this could be related to the predominance of adipocytes in the femoral samples. Indeed, it has been shown that VEGF mRNA is upregulated during the conversion of 3T3 preadipocytes to adipocytes. The VEGFR-1, VEGFR-2 and NRP-1 were measured in the same series of samples. VEGFR-1 mRNA levels were quite variable from case to case. VEGFR-1 mRNA was either absent in iliac crest and femoral bone marrow or expressed at the same level in both tissues or expressed only in femoral bone marrow or was expressed at higher level in femoral bone marrow than in iliac crest bone marrow (Figure 1B). Values for VEGFR-2 were available in five samples only: there was no case. VEGFR-1 mRNA was either absent in iliac crest and femoral bone marrow or expressed at the same level in each donor (Figure 1D) and it seems to be inversely correlated with the hematopoietic activity. The cellular origin of NRP-1 was assessed on isolated cell populations by a floatation/sedimentation procedure. By contrast to sedimented cells (hematopoietic and stromal cells), high levels of NRP-1 mRNA were detected in the adipocytic population (Figure 2A). This was confirmed by in situ hybridization (not shown) and at the protein level by immunohistochemistry (Figure 2B). This is the first report demonstrating neuropilin-1 expression in bone marrow in vivo. The capacity of adipocytes to produce NRP-1, previously suspected to play an interactive role with hematopoietic cells suggests that adipocytes may contribute to the regulation of hematopoiesis and/or that NRP-1 may be a novel regulator of adipocyte activity in the bone marrow, possibly as a receptor for VEGF. Although this study does not provide a definitive link between NRP-1, adipocyte function and hematopoiesis, such a relationship may exist and deserves further studies.

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References
Letters to the Editor

Present study was to test whether this observation in vitro had any clinical relevance in vivo. To address this issue, ET-1 plasma levels were measured in 80 homozygous SS children, treated or not with HU. These children were recruited from among the 800 SCD children followed at the Sickle Cell Center of the Robert Debré pediatric hospital in Paris. The results are shown in Table 1. Circulating ET-1 levels were not different in SS children in steady state than in an age and race-matched group of healthy AA controls. As previously reported, children with VOC, bacterial or viral acute infection, had indeed significantly higher levels of circulating ET-1, independently of their therapeutic status (HU-treated or not). However, the striking observation was that the levels of circulating ET-1 in children treated with HU were almost two times lower than those in untreated children in a steady state or in controls. Of course this decrease in ET-1 levels might not result directly from the effect of HU on endothelial cells but could simply be the indirect consequence of the HU-induced HbF increase. However, three lines of evidence argue against this single possibility. First, we compared ET-1 and HbF levels in the SS children at steady state, treated or not with HU, and found no correlation between these two parameters. Second, levels of circulating ET-1 were followed over 14 and 16 months from the onset of therapy in 2 children (Figures 1A-1B). In both cases the levels of circulating ET-1 underwent a similar sharp decrease within the first 2 months of treatment and then stabilized. In the first child, HbF increased steadily during the whole follow-up period from 11.9% to 14.5% at 2 months, and to 20.8% at 14 months (Figure 1A). However, in the second child, HbF had not changed from its initial 2.7% value after 2 months when ET-1 had already dropped by 60%; it then increased to 5.1% at 4 months and plateaued around this value. Thus, ET-1 level does not always parallel the HU-induced changes in HbF level. Finally, to evaluate the potential relationship between circulating ET-1 and HbF further, we measured these two parameters in SS children aged from 1.2 months to 2 years, i.e. the period of the physiological neonatal decrease of HbF (Figure 1C). Unexpectedly, levels of circulating ET-1 decreased with age from 1.90 pg/mL to the normal 0.65 pg/mL value in 2 years. Although this result is surprising as the opposite might have been expected, i.e. an increase in ET-1 concurrent with the HbF decrease and the onset of the disease, it does show the absence of a relation between circulating ET-1 and HbF levels. No data have been reported regarding neonatal changes in circulating ET-1 in SCD or healthy children and, at this stage, the significance of our observation remains unclear.

Whatever the mechanism, treatment with HU is accom-

Table 1. Endothelin-1 (ET-1) plasma levels in children with sickle cell disease treated or not with hydroxyurea (HU) compared to levels in healthy AA controls.

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>ET-1 pg/mL (mean±SEM)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS children with clinical events HU-treated (n=6) and untreated (n=10)</td>
<td>2.9-13.2</td>
<td>1.32 ± 0.17</td>
</tr>
<tr>
<td>SS children in steady state untreated (n=17)</td>
<td>3.0-14.9</td>
<td>0.65±0.11</td>
</tr>
<tr>
<td>SS children in steady state HU-treated (n=16)</td>
<td>3.4-15.1</td>
<td>0.37±0.05*</td>
</tr>
<tr>
<td>Healthy AA controls (n=26)</td>
<td>2.6-15.8</td>
<td>0.65±0.07</td>
</tr>
</tbody>
</table>

All the children in the HU-treated group had been treated for at least a year. Plasma was prepared and stored at –20°C. ET-1 was measured by ELISA (Quantiglo, R&D system, Abingdon, UK). Intra- and inter-assay differences were 2.2 and 4.0%, respectively. Statistical analysis was performed with an unpaired t test using GraphPad Prism (GraphPad Software, San Diego, CA, USA). A difference between groups was considered statistically significant when p<0.05. NS: not significant.

Figure 1. Comparison of HbF and plasma ET-1 levels. A and B: longitudinal follow-up of two 8-year old SS girls for 14 and 16 months, after the onset of HU treatment. ET-1 plasma levels (open circles; dashed line) and HbF levels (full circles; full line). C: ET-1 plasma levels (dashed line) and HbF levels (full line) in 29 SS infants aged from 1.2 months to 2 years.
Molecular Hematopoiesis

Changes in expression of WT1 isoforms during induced differentiation of the NB4 cell line

The levels of expression of WT1 gene and WT1+17AA isoforms rapidly decreased during the differentiation of NB4 cells induced by all-trans retinoic acid; this decrease was conversely related to the dynamic changes of CD11b positive cells, indicating that the abnormally high expression of WT1 gene and WT1+17AA isoforms was associated with a block of NB4 cell differentiation.

References


Figure 1. Position of the primers and probe of total WT1 gene and WT1+17AA isoforms. SP1: sense primer of total WT1 gene, located on exon 6; AP1: antisense primer of total WT1 gene, located on exon 7; SP2: sense primer of WT1+17AA isoforms located on exon 5; the probe was designed to hybridize at the sense strand of exon 6/7.

Figure 2. Proline/Glutamine SP1 Zinc fingers

1 2 3 4

1 2 3 4

17aa KTS

10