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Letters

Enhancement of hematopoietic stem cell engraftment by inhibition of CXCL12 proteolysis with sitagliptin, an oral dipeptidyl-peptidase IV inhibitor: A report in a case of delayed graft failure

1. Introduction

After hematopoetic stem cell transplantation (HSCT), engraftment sometimes fails. Among the various causes, defective stem cell homing to bone marrow, a process mainly driven by the chemokine CXCL12 (also known as stromal-cell derived factor 1 (SDF-1)), has been poorly investigated.

Dipeptidyl-peptidase IV (DPP-IV; EC 3.4.14.5; CD26), is a membrane-bound exopeptidase that performs the initial and quantitatively primary catabolic step in inactivation of many proteins, including glucagon-like peptides (GLPs) and several chemokines [1,2]. DPP-IV recognizes and removes the 2 N-terminal amino acids of proteins with a penultimate proline, or less efficiently a penultimate alanine. Within bone marrow, DPP-IV is located in specialized microdomains on membranes of the connective tissue stroma. Many cytokines involved in hematopoiesis, such as IL-1β, IL-2, IL-3, IL-5, IL-6, IL-8, IL-10, IL-13, granulocyte-macrophage colony stimulating factor, granulocyte-colony stimulating factor (G-CSF) and erythropoietin contain the DPP-IV-susceptible N-terminal amino acid sequence with proline in the second position. Recent studies have shown that the biological activities of chemokines can be regulated by the DPP-IV-mediated cleavage of their N-terminal region [3–5]. Among its targets, DPP-IV readily clips the N-terminal amino acids off CXCL12 rendering it no longer chemotactic for HSCs [6–8].

"Gliptins" are a class of selective DPP-IV inhibitors that help lower post-prandial glucose by inhibiting the break down of glucagon-like peptide-1 (GLP-1) [9], an insulin secretagogue synthesized by gut wall cells in response to food. Gliptins have proved useful to treat type II diabetes mellitus and sitagliptin phosphate (a.k.a. MK-0431 and marketed as Januvia®) has been the first gliptin approved for clinical use [10,11].

We report here compassionate use of sitagliptin in a patient suffering from delayed graft failure who was not showing signs of engraftment after retransplantation and high-dose granulocyte-colony stimulating factor therapy.

2. Case report

On March 2006, a previously healthy 56 years old female was admitted at our Division with pancytopenia (white blood cell 380/μl, hemoglobin 6.9 g/dl, platelets 44,000/μl). Cytochemistry showed negative Sudan black, myeloperoxidase and alpha-naphtyl-acetate esterase, with hand-mirrored cells without Auer bodies. Immunophenotyping on bone marrow blood showed blast cells were $CD1a^-$, $CD2^-$, $sCD3^-$, $CD4^-$, $CD5^-$, $CD7^-$, $CD8^-$, $CD10^-$, $CD13^+$, $CD14^-$, $CD15^-$, $CD19^-$, $CD20^-$, $CD33^+$, $CD34^+$, $CD56^-$, $HLA\text{-}DR^+$. Immunohistochemistry on bone marrow trephine biopsy showed that blast cells were $CD34^+$ $CD79A^-$, $PAX5^-$, CD68 $(KP1)^{-/+}$, MPO^-, $CD68^-$, $BCL2^{-/+}$, with normal nuclear localization of nucleophosmin. Cytogenetics showed pericentromeric inversion in chromosome 9 and she was finally diagnosed with acute myeloid leukaemia of M1 subtype in the FAB classification. She had a poor response to standard induction chemotherapy (3 + 7 with cytarabine and idarubicin) and received salvage therapy according to the FLANG protocol and consolidation chemotherapy (3 + 3 + 5 with cytarabine, idarubicin and etoposide). Having a fully HLA-compatible, ABO-mismatched, CMV-seropositive, 65 years old brother, in September 2006 she underwent allogeneic T-cell-depleted (TCD) peripheral blood HSCT after myeloablative conditioning with thiotepa, fludarabine and melphalan (total reinfused dose: 5.72 millions $CD34^+$ cells/kg of recipient body weight). She successfully engrafted at day +11 and was discharged from hospital at day +17.

After 2 months, she suddenly developed pancytopenia with a hypocellular bone marrow aspirate. Hemophagocytic syndrome was ruled out [12–14]. Extensive virological

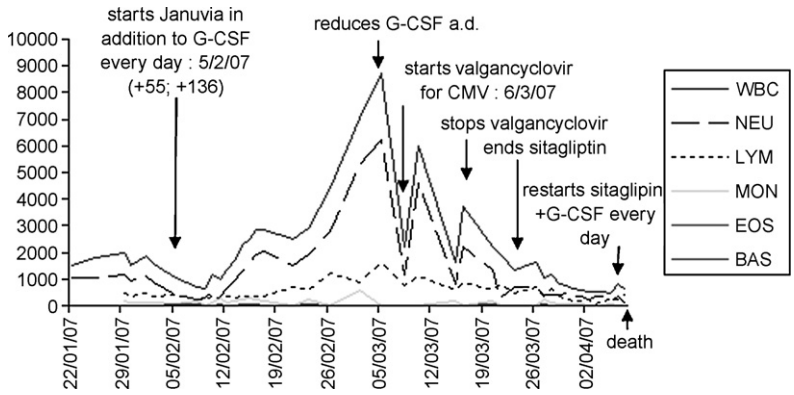

Fig. 1. Variations in white cell count (μl^{-1}) over time, in relation to concurrent infections and supportive therapies.

studies on peripheral blood and bone marrow were negative for agents commonly implicated in secondary graft failure (SGF) and bone marrow aplasia, including adenovirus, human herpesviruses [15], parainfluenzaviruses and parvovirus B_{19} [16].

After failure of supportive treatment with transfusions and high-dose filgrastim, in December 2006 she received a second myeloablative conditioning regimen including fludarabine, antithymocyte globulin and total body irradiation followed by an unselected $CD34^+$ peripheral blood stem cell boost (total infused dose: 5.12 million $CD34^+$ cells/kg) from her original donor, a strategy that had previously been effective in many cases of SGF [17–21]. At day +25 after the second HSCT, her blood group converted from recipient-type (A Rh-positive) to donor-type (0 Rh-positive), but a peripheral blood count still showed only 200 white blood cells/μl, 8 g of hemoglobin/dl, and 10,000 platelets/μl, suggesting poor engraftment. Once again, her white blood cell count did not increase during a 5-day course with filgrastim (30 million units a day).

On the basis of preclinical data [22], since February 2007 the patient was given the oral DPP-IV inhibitor sitagliptin 100 mg bid. No hypoglycaemia or other side effects were observed at this dose and after 4 weeks of treatment her white blood cell count rose to 8700/μl, as shown in Fig. 1, and her transfusion needs decreased from 2 to 1 unit of packed red cells and platelet concentrates per week.

Unfortunately after several weeks her white cell count (WCC) dropped again: the patient was diagnosed with human cytomegalovirus (HCMV) reactivation and started oral valgancyclovir treatment. After a transient rise, the WCC dropped once more: HCMV tested negative at this stage and valgancyclovir was withdrawn, followed by a new rise in the WCC, suggesting myelotoxicity.

Some days later we had to stop sitagliptin because of drug unavailability. Since then her WCC began dropping down. She was admitted again to our Division with fever and abdominal pain. Faecal tests were positive for astrovirus and calicivirus antigens. Despite intensive supportive treatment, the patient died 2 weeks later due to acute renal failure and disseminated intravascular coagulopathy.

A retrospective analysis of CXCL12 levels in serial peripheral blood samples was performed using the Quantikine DSA00 Human SDF1 Immunoassay. We found significant fluctuations in CXCL12 levels which paralleled with beginning and discontinuation of sitagliptin treatment (Fig. 2). No significant variation in the serum levels of 12 other cytokines (IL-1β, IL-2, soluble IL-2 receptor, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-17, and IFN-γ) tested at the same times was observed during treatment with sitagliptin.

3. Discussion

ABO-mismatched HSCT is known to result in delayed platelet engraftment, but our patient showed a delayed graft failure rather than a lineage specific primary engraftment failure, so other underlying causes were likely to exist.

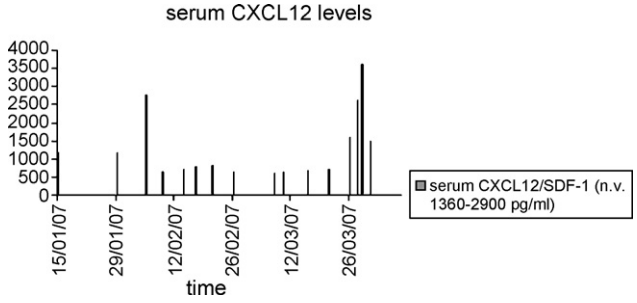

Fig. 2. Variations in serum CXCL12 levels (pg/ml) during treatment with sitagliptin.

We reasoned that the experimental conditioning regimen could have damaged her bone marrow stromal cells, leading to reduced levels of active CXCL12. Since recombinant CXCL12 is not available for human use, we tried to maximize the half-lives of endogenous CXCL12 by inhibiting its catabolism with sitagliptin. Sitagliptin is known to induce neutrophilia at high doses (Merck Co., personal communication), but the increased WCC in our patient was also due to increased lymphocyte and monocyte counts and was accompanied by reduced transfusion requirements, suggesting effective engraftment.

To explain the reverse-from expected fluctuations in serum CXCL12 levels, we suggest that the circulating CXCL12 detected while off sitagliptin was mostly the inactivated (DPP-cleaved) form, whose levels dropped during sitagliptin-mediated inhibition of DPP. Also, when on sitagliptin the excess of active CXCL12 is likely to bind CXCR4 on HSCs, triggering internalization of the ligand–receptor complex and the observed fall in concentration below the lower limit of normal. Unfortunately at the top of our knowledge none of the commercially available monoclonal antibodies can effectively discriminate between the active (full) and the inactive (DPP-cleaved) form of CXCL12, partly because it is very unusual for a diagnostic monoclonal antibody to have the two terminal amino acids as part of its cognate epitope. In this scenario, column chromatography remains the best approach to distinguish between full and truncated CXCL12 isoforms (both represented at very low concentrations) in peripheral blood samples. Alternatively it could be possible to use a specific anti-CXCL12 monoclonal antibodies for immunohistochemistry on bone marrow biopsies: since CXCL12 bound to stromal cell is invariably active (uncleaved), the degree of surface saturation could eventually be used as a less surrogate marker and this would ideally be a "gold-standard" assay, although far more invasive.

In conclusion, this preliminary result encourages the systematic study of sitagliptin and related agents, such as vildagliptin (a.k.a. LAF-237, marketed as Galvus®) and saxagliptin, in the setting of randomized, placebo-controlled clinical trials investigating the reduction of engraftment times in homogeneous cohorts of transplant recipients.

Conflicts of interest

We declare that we have no conflict of interests.

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A case of myelodysplastic syndrome associated with CD14$^+$CD56$^+$ monocytosis, expansion of NK lymphocytes and defect of HLA-E expression

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of stem cell characterized by dysplasia, ineffective haematopoiesis and frequent progression to acute myeloid leukemia [1]. Hyper-cellular bone marrow (BM) and peripheral cytopenias are the hallmarks of MDS [1,2].

Physiopathology of BM failure is complex: occurrence of an intrinsic stem cell defect and immune-mediated mechanisms has been suggested [3,4]. A number of data refer the involvement of pro-inflammatory mechanisms. Indeed, the up-regulation of interferon-gamma (IFN)-γ-induced genes (like CD95/Fas) in MDS CD34$^+$ BM cells and the pathogenetic role for IFN-γ producing cells like large granular lymphocytes (LGL), belonging to T or natural killer cell (NK) lineages, has been largely described [4–6]. Apoptosis and altered Fas (CD95)/FasL (CD178)-mediated pathways have been also referred in MDS [4,5].

NK cells are crucial for innate immunity and for the regulation of the immune response [7]. The expression of NK ligands during myeloid maturation [8] suggests an involvement of NK in the control of haematopoiesis. Human NK express two families of human leukocyte antigen (HLA) class I recognizing receptors: the killer immunoglobulin-like (KIR) and the lectin-like receptors, like CD94/NKG2 molecules [9,10]. Both inhibitory and activating signals have been described for these molecules. An altered expression of CD94/NKG2 receptors was correlated to chronic NK lymphocytosis and to other LGL disorders [11,12]. A major role for HLA-E, a non-classical HLA molecule in regulating NK activity was described [10,13]. This molecule is expressed by several immune and non-immune cells, in both physiological and pathological conditions [14,15]. The involvement of HLA-E alterations in haematopoietic diseases has been proposed [16].

CD56$^+$CD14$^+$ cells are a nearly detectable peripheral blood monocyte population that produce a variety of cytokines, present antigens and mediate regulatory functions [17]. Such cells represent one of the major BM phenotype aberrations in MDS [18].

We describe an MDS patient showing neutropenia, CD14$^+$CD56$^+$ monocytosis and polyclonal expansion of NK cells. A defective HLA-E expression on myeloid lineage, the constitutive presence of CD178 on the CD14$^+$CD56$^+$ monocytes combined with expansion of CD94/NKG2A$^+$ CD94/NKG2C$^+$ NK cells are proposed to be relevant for the pathogenesis of this haematopoietic disorder.

2. Materials and methods

2.1. Case history

A 73-year-old man presented with mild anaemia and neutropenia. The first record of cytopenia was referred to few months before. The Hb level was 10.9 g/dl, MCV 107.3 fl, reticulocyte count 30×10^9 l, white blood cell count 2.95×10^9/l with 0.61×10^9/l polymorph nuclear cells (PMN), 0.82×10^9/l monocytes and 1.52 lymphocytes with predominant features of LGL, platelet count was 174×10^9/l. Flow cytometry analysis revealed in peripheral blood the presence of 54% of T lymphocytes (CD3$^+$CD56$^-$) with a CD4/CD8 ratio of 4.2, 37% of NK cells (CD56$^+$CD3$^-$), 3% of B cells (CD20$^+$) while the monocytes mainly co-expressed CD14 and CD56 molecules.

Good cellularity with dysmyelopoiesis, normal erythroid/myeloid series ratio, normal megakaryocytic number with blast cell count less than 5% characterized BM aspirate.

Flow cytometry analysis of the BM cells revealed 18% of lymphocytes (52% CD3$^+$CD56$^-$ with a CD4/CD8 ratio of 4.27, 40% CD56$^+$CD3$^-$, 3% CD20$^+$), 12% of monocytes (all CD14$^+$CD56$^+$), 52% of granulocytes (CD66b$^+$), 5% of blasts and 12% of the cells in the erythroid region (CD45$^-$).

Cytogenetic analysis showed an aneuploid karyotype (40–45 XY, with random losses) in CD14$^+$CD56$^+$ cells, while none alteration was found in lymphocytes.