutation reduced by 20% - 49%.

Bone marrow aspiration and detection was conducted before and after treatment and the procedure was in line with method from reference (Bai et al., 2011), genomic DNA prepared from 5 ml bone marrow extraction was used for JAK2V617F mutational test by nested quantitative PCR. Calculation was performed according to the following formula.

JAK2V617F load (%V617F) = DNA (JAK2V617F) /DNA (JAK2WT + JAK2V617F)

△Ct = CtJAK2V617F - CtJAK2WT

JAK2V617F load (%V617F) as horizontal ordinate and △Ct value as vertical coordinates to draw a standard curve. All samples were repeatedly tested for 3 times. According to average from three △Ct, calculate the corresponding JAK2V617F load from the standard curve.

Statistical analysis

Measurement data were analyzed by mean±standard deviation (x±s), using SPSS 17.0 software. Comparision between groups was conducted by analysis of variance. Statistically significance was set as p<005.

Results

Two groups of MPN patients with hematologic response rate

In Table 1, hematological total response rate (CRH + PHR) for patients with PV and treated by IFNα-2b was 88.9%, time of CRH was 4.5±1.2 months, and in hydroxyurea treatment group these figues were 46.7% and 6.0±1.5 months, respectively. The total response rate was significantly higher in IFNα-2b group than that in hydroxyurea group (p<0.05), and time of CRH was significantly shorter than that in hydroxyurea group (p<0.05). Hematological total response rate (CRH + PHR) for patients with ET and treated by IFNα-2b was 90.0%, time of CRH was 3.6±1.8 months, and in hydroxyurea treatment group these figues were 44.4% and 5.4±1.6 months, respectively. The total response rate was also significantly higher in IFNα-2b group than in hydroxyurea group (p<0.05), and time of CRH was also significantly shorter than that in hydroxyurea group (p<0.05).

Comparision of molecular remission rate in two groups of MPN patients

In Table 2, for patients with PV, the rate of molecular response (CMR + PMR) was 77.7% in IFNα-2b group and 5% in hydroxyurea group (p< 0.05). For patients with ET, the rate of molecular remission was 60% in IFNα-2b group and 0% in hydroxyurea group (p< 0.05). After patients with PV and ET were treated with IFNα-2b, time to CMR was 18.7±6.3 months and 21.0±6.0 months, respectively. Two patients with PV stoped IFNα-2b after CMR is reported, then diagnosed with molecular relapse at 6 and 12 months respectively, were treated with IFNα-2b for 6 months and achieved CMR again.

Comparison of complications in two groups of patients with MPN

In table 3, in two groups of patients with MPN, incidence of thrombus was lower in IFNα-2b group than that in hydroxyurea group, p < 0.05; the incidence of haemorrhage was not different in IFN and in group, p>0.05.

Adverse reactions of interferon treatment

After first dose of interferons alpha 2 b, flu-like symptoms, eg., fever (37.8 ~ 39.9 °C), headache, arthrodynia were detected. And could be relieved by nonsteroidal anti-inflammatory drugs. After 3 ~ 6 administration, fever was not reported. Three patients complained weakness, loss of appetite , abdominal distension, 2 patients reported lethargy and depression, and alopecia was recorded in one patient.

Discussion

According to 2008 World Health Organization (WHO) classification for hematopoietic and lymphoid tissue disease, PV and ET were defined as malignances originated from hematopoietic stem cells. Pathologic characteristic is myelodysplastic activity, peripheral blood cell augmentation and immaturity. Inhibition on cell proliferation in bone marrow is considered the key of treatment. Targeted therapy is possible after the discovery of JAK2V617F mutation for this disease. In some countries, JAK kinase inhibitors are currently tested in clinical trials, but not available in clinical applications due to lack of data on effectiveness and side effects. At present, IFNs application is still prescribed in first line treatment. This is because IFNs demonstrated following biological effects: 1 inhibiting cell proliferation in bone marrow. Previous study suggested that IFN alpha could downregulate expression and activation of cyclin E, A, cyclin cdc25A, cyclin D - cdk4, and cyclin - cdk6, suppressing protein phosphorylation of Rb, and further inhibiting cells from G1 phase to S phase, and delaying cell proliferation and division (Hasselbalch HC, et al., 2011). Previous study also suggested that IFN alpha could inhibit production of hematopoietic growth factors, eg., granulocyte colony stimulating factor (G-CSF), megakaryocyte colony stimulating factor (Mk - CSF), promote the platelet hormone (TPO), and promote secretion of cytokines that is hematopoieticaly inhibitory, eg., transforming growth factor (TGF), tumor necrosis factor (TNF), and thereby inhibiting proliferation and differentiation of megakaryocyte, red blood cells and granulocyte (Wang Q, et al., 2000; Dai CH et al., 1998). 2 promoting cell apoptosis. IFNs could cause structural change of cell membrane and cytoplasm, DNA fragmentation and formation of apoptotic body; IFNs could upregulate TRAIL and FasL, and after conbined with corresponding receptor, is able to activate death domain structure that is associated with Fas, and induce apoptosis by activating downstream proteases, eg., Caspase - 4 and Caspase - 8 (Chawla-Sarkar et al., 2003; Guo et al., 2012; Dirican et al., 2013; Zhao et al., 2013; Laljee et al., 2013). 3 modulating immunological function. IFNs could promote differentiation of immature cells, eg., T lymphocytes, NK and other mononuclear cells. Mononuclear cells differentiate into antigen-presenting cell and further activate T and B cells, T and NK cells are anti-cancer cells (Rizza et al., 2010). 4 inhibiting myelofibrosis. Although controversial, previous study demonstrated that TGF - beta could stimulate fiber cell proliferation in a variety of organs and tissues, and regulate the secretion of platelet derived growth factor (PDGF), thus is an important regulatory factor to promote synthesis and deposition of collagenous fiber. IFN - gamma could regulate secretion of TGF -beta through STAT pathway, and inhibit deposition of collagenous fiber in bone marrow(Eickelberg et al., 2001). 5 inducing cytogenetic response. For patients with MPN, who are frequently detected with chromosome abnormality, IFNs as a kind of biological treatment could induce cytogenetics response without side effects of leukemia (Kiladjian et al., 2008).

Our study suggested that compared with hydroxyurea, IFN-αwas associated with higher hematologic remission, lower incidence of thrombosis, and could induce molecular remission, and had no side effects of leukemia. These characteristics of IFN-α, make it be used as a suitable agent of biological treatment for patients with MPN. But IFN-αis linked with side effects, eg., fever, chills, muscle aches, fatigue, loss of appetite, nervous system, endocrine and reproductive systems. Another concern on IFN-αis the long-term administration, so that some patients are not able to tolerate the injection. Our study demonstrated that at the beginning of IFN-αtherapy, JAK2V617F copy number was increased, and decreased after continuation of therapy. Therefore, interferons alpha treatment should be maintained for a long period, although standardized dose and continuation period are not established. Polyethylene glycol interferon (peg IFN) that is originally used to treat patients with hepatitis b virus infection, is characteristics of long half-life and low incidence of side effects compared with conventional IFN, and is injected once a week with good tolerance. Recent study reported that peg IFN - MPN also achieved satisfactory treatment effect for patients with MPN (Quintás-Cardama et al., 2013). Currently, randomized controlled trial is initiated to compare peg IFN and hydroxyurea in the treatment of PV and ET (registration number: NCT01259856), and peg IFN is expected to be an option for patients with Ph chromosome negative MPN.

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