Endostatin serum level in acute myeloid leukemia

T. WRÓBEL, G. MAZUR, K. KAPELKO, K. KULICZKOWSKI

Department of Haematology, Blood Neoplasms and Bone Marrow Transplantation, e-mail: wrobelt@hemat.am.wroc.pl, Wroclaw Medical University, 50-367 Wroclaw, Poland

Received July 28, 2004

Increased levels of tumor angiogenesis have been demonstrated in variety of solid tumors and hematological malignancies including acute myeloid leukemia (AML). The aim of the study was to evaluate serum level of endostatin in newly diagnosed patients with AML before chemotherapy and after achieving complete remission (CR). Serum samples from 68 adult patients (28 females and 40 males, median age 42 years, range 21–83 years) with AML had been taken before chemotherapy was administered. In addition 21 out of 68 patient were analyzed again after achieving CR. Endostatin levels were measured using ChemiKine sandwich ELISA kit (Chemicon International). Twelve samples from healthy volunteers (5 females and 7 males, median age 40 years; range 35–65 years) were evaluated as the control. Endostatin serum levels were significantly higher in untreated AML patients than in the normal controls. In AML patients baseline endostatin levels were significantly lower than in CR. We did not found any correlation between white cell count or percentage of blasts in the bone marrow and endostatin level. Moreover endostatin levels did not differ statistically among AML FAB subgroups. Increased endostatin plasma levels may reflect intensity of inhibition of angiogenesis and may by useful in prognosis of CR in AML. Chemotherapy can modulate the regulation of angiogenesis in AML patients.

Key words: endostatin, angiogenesis, acute myeloid leukemia

Angiogenesis refers to the formation of new capillaries from preexisting vessels. There are several reports that angiogenesis plays important role in hematologic malignancies including acute myeloid leukemia (AML). The initiation of angiogenesis and the switch to the angiogenic phenotype requires a change in the balance between proangiogenic factors and angiogenic inhibitors. There is an increased evidence that down-regulation of angiogenesis inhibitors is important in the angiogenic switch of the tumor [5]. Endostatin, C-terminal fragment of collagen XVIII, is one of the most potent and specific inhibitors of angiogenesis. Endostatin, originally isolated from medium of hemangiogendothelioma, is generated from collagen XVIII through cleavage of an Ala-His linkage. On the cellular level, endostatin was shown to inhibit endothelial cell proliferation and migration and to induce apoptosis of endothelial cells [6, 15, 16]. The clinical significance of endostatin in hematologic malignancies is not clear and data concerning with endostatin serum/plasma levels in AML are limited [7, 12, 13].

The aim of the study was to evaluate serum level of endostatin in newly diagnosed patients with AML before chemotherapy and after achieving complete remission (CR).

Material and methods

Serum samples from 68 adult patients (28 females and 40 males, median age 42 years, range 21–83 years) with AML were taken before chemotherapy was administered. In addition 21 out of 68 patients were analyzed again after achieving complete remission. Endostatin levels were measured using ChemiKine sandwich ELISA kit (Chemicon International) following the manufacturer instruction. Analyses and calibrations were carried out in duplicate, intra- and interassay variations were within the range given by the manufacturer.

Twelve samples from healthy volunteers (5 females and 7 males, median age 40 years; range 35–65 years) were evaluated as the control. The data were analyzed statistically by using Wald-Wolfowitz test, Spearman test and Anova test rang Kruskall Wallis ($p<0.05$).

Results

The results are shown in the Figure 1 and the Table 1.

There was significant difference in endostatin levels between the untreated AML patients and the normal control. In

Table 1. Serum endostatin concentration in AML patients (untreated and in complete remission, CR) and healthy control

	AML untreated versus Control	AML in CR versus Control	AML untreated versus AML in CR
Endostatin pg/ml	21.2± 9.3 v 7.6±4.0	27.9±10.7 v 7.6±4.0	21.2±9.3 v 27.9±10.7
p	0.001	0.001	0.036

AML patients baseline endostatin level was significantly lower than in the complete remission. We did not found any correlation between white cell count or percentage of blasts in the bone marrow and endostatin level. Moreover, endostatin level did not differ statistically among AML FAB subgroups.

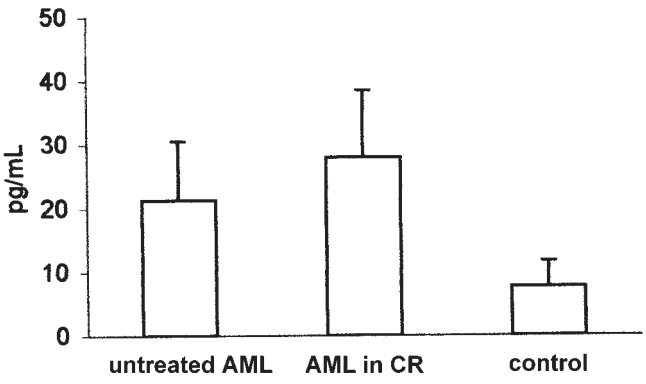

Figure 1. Serum endostatin concentration in AML patients (untreated and in complete remission, CR) and healthy control.

Discussion

Acute myeloid leukemia is an aggressive malignancy with overall disease free survival lower than 50 %. There is increasing evidence that angiogenesis is involved in pathogenesis of AML. Several studies have demonstrated increased microvascular density of AML bone marrow and increased expression of proangiogenic cytokines such as vascular endothelial growth factor (VEGF). In view of these results angiogenesis has been suggested to be involved in pathogenesis of AML [1, 9, 14]. Inhibitors of angiogenesis are more often fragments of extracellular matrix. Endostatin, a C-terminal proteolytic fragment of typ XVIII collagen, specifically inhibits proliferation of endothelial cells. Administration of recombinant endostatin resulted in regression of various tumors in xenograft model. Both systemic and local endostatin therapy is now investigated for the treatment of malignant disorders. Systemic therapy with endostatin has been shown to suppress tumor induced angiogenesis. However *in vitro* endostatin is able to inhibit growth of new vessels only in high concentrations [4, 6, 10, 15, 16]. The role of angiogenesis inhibitors in hematological malignancies is not clearly established. There are some reports that in multiple myeloma loss of marrow angiogenesis inhibitory activity is correlated with disease progression. In non-Hodgkin's lymphoma (nHL) patients no difference was found between endostatin plasma levels in complete remission and progressive disease. Moreover endostatin level did not correlate with disease-free survival. However, endostatin is able to induce tumor stabilization after chemotherapy or rituximab therapy in animal nHL model. In contrast to solid tumors there is no association between specific endostatin polymorphism and the risk of leukemia [2, 3, 6, 11]. The data concerning endostatin serum levels in leukemia patients are confusing. LAI et al [12] did not find significant difference in the median plasma endostatin level between AML/MDS patients and normal controls. On the other hand GLENJEN et al [7] observed increased endostatin serum levels in untreated AML. Furthermore endostatin levels remained high even after chemotherapy [7, 12]. In chronic lymphocytic leukemia endostatin serum concentration is not elevated when compared with the control [8]. In our study serum levels of endostatin in the untreated AML patients were significantly higher than in the healthy controls. Elevated baseline endostatin levels in AML may be the result of increased angiogenesis in the bone marrow and reflecting tumor burden. In solid cancers endostatin levels were raised after surgical removal of the primary tumor [17]. Similarly we have demonstrated that in CR AML group endostatin level was higher than in untreated AML patients. This suggests that chemotherapy can modulate the systemic component of angiogenic regulation and raises the possibility that endostatin may be used in AML therapy.

We conclude that increased endostatin plasma level may reflect intensity of inhibition of angiogenesis and may by useful in the prognosis of CR in AML. Chemotherapy can modulate the regulation of angiogenesis in AML patients.

References

[1] AGUAYO A, ESTEY E, KANTARIJAN H, MANSOURI T, GIDEL C et al. Cellular vascular endothelial growth factor is a predictor of outcome in patients with acute myeloid leukemia. Blood 1999; 94: 3717–3721.

[2] BERTOLINI F, PAOLUCCI M, PECCATORI F, CINIERI S, AGAZZI A et al. Angiogenic growth factors end endostatin in ino-Hodgkin's lymphoma. Br J Haematol 1999; 106: 504–509.

[3] BERTOLINI F, FUSETTI L, MANCUSO P, GOBBI A, CORSINI C et al. Endostatin, an antiangiogenic drug, induces tumor stabilization after chemotherapy or anti-CD20 therapy in a NOD/SCID mouse model of human high-grade non-Hodgkin lymphoma. Blood 2000; 96: 282–287.

[4] BRUSERUD O, GLENJEN N, RYNINGEN A. Effects of angiogenic regulators on in vitro proliferation and cytokine secretion by native human acute myelogenous leukemia blasts. Eur J Haematol 2003; 71: 9–17.

[5] FOLKMAN J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1: 27–31.

[6] FOLKMAN J. Cancer without disease. Nature 2004; 427: 787–789.

[7] GLENJEN N, MOSEVOLL KA, BRUSERUD O. Serum levels of angiogenin, basic fibroblast growth factor and endostatin in patients receiving intensive chemotherapy for acute myelogenous leukemia. Int J Cancer 2002; 101: 86–94.

[8] GORA-TYBOR J, BLONSKI JZ, ROBAK T. Circulating pro-angiogenic cytokines and angiogenesis inhibitor endostatin in untreated patients with chronic lymphocytic leukemia. Mediators of Inflammation 2003; 12: 167–171.

[9] HUSSONG JW, RODGERS GM, SHAMI PJ. Evidence of increased angiogenesis in patients with acute myeloid leukemia. Blood 2000; 95: 309–313.

[10] JUNG SP, SIEGRIEST B, HORNICK CA, WANG YZ, WADE MR et al. Effect of human recombinant endostatin protein on human angiogenesis. Angiogenesis 2002; 5: 111–118.

[11] KUMAR S, WITZIG T, TIMM M, HAUG J, WELLIK J, KIMLINGER TK et al. Bone marrow angiogenic ability and expression of angiogenic cytokines in myeloma: Evidence favoring loss of marrow angiogenesis inhibitory activity with disease progression. Blood 2004 May 6 [Epub ahead of print].

[12] LAI R, ESTEY E, SHEN Y, DESPA S, KANTARIJAN H ET AL. Clinical significance of plasma endostatin in acute myeloid leukemia/myelodysplastic syndrome. Cancer 2002; 94: 14–17.

[13] LIU TC, PENG CT, LIN SF, CHANG CS, CHEN TP, CHANG JG. Association of endostatin D104N with leukemia. Kaohsiung J Med Sci 2003; 19: 1–5.

[14] LOVENBERG B, DOWNING JR, BURNETT A. Acute myeloid leukemia. N Engl J Med 1999; 341: 1051–1062.

[15] O'REILLY MS, BOEHM T, SHING Y, FUKAI N, VASIOS G et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell 1997; 88: 1–20.

[16] SCHUCH G, HEYMACH JV, NOMI M, MACHLUF M, FORCE J et al. Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. Cancer Res 2003; 63: 8345–8350.

[17] TEH SH, HILL AD, LEE AW, FOLEY D, KENNEDY S et al. Raised plasma endostatin leves correlate inversely with breast cancer angiogenesis. J Surg Res 2004; 116: 165–171.