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Endostatin variations in childhood acute lymphoblastic leukaemia—Comparison with basic fibroblast growth factor and vascular endothelial growth factor

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Abstract

Angiogenic factors such as basic fibroblast growth factor (bFGF) or vascular endothelial growth factor (VEGF) were previously studied in childhood acute lymphoblastic leukaemia (ALL) but little is known concerning the anti-angiogenic response in ALL. At diagnosis, the plasma levels of the anti-angiogenic factor endostatin were significantly higher in 33 children with ALL than in controls (median values 17.7 and 7.6 ng/ml, respectively, $p = 0.0192$) but no relationship was observed with plasma bFGF or VEGF levels. The highest levels were observed in patients with an hyperdiploïd karyotype. Expression of mRNA for collagen XVIII/endostatin in lymphoblasts was detected in 19/24 cases but protein secretion was found only in 14/28 supernatants of cultured lymphoblasts. No direct relationship appeared between secretion of endostatin by lymphoblasts and plasma levels. In addition, endostatin levels remained elevated in remission, suggesting that endostatin could have a stromal origin as well. No prognostic value of plasma endostatin could be assessed. In conclusion, the present data indicate that an anti-angiogenic response is observed in some ALL children, but its physiopathological importance remains to be established.

Keywords: Acute lymphoblastic leukaemia; Angiogenesis; Childhood; Endostatin; Microenvironment

1. Introduction

The role of angiogenesis in growth and metastasis of solid tumours lead to the understanding of its implication in the pathogenesis of haematological malignancies. An increased microvessel density of the bone marrow was detected in adult acute myeloblastic leukaemia (AML) as well as in childhood acute lymphoblastic leukaemia (ALL) [1,2]. High cellular and plasma vascular endothelial growth factor (VEGF) levels were described as a poor prognostic marker in adult AML [3,4]. However, high plasma VEGF levels were not found in all studies [5–7]. An increase of other angiogenic factors such as hepatocyte growth factor, angiogenin, basic fibroblast growth factor (bFGF) was reported as well [5–7]. In childhood ALL, the different studies focused mainly on bFGF and VEGF. Perez-Atayde et al. first noted that the level of urinary bFGF was increased at diagnosis [2]. Yetgin et al. observed that serum bFGF and VEGF levels were increased in remission of ALL when compared to the diagnostic values, likely because of a renewal of normal haematopoiesis [8]. In addition, an elevation of VEGF mRNA in bone marrow appeared to be associated with a significantly lower probability of event-free survival at 3 years [9]. In a prospective

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study measuring urinary bFGF and VEGF, we described that a low urinary bFGF elimination is a poor prognosis marker in childhood ALL [10].

The importance of angiogenesis in acute leukaemia was emphasized by some recent studies suggesting a possible disease control by anti-angiogenic therapies in AML [11,12]. Angiogenesis is regulated by a wide range of systemic components in a complex balance of pro- and anti-angiogenic factors. Recently, it was reported that endostatin could inhibit AML progression in mice [13]. Endostatin is a fragment of collagen XVIII having anti-angiogenic effect through anti-migratory and anti-proliferative properties [14]. Endostatin is released from the collagenous domain by cleavage within a protease-sensitive hinge by enzymes, such as elastase [15] and cathepsins [16]. The detection of numbers of endostatin-like fragments in tissue and serum, with sizes ranging from 20 to 40 kDa, indicates that collagen XVIII is sensitive to proteolytic degradation at various sites. This suggests that a number of proteolytic pathways may exist for the generation and degradation of endostatin.

If the role of pro-angiogenic factors begins to be well documented in leukaemia, less is known about anti-angiogenic factors. In the present work, we then focused our attention on the anti-angiogenic factor endostatin in children with ALL. We measured plasma endostatin levels in children with ALL and controls and compared them with plasma bFGF and VEGF levels. In addition we investigated if lymphoblasts could be a source of endostatin by analysing mRNA expression and quantification of the protein in the supernatants of cultured lymphoblasts. We observed that the median serum level of endostatin was significantly higher in patients than controls at diagnosis and remained similarly elevated in remission. The highest levels of endostatin were observed in patients with an hyperdiploïd karyotype. The mRNA of endostatin, as well as the protein was detected in lymphoblasts, suggesting that leukaemia cells are a possible source of endostatin. In contrast to urinary levels of bFGF and VEGF, no prognostic value of plasma endostatin can yet be considered.

2. Patients and methods

2.1. Patients

Thirty-three children, aged from 6 to 191 months (median 57), with newly diagnosed ALL were analysed, including 32 B-phenotypes (4 preB1, 20 preB2, 7 preB3, 1 preB4) and one biphenotypic ALL. Diagnosis was based on complete blood cell counting, bone marrow aspiration, usual immunophenotyping and cytogenetic analysis. The ALL classification was made according to the GEIL classification [17]. Clinical and main laboratory features are shown in Table 1. The median follow-up time for the 29 alive children is 38 months (range: 13–93).

Samples of 10 patients in complete remission (2 preB1, 7 preB2, 1 biphenotypic) were also analysed. Seven of these children were investigated at diagnosis (LP, AP, CR, YY, PH, GF, CD) as well.

In addition, another group of seven patients at relapsed were studied (three preB2, three preB3, one preB4). Newly diagnosed and recurrent ALL cells originated from the same patient in only two cases (AJ, LS).

2.2. Controls

The control group was composed of two different subgroups of children according to the type of analysis performed (plasmatic or cellular). All of them had blood tests for other reasons than haemopathy.

Normal values of endostatin, VEGF and bFGF were determined on citrated plasma from 17 healthy children (median 84 months, range 15–186) who were referred to our institution for the research of a familial haemostasis abnormality, and who were exempt of the coagulation disorder.

In the second group ($n = 16$) mononuclear cells (MNC) were separated and performed a RT-PCR for the three (anti)angiogenic factors. We also quantified endostatin and VEGF in the supernatants of MNC for the 16 controls.

2.3. Cell preparation and cultures

Leukaemic cells or mononuclear cells from controls were isolated by Ficoll-Hypaque (Eurobio, Les Ulis, France) density gradient centrifugation as previously described [18]. Monocytes were eliminated from leukaemic population by their capacity to adhere on plastic (Nunc, Roskilde, Denmark). Then 10^6 cells (mononuclear cells or leukaemic cells) were incubated in 1 ml of serum free medium (Stem Span, StemCell Technologies, Meylan, France) for 4 days at 37 °C in 5% CO_2, 95% water–saturated air mixture. Supernatants were collected, centrifuged, and stored at −80 °C until ELISA analysis.

2.4. Quantification of endostatin, bFGF and VEGF by ELISA

ELISA analysis of citrated plasma samples, urine or culture supernatants were performed according to the manufacturers' instructions. Only morning plasma samples were analysed, since circadian variations were previously described for endostatin [19]. The samples were centrifuged twice at 4500 rpm in order to avoid platelet contamination and were kept frozen at −80 °C until use. A sample of a 24 h urine collection was obtained at diagnosis, and the results were reported to the quantity of urinary creatinine eliminated. bFGF and VEGF kits (Quantikine) were from R&D Systems (Abington, UK), endostatin kit was from TEBU-Bio (Le Perray en Yveline, France).