this protein is important in maintaining normal transferrin saturation, possibly by regulating hepcidin, as recently proposed.[10] Indeed the inactivation of both proteins causes early iron overload, indicating that *TFR2*, like hepcidin, is crucial for maintaining iron balance in the first decades of life.

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Key words: transferrin receptor-2, iron overload, Y250X mutation

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Sickle Cell Anemia

Circulating endothelin-3 levels in patients with sickle cell disease during hydroxyurea treatment

Abnormal adhesion of red blood cells to the endothelium and the production of cytokines and vasoactive substances, such as endothelin-1 contribute to the pathogenesis of microvascular occlusion in sickle cell disease (SCD), even during the steady state. Endothelin-3 (ET-3) is a vasoconstrictive agent, which has not yet been studied in SCD.

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Sicke cell disease (SCD) is a heritable hemoglobinopathy characterized by chronic hemolysis, frequent infections and recurrent occlusion of the microcirculation that cause painful crises. Subclinical vaso-occlusion occurs even during the steady state and results in chronic organ damage.[1] Vaso-occlusion involves not only the polymerization of hemoglobin S but also abnormal adhesion of young deformable red blood cells to the endothelium and the production of cytokines and vasoactive substances, such as endothelin-1 (ET-1) by the activated endothelial cells.[1,2] ET-1 is a potent vasoconstrictor and pro-inflammatory agonist whose levels have been shown to be elevated in SCD both during crises and in steady state.[3] ET-1 production is regulated by cytokines, growth factors and other vasoactive substances, such as endothelin-3 (ET-3).[4] ET-3 is principally produced by the endothelium and functions not only as a vasoconstrictor, but also as a mediator of inflammation by inducing interleukin-6 (IL-6) production by endothelial cells.[5]

ET-3 has not yet been studied in SCD. Since the actions of ET-3 could be involved in endothelial dysfunction and the pathophysiology of vaso-occlusion in SCD, we decided to measure plasma ET-3 levels in steady state SCD and to investigate their relationship with hydroxyurea (HU) treatment. We studied 12 patients with sickle/β^0 thalassemia, 5 with sickle/β^+ thalassemia and 2 with homozygous SCD. These 19 patients were aged from 24 to 60 years; there were 8 males and 11 females; HU (25 mg/kg of body weight daily) was administered over a 5-month period. All the patients had experienced at least three painful crises necessitating hospitalization in the preceding year. None of the patients had had a painful crisis in the 4 weeks prior to starting HU treatment, nor had they received a transfusion within the 3 months prior to the start of the study. HbF and ET-3 levels were determined before the start of HU treatment after 1 month and 5 months of treatment. HbF was quantified by cation exchange high-performance liquid chromatography. ET-3 was assayed using an ELISA technique according to the manufacturer's guidelines (ET-3: Assay Designs, Inc.). The cross reactivity with ET-1 (1-21) and ET-1 (1-31) was 1.8% and less than 0.1%, respectively. Forty healthy volunteers served as controls. Data were analyzed by Wilcoxon's signed rank test and the Mann-Whitney U test.

The study results are summarized in Table 1. Hematologic changes occurring during HU treatment included a non-significant increase in hemoglobin concentration and a non-significant reduction in white blood cell and reticulocyte counts. HbF levels did not increase significantly by one month after the start of the treatment although they did increase significantly after 5 months. None of the patients had painful crises, required transfusion, or developed serious myelotoxicity. Steady state ET-3 levels were found to be significantly higher in patients than in healthy controls and dropped significantly during HU treatment, even after one month.

To our knowledge this is the first report of high ET-3 levels in SCD. One possible source of ET-3 production is the activated and injured endothelium due to its abnormal interactions

Table 1. Hematologic parameters, fetal hemoglobin (HbF) and endothelin-3 (ET-3) concentrations in 19 patients with sickle cell disease before and during hydroxyurea treatment.

	Before treatment (mean±SD)	*1st month (mean±SD)*	*5th month (mean±SD)*	*Controls (mean±SD)*	*Mann-Whitney (p)*	*Wilcoxon (p)*
Hb (mmol/L)	1.10±0.28	1.18±0.19	1.32±0.28			NS
WBC ($\times 10^9$/L)	12.2±2.7	11.9±2.4	11.1±3.8			NS
Reticulocytes ($\times 10^9$/L)	335±155	280±135	210±180			NS
HbF (%)	7.6±3.8	8.1±2.9	15.8±7.2			< 0.05
ET-3 (pmol/L)	5.66±2.23	2.16±1.21	1.05±0.46	0.99±0.58	< 0.005	< 0.005

Hb: hemoglobin; WBC: white blood cell count; NS: non significant.

with red blood cells. ET-3 could play a role in the cycle of ischemia and inflammation by deregulating vascular tone, and by increasing ET-1 and IL-6 production. The early decrease of ET-3 levels, at a time when HbF elevation was not evident in most patients, is a particularly interesting finding of this study. It indicates that increased HbF is not the only mechanism by which hydroxyurea can affect SCD and this is in concert with previous studies, as it has been shown that the beneficial clinical effect of HU precedes any significant increase in HbF and is not correlated with the achieved level of HbF.[6] At a molecular level, HU reduces the expression of adhesion molecules on the surface of red blood cells, lymphocytes, monocytes and neutrophils before HbF increases.[7,8,9] In addition, it downregulates ET-1 gene expression on endothelial cells.[10]

In summary, the elevated steady state plasma ET-3 levels and their early decrease during HU treatment, indicate that ET-3 may have a role in endothelial deregulation and inflammation in SCD patients. More studies are needed to determine ET-3 levels and their correlation with the extent of vascular damage and the severity of SCD.

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Phagocytes

High incidence of neutropenia in patients treated with rituximab after autologous stem cell transplantation

We report a high incidence of neutropenia in patients treated with rituximab prior to and following autologous stem cell transplantation (ASCT). Fourteen patients with follicular or mantle–cell lymphoma were treated with high dose (HD) therapy followed by an *in vivo*–purged autologous graft. Ten of these patients received two additional courses of rituximab after the transplant. Seven experienced severe neutropenia after the second administration. Our data suggest that early administration of rituximab following a transplant may favor the onset of neutropenia.

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Rituximab is a chimeric IgG-1 monoclonal antibody which binds to the CD20 antigen with high affinity.[1] When used in the pretransplant regimen, it can be considered an *in-vivo purging* agent that can remove CD20+ lymphoma cells from the graft.[2] The occurrence of neutropenia has rarely been reported in patients exposed to rituximab,[3-6] although this complication seems more frequent in the setting of autologous stem cell transplantation (ASCT).[7] From 1999 to 2003, 14 consecutive patients with confirmed histologic diagnosis of follicular (n=9) or mantle cell (n=5) lymphomas were referred to our institution and treated with a protocol integrating chemotherapy, rituximab and ASCT[8] (Table 1). After a phase of debulking with VACOP-B, the patients received rituximab, vincristine and cyclophosphamide and underwent mobilization and harvest of CD34+ cells after high dose cytarabine and rituximab. The final phase of the protocol consisted of ASCT, using BEAM as the conditioning regimen, followed by two courses of rituximab (375 mg/m^2).

All 14 patients underwent ASCT using peripheral blood as the source of stem cells. In 13 cases the apheresis products