Case Report
Autoimmune thrombocytopenia: a complication of fludarabine therapy in the treatment of Waldenstrom's macroglobulinemia

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Abstract: Fludarabine is an effective purine analogue which has become extensive used in lymph proliferative malignancies. However, an increased incidence of autoimmune disorders including autoimmune hemolytic anemia (AHIA) and idiopathic thrombocytopenia (ITP) is reported with the use of fludarabine. The exact mechanism for fludarabine to exacerbate thrombocytopenia is not distinct. In our report, we describe a patient with WM developed a refractory, life-threatening and fludarabine-associated thrombocytopenia which could not be explained by the cytotoxic effects of fludarabine. Possible mechanisms of fludarabine on autoimmune disorders are discussed.

Keywords: Fludarabine, Waldenstrom's macroglobulinemia, thrombocytopenia

Introduction

Fludarabine phosphate (9-B-D-arabinofuranos-yl-2-fluoradenine 5'-monophosphate) is a purine analogue which has become extensively used in lymphoproliferative malignancies such as chronic lymphocytic leukemia (CLL), non-Hodgkin's lymphomas (NHL) and Waldenstrom's macroglobulinemia (WM) [1-2]. WM is a disease of the elderly with a median age of 63-68 years and a male predominance [3-4]. Fludarabine is thought to be more effective as a single agent than chlorambucil and has been used as one of the first line treatment options in European Society for Medical Oncology (ESMO) guideline [3]. Myelosuppre-ssion and T-cell immunosuppression are the main toxicities observed with fludarabine. These toxicities account for high incidence of infectious and immunosuppressive features [5]. Furthermore, with the current beliefs that fludarabine may cause autoimmune complications [6]. An increased incidence of autoimmune disorders such as autoimmune hemolytic anemia (AHIA) and idiopathic thrombocytopenia (ITP) had been reported in patients with CLL and NHL when treated with fludarabine [6-7]. AIHA and ITP are observed in

approximately 20% to 35% and 2% to 4% in CLL patients, respectively [8]. However, autoimmune disorders occurring associated with fludarabine are less common in patients with WM than those with CLL. The direct responsibility of fludarabine in these autoimmune disorders remains to be established. Here we report a case of severe ITP after two courses of fludarabine in a patient with WM. For this patient, no autoimmune disorder was previously noticed.

Case report

A 64-year-old man presented in 2012 with debility, shortness of breath and yellowish complexion. On physical examination, multiple lymphadenectasis were found without splenomegaly. The patient gave a history of "anemia" for three years. The peripheral blood cell count showed anemia (hemoglobin 56 g/L) with the leukocytes and platelets were almost in normal range. Total protein was 106.6 g/L (66-83) and globulin and albumin were 81.4 g/L (20-35) and 25.2 g/L (35-52) respectively. An obvious sharp level of IgM quality was 70.2 g/L (0.4-2.3) and that of the light-chain κ was 10 g/L (1.7-3.7). Microscope examination of a bone marrow

Table 1. Patient characteristics

Baseline characteristics	Patient
Age/Sex	64/M
Symptom of WM	Anemia, multiple lymphadenectasis
Previous treatment	None
Baseline CBC before fludarabine	
WBC	3.56×10^9/L
Hemoglobin	54 g/L
Platelets	129×10^9/L
Current fludarabine cycles before ITP	2
Serum IgM/κ	
Before fludarabine	70.2/10
After 1 cycle of fludarabine	47.9/8.5
After 2 cycle of fludarabine	44.7/10.3
Platelet nadir	0
ITP clinical manifestations	Epistaxis, buccal hematoma, palatal and skin petechiae
ITP treatment	Methylprednisolone, IVIG, IL-11, rhTPO and irradiated platelet transfusion
Response	None

Note: the quantitative referent range of IgM is from 0.4 g/L to 2.3 g/L. The quantitative referent range of κ is 1.7 g/L to 3.7 g/L.

aspirate and biopsy showed 5% mature plasma cell and a phenomenon of rouleaux red cells could be detected. Flow cytometry showed 1.22% plasma cells with phenotype: CD38+, CD138+, HLA-DR+, CD19+, and ckappa+. A computed tomographic (CT) scan showed armpit, mediastinal, and groin lymphadenopathy. Pathologic and immunohistochemical studies of his right cervical lymph node showed major plasma cells and lymphocytes without tumor cells being found. A diagnosis of Waldenstrom's macroglobulinemia (WM) was made from the above investigations.

A protocol was made with single agent of fludarabine at our department which was approved by the institutional review board and informed consent was provided according to the Declaration of Helsinki. The patients received 42 mg (25 mg/m^2) fludarabine as single agent daily for 5 days every 4 weeks. After the first course of treatment, the hemoglobin was increased to 98 g/L and the turgescent lymphonodes were smaller than before. The second cycle of fludarabine (the same with before) was given and no obvious chemotherapeutic side-effect was observed till 2 weeks after dosage. The patient developed a transient decrease in platelets to 2*10^9/L on day 14 of cycle 2. The patient has not massive splenomegaly and his blood biochemistry was almost in a normal range. Soon epistaxis, buccal hematoma, and

palatal petechiae showed up, and he did not respond to multiple irradiated platelet infusions.

In order to differentiate between thrombocytopenia due to prolonged effects of chemotherapy and thrombocytopenia as a complication of marrow infiltration associated with WM disease process, a bone marrow biopsy and aspirate were undertaken. The presence of adequate megakaryocytes without platelet production and no evidence of microangiopathic hemolysis on examination of the marrow aspirate and biopsy suggested a peripheral consumptive process. Ethylene diamine tetraacetic acid (EDTA) associated thrombocytopenia was also be depleted. For its low specific or sensitive, antibodies test such as immunogenic platelet glycoproteins GPIIb/IIIa, GPIb/Ix and GPIa/IIa were not detected in this case. The immune function of T cells detected by flow cytometry showed that: T cell subset detection indicated a decrease in the CD4/CD8 ratio. Furthermore, the normal platelet count 2 weeks prior to presentation also indicated a diagnosis of ITP.

Standard treatment with 1 mg/kg intravenous methylprednisolone per day and 0.4 g/kg per day intravenous immunoglobulin (IVIG) for 5 days (Shandong Taibang Biological Products Co., Ltd.) was begun and fludarabine in schedule was discontinued. Irradiated platelet infu-

sions were given to him to avoid vital hemorrhage. In addition, IL-11 was given to stimulate the production of platelets. Ten days later no response was observed in increasing the platelet count. Then rhTPO (Recombinant human thrombopoietin) was administrated instead of IL-11 and showed any response at all. The platelet count was 0-1*10^9/L at the time and petechiae or mucosal bleeding could be often observed. At the same time, the pneumonic infect occurred and aggravated quickly, so we had to decrease the dose of methylprednisolone. Sputum was cultured for bacteria and fungi. Empirical antibiotics and antifungal therapy were carried out. However, the patient became increasingly deteriorated with fever and respiratory failure. Rituximab was suggested to the patient but his family member declined it. Unfortunately, the patient eventually died of progressive mixed pattern pneumonia infection and development of disease-related thrombocytopenia. The patient's clinical characteristics and treatment information was shown in **Table 1**.

Discussion

WM is a rare disease and monochemotherapy with fludarabine is frequently used with low toxicity. As an effective and relative safe agent, the most obvious side effects of fludarabine are myelosuppression and high incidence of infectious. Here we report a case of severe ITP after two courses of fludarabine in a patient with WM. In this patient, thrombocytopenia was first observed 14 days after following the administration of fludarabine. The temporal association between fludarabine therapy and thrombocytopenia supported a pattern suggestive of marrow suppression from chemotherapy. However, ITP was suspected when the patient's platelets were continued to be measured at level < 10*10^9/L since leukocytes count was normal. The presence of adequate megakaryocytes on bone marrow aspiration was taken as confirmation of peripheral destruction. So a diagnosis of ITP associated with fludarabine was confirmed. Patient was begun on steroids, IVIG, IL-11, and TPO, and fludarabine treatment was discontinued. However, he continued to have bleeding complications without platelet recovery. Patient and his relatives rejected to use rituximab and ultimately died of serious bleeding and infection.

Thrombocytopenia is not a common feature of WM and fludarabine associated ITP in WM is further uncommon [4]. There are a few reports of ITP in association with the treatment of CLL with single-agent fludarabine, but to our knowledge this is the first case documented in a patient with WM [8, 9]. Former studies have indicated that fludarabine might induce obvious autoimmune disorders such as severe AIHA or ITP. The original case report of Evans syndrome in a patient with CLL post fludarabine has been reported by Schvidel et al. in 1997 [10]. In their report, after 6 cycles of fludarabine (50 mg/d for 5 days), the state of the illness was controlling and the Hb level and the platelet count remained stable. One year later the illness of CLL relapsed and two cycles of fludarabine (the same dose as before) combined 500 mg cyclophosphamide were give. After six weeks the patient presented with severe anemia and bleeding. The diagnosis of Evans syndrome was made for DAT (Coombs test) was positive and immature megakaryocytes in bone marrow. With the use of prednisone and cyclosporine, the patient recovered from the Evans syndrome. The author believed that T-lymphopenia associated with fludarabine might induce dysregulation in T-cell function and produce autoantibodies directed against red cells and platelet antigens. A retrospective review of the case notes of 45 patients with lymphoproliferative disorders treated with fludarabine over the past 6 years indicated the development of autoimmune thrombocytopenia in 4.5% (two out of 45) and autoimmune hemolytic anemia in 6.7% (three of the 45) [11]. Churn M et al [12] reported a case of ITP occurring in a patient with relapsed low-grade NHL treated with fludarabine as second-line chemotherapy. Only one week after completion of his first cycle of fludarabine the platelet count of the patient dropped to 2*10^9/L. With the help of intravenous steroids and immunoglobulin, the platelets count rose to 70*10^9/L. However, the patient died of serious infection with respiratory failure. Leach et al. recently described two further cases with CLL, both of these patients responded to a combination of high-dose steroids and intravenous immunoglobulin. They suggested that the incidence of fludarabine-induced ITP may be in the region of 4.4% [11]. All these reports suggest that fludarabine may play a role in triggering the immune disorders including AIHA and ITP. However, the

exact mechanism of that have been undefined.

The possible anti-tumor mechanism of fludarabine has been reported former [13]. It is worth paying attention that the following inhibition of suppressor T-cell effects is thought to be the mechanism that leads to the emergence of autoimmune diseases [14]. Meanwhile, as a result of this process, normal host lymphocytes are also depleted, particularly the CD4 subset of T cells, with a decrease in the CD4/CD8 ratio [15]. A hypothesis raised by Lewis et al preferred that an imbalance in CD4-helper and CD8-suppressor cells might produce immune dysregulation and autoimmune complications [16]. Caligaria-Cappio and Hamblin hypothesized that profound suppression of CD4 cells by fludarabine may cause aberrations of the immunoregulatory circuits involving malignant B-cells. CLL and normal B cells can coexpress CD40, which, when stimulated by CD40 ligand on T cells, may up-regulate CD80 and CD86 costimulatory molecules and acquire the ability to present hematopoietic antigens to normal bystander B cells and invoke an immune response [17]. It has also been suggested that the immune suppression from fludarabine may depress an antibody response against hematopoietic antigens. Some researchers suggested that quantity and function of regulatory T cells (Treg), which are a subset of $CD4+CD25^{hi}FoxP3$ T cells with a role in maintaining peripheral tolerance, have been shown to be decreased after treatment with fludarabine, resulting in a loss of self-tolerance and a high risk for the development of autoimmune disorders [18]. Standpoint of that defective Treg function should be a factor in the development of autoimmune cytopenia should be investigated further. In addition, lymphoid malignancies are already characterized by hypogammaglobulinemia, which may result in a lack of anti-idiopathic antibodies to antagonize autoimmune clones [19]. However, it is worthy of reconstruction that in some lymphoproliferative disorders which incorporate AIHA or ITP, fludarabine showed much faculty to overcome the anemia and thrombocypenia successfully. For example, Tiffany et al reported a case of autoimmune thrombocytopenia due to CLL treated with fludarabine successfully [20]. From the former studies we presume that fludarabin may be double-blade-sword when used in lymphoproliferative diseases. It not only can trigger autoimmune disorders, but also reverse

immunologic derangement. How it works in different immune background and which result it may lead to have not been interpreted distinctly. Thus, randomized trials are however needed to study this issue.

All patients acquire severe thrombocytopenia following fludarabine treatment and represent variable responses to standard interventions. In another hand, the time when thrombocytopenia develops is also different. Once thrombocytopenia associated with fludarabine happened, most of the experts suggest that fludarabine should be discontinued. The goal of treatment of refractory ITP is to maintain a 'safe' platelet count of 20-50*10^9/L [21]. Corticosteroids and immunoglobulin are the most frequently used agents in overcoming the thrombocytopenia and work well in bulk cases [11]. IVIG usually results in rapid increase in platelet counts but these are often short duration. However, considerable patient failed to respond to above therapeutic strategies. Leach et al reported that a patient with CLL developed a fludarabine-associated ITP. High dose dexamethasone (40 mg daily for 4 days) given monthly was then found to be effective in recovering, then maintaining, the platelet count in the normal range over the next six months. However, when fludarabine was recommenced in view of progressive adenopathy, severe thrombocytopenia recurred within 2 weeks and this time was refractory to dexamethasone. The patient died of diffuse gastrointestinal haemorrhage. So it is more safe to avoid give the same agents again which are responsibility for the therapy-related thrombocytopenia.

Other agents such as cyclosporine, vincristine, combination chemoimmunotherapy and monoclonal antibodies including Alemtuzumab and Rituximab are also presented good effect [8, 22, 23]. Montillo et al. reported ITP recurring in a patient with CLL treated with fludarabine who had a previous episode of ITP with a positive test for auto-antibodies against platelets earlier in the illness. The initial episode of ITP had responded to corticosteroids but this subsequent event was eventually successfully treated with cyclosporine after failure with both corticosteroids and intravenous immunoglobulin [24]. Splenectomy can be a choice for refractory or corticosteroid-dependent ITP in the management of ITP-complicating CLL [25]. An additional diagnostic consideration in these pa-

tients is cytomegalovirus (CMV) reactivation. CMV infection of hematopoietic cells can increase immune destruction of platelets and megakaryocytes [21]. Other supportive treatment options include IL-11, TPO, irradiated platelet transfusion, etc. however, the curative effect of the later agents are negative precisely yet.

Rituximab, a monoclinic anti-CD20 antibody which is widely used in CLL, WM and NHL, has also shown its powerful effect in overcoming the refractory ITP and other autoimmune cytopenia. Former studies illustrated that rituximab can be an effective and safe therapy for the induction of remission from refractory fludarabine-associated immune thrombocytopenia. Hegde et al. reported 3 CLL patients with fludarabine-associated ITP who responded to rituximab. They concluded that the rapid increase in platelet count in their case was also probably attributable to rituximab and not to the delayed effect of steroid therapy [8]. Yutaka Tsutsumi reported that a case complicated fludarabine-associated thrombocytopenia recovered by rituximab, too. It is interesting that although fludarabine was administered again, the platelet count did not decrease when combined with rituximab [7]. In the forthcoming studies, most responding patients showed a rapid improvement in platelet count even within 24 hours of rituximab therapy, suggesting other mechanisms different than depletion of antiplatelet antibodies. This rapid increase in platelet count provides support for the speculation that rituximab causes opsonization of B cells that inhibit macrophage Fc receptor function and clearance of IgG-coated platelets. This action may lead to the eventual suppression of autoreactive B cells and the sustained remissions observed in some patients [8]. In our report, it is regret that we could not treat this patient with rituximab to retrieve the thrombocytopenia associated with fludarabine. Further explorations are needed to determine the exact mechanism of rituximab for the therapy-related thrombocytopenia.

Summary

Fludarabine-associated ITP has previously been reported in several patients with CLL. To our knowledge, this is the first reported case of WM associated with ITP caused by administration of fludarabine. We speculated that immune dysregulation induced by fludarabine played an important role in the development of ITP in our case. From our case we consider that it is important to generate serious concerns that fludarabine may cause autoimmune complications such as ITP. Once ITP is thought to be caused by fludarabine, it is much safe to discontinue or defer it. Vigilant blood monitoring should be recommended in the administration of fludarabine. Furthermore, as corticosteroids might increase the risk of systemic opportunistic infections, necessary monitoring of bellows including CT, sputum culture are requested. Rituximab may be a promising agent to treat ITP associated with fludarabine. Clinical doctors must monitor patient those are treated with fludarabine, pay more attention to the probability of autoimmune diseases induced by fludarabine.

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Disclosure of conflict of interest

None.

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