Pure Red Cell Aplasia Associated with Imatinib-Treated FIP1L1-PDGFRA Positive Chronic Eosinophilic Leukemia

Hideo Tanaka, Koji Iwato, Hiroya Asou and Akiro Kimura

Abstract

A 28-year-old man with marked eosinophilia is described. FIP1L1/PDGFRA mRNA showed multiple alternatively-spliced fusion transcripts. Sequencing analysis showed that the deduced DNA breakpoints were intron 10 in the FIP1L1 gene and exon 12 in the PDGFRA gene. Then, a diagnosis of chronic eosinophilic leukemia (CEL) was made. Whereas the response to the treatments with prednisolone and hydroxyurea were unsatisfactory, treatment with imatinib showed a rapid decrease of eosinophils. The hemoglobin level also dropped and bone marrow examination showed pure red cell aplasia. Continued administration of very low dose imatinib (100 mg every 5 days) led to and maintained complete molecular remission, with good tolerability.

Key words: CEL, imatinib, PRCA, FIP1L1, PDGFRA

(Inter Med 49: 1195-1200, 2010)  
(DOI: 10.2169/internalmedicine.49.3178)

Introduction

Hypereosinophilic syndrome (HES) was first characterized in 1975 (1). At that time, the molecular mechanisms of HES had been unclarified, though those patients with clonal disorders were diagnosed as chronic eosinophilic leukemia (CEL). The first report of treatment of HES patients with imatinib mesylate (imatinib) was in 2001, and then some case reports and small case series followed; excellent effects were observed in a high proportion of the patients (2-4). In 2003, an epoch-making discovery was reported, in which a fusion of FIP1-like 1 gene (FIP1L1) to the platelet derived growth factor receptor α gene (PDGFRA) was formed, as a result of chromosome 4q12 interstitial deletion in HES patients with normal karyotype (5, 6). These patients are now recognized as FIP1L1-PDGFRA (F/P)-positive CEL patients. The emergence of genetically assigned eosinophilia led the World Health Organization (WHO) to adopt a subcategory named “myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1”, and patients with F/P-associated disease are now recognized as myeloid neoplasms (7). Imatinib was approved for the treatment of HES/CEL patients by FDA in the US in 2006, and has undisputedly become the first line therapy for F/P-positive patients.

Here, we describe a F/P-positive CEL patient who achieved rapid hematological remission and long-term complete molecular remission (CMR) with a very low dose of imatinib maintenance treatment. One of the notable clinical features of this patient was that he underwent pure red blood cell aplasia (PRCA) after the initiation of the imatinib therapy. We also performed molecular analysis of the F/P fusion gene which revealed that the deduced DNA breakpoints for both genes were determined, and that multiple fusion mRNAs with a variety of splicing variants existed. Our study was performed in compliance with the Helsinki Declaration.

Case Report

A 28-year-old man was referred to another hospital in...
September 2004 because of cough and mild fever. He had a negative history for allergic diseases and parasitic infections. Chest X-ray showed diffuse mild infiltration shadows in both lungs. A peripheral blood (PB) hematological examination showed leukocytosis with eosinophilia (WBC 37.9×10^9/L, eosinophils 81.6%), and the patient was treated with 30 mg/day of prednisolone, without satisfactory response. In December 2004, the patient was referred to our hospital. Urticaria was easily formed by stimuli on the skin. White blood cell (WBC) count was elevated to 63.7×10^9/L (eosinophils 92%), hemoglobin (Hb) 13.1 g/dL, and platelet count 129×10^9/L. Serum lactate dehydrogenase (LDH) was elevated to 552 U/L (normal range 119-229). Computed tomography showed mild splenomegaly. In early December, bone marrow (BM) examination revealed hyperplastic marrow with 81.0% eosinophils, and slightly increased myeloblasts (2.0%) with nuclear dysplasia (Figs. 1A, 1B). No increase of bone marrow mast cells was observed. Chromosomal analysis of the BM cells showed normal karyotype (46, XY [20/20]).

His clinical course is shown in Fig. 2. The patient was first treated with 40 mg/day of prednisolone, and then the WBC count was reduced to 38.1×10^9/L (eosinophils 82%). After 500 mg/day of hydroxyurea was started, the WBC count was further reduced to 19.8×10^9/L (eosinophils 69%), but it increased again to 24.3×10^9/L (eosinophils 86%). In early December 2004, reverse transcribed polymerase chain reaction (RT-PCR) was performed using polymorphonuclear cells (PMNs) in the BM aspirate (8, 9), and revealed that the F/P fusion mRNA was positive, thus a diagnosis of F/P-positive CEL was made. Based on this information, in mid-January 2005, imatinib (100 mg daily) was started, after informed consent was obtained from the patient and his family. Four days after the initiation of the imatinib treatment, both the WBC count and eosinophil count were dramatically decreased to 2.1×10^9/L (eosinophils 1%), and Hb was 12.2 g/dL. In late January the Hb level decreased to 8.9 g/dL and reticulocyte count decreased to 0‰; then imatinib administration was discontinued. In early February, Hb level further decreased to 6.9 g/dL (WBC count was 3.4×10^9/L), and then red blood cell transfusion was performed. BM examination showed the disappearance of eosinophils, and a
Figure 2. Clinical course of the patient. WBC: white blood cell count, Hb: hemoglobin level, Plt: platelet count, Eo: eosinophils, Ebl: erythroblasts, Neuto: neutrophils, RBC: red blood cells. Vertical bars indicate bone marrow aspirations and black portion in each bar indicate erythroblasts.

Figure 3. Detection of the F/P fusion mRNA by RT-PCR. Mononuclear cells (MNCs) and polymorphonuclear cells (PMNs) were separated from the BM aspirates in early December 2004, according to the method previously described (8). The PMNs were used to synthesize cDNA from the total RNA. Peripheral blood PMNs from a normal individual was also used as a negative control. Primers for nested-RT-PCR were synthesized with the location in the exon 7a of the FIP1L1 gene, and in the exon 13 of the PDGFRA gene as previously reported (5). Arrows indicate multiple F/P fusion mRNAs. Lane 1: 100 bp ladder marker, Lane 2: normal individual, Lane 3: this patient.

marked reduction of erythroblasts (Fig. 1C). In early March, Hb level recovered to 13.7 g/dL, and WBC count increased again to 14.0×10^9/L (eosinophils 52%), and thus imatinib treatment was resumed. In late March, BM showed recovery of erythroblast (Fig. 1D), while RT-PCR analysis showed that the F/P fusion mRNA was still positive. Since that time, low dose imatinib administration (100 mg per every 5 days) has been continued. He achieved CMR in late November 2006, as nested-RT-PCR analysis for the F/P mRNA became undetectable using PMNs of the BM sample. To date (July 30, 2009), CMR has remained for 32 months.

We further examined the detailed structure of the F/P-fusion mRNA. The RT-PCR products showed multiple bands in the agarose gel (Fig. 3), and then each sequence was determined. The deduced DNA breakpoint of the FIP1L1 gene was assumed to be in intron 10, and the deduced DNA breakpoint of the PDGFRA gene was at 81 bp downstream from the first nucleotide in exon 12 (Fig. 4). The detailed sequence analyses of the multiple RT-PCR products revealed that there were a variety of splicing variants, and among them, five variants were analyzed. Briefly, they utilized sequences which end with “ag” in the FIP1L1 gene as new splicing acceptor sites, and some exons in the FIP1L1 gene and some of the PDGFRA gene were skipped. Taken together, 4 out of 5 fusion products retained open reading frames.

To evaluate the effects of imatinib on the growth of the eosinophils, we performed a cell viability assay by trypan blue dye exclusion method (9). In addition to imatinib, PKC412 which is a staurosporine derivative possessing capability of inhibition of multiple kinases (10), 17-AAG was also used. Both imatinib and PKC412 decreased the viable cell counts by 60% and by 20%, respectively, whereas 17-AAG (as a negative control) did not decrease the viable cell count (Fig. 5). These results confirmed that tyrosine kinase
Splicing variants of the fusion mRNAs revealed by sequence analyses of the multiple PCR products. The RT-PCR products were cloned into a T/A-cloning vector, and the sequence was determined for each of them. Positions of original exons are shown by rectangles. The number in each rectangle is the number of nucleotides in each exon. Clones 16, 14, 23, and 15 retained open reading frames (indicated by open circles).

**Figure 4.**

Inhibitions of the growth of eosinophils by tyrosine kinase inhibitors assessed by cell viability assay. PMNs (mostly eosinophils) in the BM (December 2004) (i.e. before imatinib treatment) were used. PMNs were cultured either without or with indicated concentration of imatinib (kindly provided by Novartis Pharma Inc.) (0.1 μM), or with PKC412 (kindly provided by Novartis Pharma Inc.) (0.1 μM), or with 17-AAG (as a negative control) (Alexis Biologicals, San Diego, CA, USA) (0.1 μM), in culture medium RPMI 1640+10%FCS for 48 hrs, and then the viable cells were counted by microscope after trypan blue staining. Each bar indicates the relative viable cell number, in which the cell number without any drug (-) are supposed to be 100%. Each assay was done in triplicate, and mean±SD is shown.

**Figure 5.**

Discussion

Since the discovery of the F/P-fusion gene (5), a comprehensive picture has emerged regarding several characteristics of the fusion, its incidence, biological features, and the clinical profiles of the F/P-positive CEL patients (11).

In the present patient, we performed a molecular analysis of the F/P fusion mRNAs. It seems unlikely that the RT-PCR products were pseudo-PCR products as a previous report warned (12). We performed the RT-PCR for 50 hypereosinophilia patients and 2 normal individuals and among them, RT-PCR product was observed only in the present case, i.e., F/P-positive rate was 2% (1/50). Based on the sequencing analysis, we assumed the deduced DNA breakpoints in the *FIP1L1* gene and the *PDGFRA* gene. The deduced DNA breakpoint in the *PDGFRA* gene in our patient was unique as compared with those previously reported (5, 12, 13). Our results were also in accordance with previous reports that the DNA breakpoints in *FIP1L1* gene were diverse but those in the *PDGFRA* gene were tightly clustered within the exon 12 (5, 12, 13), indicating that interruption of the juxtamembrane domain of PDGFRα is indispensable for kinase activation in the context of F/P fusion protein (14). The RT-PCR analysis of the F/P fusion mRNA in the present patient showed diversely-spliced transcripts. The pattern of the fusion mRNAs found in our patient was similar but not at all the same from those reported before (5, 12, 13), and this diversity seems to be one of the characteristics of the F/P fusion mRNA.

From the clinical aspect of the present patient, the hema-
tological response to imatinib was very rapid, as reported in the literature (15). Furthermore, the maintenance dosage of the imatinib was as low as 100 mg per every 5 days, which was very low as compared to the dose usually used in chronic myelogenous leukemia (CML) patients. Regarding low-dose imatinib therapy, the minimal imatinib dose required for maintenance of remission in CEL has not been established yet. There are a few reports which showed sustained response in CEL patients treated with low-doses of imatinib, i.e. administered bi-weekly and/or weekly (16, 17).

One report described six F/P-positive cases who were treated with initial daily doses ranging 100 mg to 400 mg, and then the remission was maintained with a single imatinib dose ranging 100 mg to 200 mg weekly (18). With a median follow-up of 30 months, all patients remained in hematological remission and five of the six patients achieved CMR (18, 19). These studies also revealed both long-term efficacy including molecular remission and good safety. On the other hand, it became clear that discontinuation of imatinib leads to relapse, indicating that imatinib can suppress, but may not be able to eradicate the F/P-positive clone. Thus, continuation of imatinib therapy seems to be required, and in this regard, it is important to define a minimal imatinib dose required to maintain the remission.

One of the important clinical features to note in our patient was occurrence of PRCA after initiation of the imatinib therapy. The occurrence of PRCA following imatinib therapy is a rare event, and to our knowledge, only one case in CML in chronic phase patient who showed imatinib-induced PRCA has been reported to date (22). According to their report, PRCA in the patient was likely associated with imatinib therapy, because imatinib was exclusively used before the occurrence of PRCA, and the withdrawal of imatinib induced recovery of both anemia and PRCA status (22). To our knowledge, the present case is the first one that imatinib therapy induced PRCA in CEL patient, though the clear mechanism is uncertain. Other previous reports, not with imatinib therapy, discuss that some CML patients underwent PRCA associated with interferon-α (IFN-α) therapy (23, 24), though the mechanism of PRCA induced by IFN-α also remained unclear.

In conclusion, we have confirmed the long efficacy and good drug tolerance with a low dose of imatinib in the treatment of F/P-positive CEL patients. It is important to determine the optimal imatinib dose required to maintain the disease in molecular remission. Imatinib-induced PRCA is a rare event, but it is important to bare it in mind when severe anemia occurs during imatinib therapy.

Acknowledgement

We thank Ms. Sumida for her excellent technical support. We also thank Ms. Matsumoto, Ms. Fukumoto, and Ms. Nakaju, for their excellent laboratory work.

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