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RESEARCH ARTICLE

The effect of imatinib mesylate on the erythroid colony formation from patients with polycythemia vera in the presence of different cytokines

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ABSTRACT

It has been shown that imatinib mesylate, a drug used in the treatment of chronic myelogenous leukemia, inhibits the effect of stem cell factor, which has a central role in erythropoiesis. In some polycythemia vera (PV) patients, it has inhibited autonomous erythroid colony growth in vitro and decreased the need for phlebotomy. In this study we have investigated the effect of insulin like growth factor (IGF)-I, stem cell factor (SCF) and erythropoietin (Epo) with interleukin (IL)-3, granulocyte macrophage-colony stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF) in the presence of imatinib mesylate on the erythroid progenitors derived from peripheral blood mononuclear cells of three patients with PV and four healthy controls in semisolid medium. Erythroid colony formation from hematopoietic progenitors obtained from healthy controls was observed only in the presence of all cytokines. However, the number of erythroid colonies could not reach that of patients with PV. Inhibition of imatinib mesylate on erythroid colony growth was evident. Hematopoietic progenitors of patients with PV displayed two types of colony formation: the first type was exogenous cytokine-independent and was hypersensitive to current cytokines, and the second displayed hypersensitivity to current exogenous cytokines, but was exogenous cytokine-dependent. For both types, the inhibitory effect of imatinib mesylate was striking in the presence of all cytokines including IL-3, GM-CSF and Epo. There is no direct evidence yet that imatinib mesylate could inhibit the effect of IL-3, G-CSF, GM-CSF, Epo and IGF-I on erythropoiesis. Considering former studies together with results of this study, it can be argued that imatinib mesylate is effective in PV on the intersecting signal transduction mechanisms in which stem cell factor and its receptor may have a part.

Key Words: Polycythemia vera, imatinib mesylate, BFU-E, SCF, IGF-I, erythropoiesis.

ÖZET

Polisitemia veralı hastalarda farklı sitokinler varlığında eritroid koloni gelişimi üzerine imatinib mesylate'ın etkisi

Kronik myeloid lösemi tedavisinde kullanılan imatinib mesilat aynı zamanda kök hücre faktörünü de inhibe etmektedir. Imatinib mesilatın bazı polistemia veralı hastalarda otonom eritroid koloni gelişimini inhibe ettiği ve flebotomi ihtiyacını azalttığı da gösterilmiştir. Bu çalışmada, imatinib mesilat varlığında, polistemia veralı üç hasta ile sağlıklı dört kontrolden elde edilen periferik kan eritroid öncül hücreleri üzerine yarı-katı ortamda insülin benzeri büyüme faktörü-I (IGF-I), kök hücre faktörü (SCF) ve eritropoietin (EPO) ile interlökin-3 (IL-3), granülosit-koloni stimule edici faktörün (GM-CSF) ve granülosit-koloni stimule edici faktörünün (G-CSF) etkisi araştırıldı. Sağlıklı kontrollerden elde edilen hematopoietik öncü hücrelerden eritroid koloni gelişimi sadece tüm sitokinlerin varlığında gözlendi. Bununla birlikte eritroid kolonilerin sayısı polistemia veralı hastalardan elde edilen sayılara ulaşamadı. Imatinib mesilatın eritroid koloni gelişimi üzerindeki inhibe edici etkisi belirgindi. Polistemia veralı hastaların hematopoietik öncüllerinden iki tip eritroid koloni gelişimi gözlendi; ilki eksojen sitokinlerden bağımsız ve sitokinlere aşırı duyarlılık gösteren, ikincisi ise eksojen sitokinlere aşırı duyarlılık gösteren tip idi. Her iki tipte de IL-3, GM-CSF ve EPO de dahil olmak üzere, sitokinlerin varlığında imatinib mesilatın inhibe edici etkisi belirgindi. Imatinib mesilatın bu etkisinin IL-3, G-CSF, GM-CSF, EPO ve IGF-I üzerine olduğunu söylemek için henüz yeterli kanıt yoktur. Bu çalışmanın sonuçları daha önceki çalışmalardan elde edilen bilgilerle birlikte değerlendirildiğinde, imatinib mesilatın eritroid koloni gelişimini inhibe edici etkisinin kök hücre faktörünün ve reseptörlerinin rol aldığı sinyal ileti yolları üzerinden olduğu söylenebilir.

Anahtar Sözcükler: Polisitemia vera, imatinib mesylate, BFU-E, SCF, IGF-I, eritropoiezis

INTRODUCTION

Polycythemia vera (PV) is characterized by an increased proliferation of all three myeloid lineages. The increase in red cell mass is the clinically predominant feature. Bone marrow cells from PV patients are able to form erythroid colonies (ECs) in vitro without the addition of exogenous erythropoietin (Epo) [1,2]. Apart from Epo, PV hematopoietic progenitors are hypersensitive to hematopoietic growth factors including insulin-like growth factor-I (IGF-I), interleukin-3 (IL-3), granulocyte/macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF) [3-8]. Epo has synergism with IGF-I on DNA synthesis of erythroid progenitor cells [9]. SCF has a similar synergism with Epo and IGF-I [10]. The number of receptors for the growth factors and their dissociation constants are normal. These observations suggest that signal transduction pathways may be altered in these cells [4,11-13].

Imatinib mesylate is a potent inhibitor of Bcr-Abl tyrosine kinase activity. Imatinib mesylate inhibits other tyrosine kinases including SCF. SCF is described as a ligand for receptor tyrosine kinase that is encoded by c-kit protooncogene [14-16]. It is well known that SCF has a central role in normal erythropoiesis [17-19].

It has been shown that imatinib mesylate significantly decreased SCF-dependent growth of M-07e cells in a dose-dependent manner and blocked the antiapoptotic activity of SCF [20]. Imatinib mesylate has a potent inhibitory effect on the kinase activity of a human mast cell leukemia cell line (HMC-1), which has an activated mutant form of c-kit [20]. It has been shown that imatinib mesylate, from 0.01 μM to 10 μM concentrations, markedly reduced autonomous burst forming units-erythroid (BFU-E) growth of PV hematopoietic progenitors in semisolid medium. The amount of suppression was dose-dependent and was more pronounced in the absence of IL-3, GM-CSF, and Epo [21].

These observations provided us the rationale to test the effect of imatinib mesylate on BFU-E growth of PV hematopoietic progenitors in semisolid medium in the presence or absence of SCF and IGF-I with Epo, G-CSF, GM-CSF and IL-3.

PATIENTS and METHODS

Patients:

Four healthy controls who had no known disease and were not smokers, with normal hematocrit, leukocyte and platelet counts, and three patients with PV as established by the diagnostic criteria of the Polycythemia Vera Study Group were studied [22]. Patients were treated with phlebotomy at the time of study, and none of them had previously received cytostatic drugs or radioactive phosphorus. None of the healthy controls or patients had been taking theophylline, β-blocker, angiotensin converting enzyme inhibitor or angiotensin-II receptor type-1 blocker for at least three months. The study was approved by the Ethics Committee of Dokuz Eylül University Medical Faculty. Informed consents were obtained from all healthy controls and patients.

Colony assay:

After an overnight fasting period, peripheral blood samples were obtained by venipuncture from healthy controls and patients in the morning and were immediately placed into polypropylene tubes containing sodium heparin. Mononuclear cell (MNC) fraction was isolated by Ficoll-Hypaque (1.077 g/ml; Sigma Diagnostics, USA) density gradient centrifugation. The interphase layer was carefully collected, centrifuged at 250x*g* for 10 min at room temperature in Iscove's Modified Dulbecco Medium (IMDM; Biological Industries, Kibbutz Beit Haemek, Israel). After double washing procedure, the non-adherent (NA) fraction was separated by adherence technique. First, MNC pellets were diluted in 10 ml of 10% fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel) in IMDM. Then, MNC suspension was placed into t-25 flasks and incubated at 37^0C for 2 h. NA MNCs were washed with IMDM. MNC pellets at the bottom of the tubes were resuspended in 10 ml of 10% FCS in IMDM. MNCs were cultured in methylcellulose based semisolid culture mediums in 24-well flat bottom flasks. Each well contained 200 μL methoculth (Methoculth; Stemcell Technologies Inc, Vancouver, Canada) and 300 μL IMDM (final volume 500 μL with cytokines). Cytokines were added to each well according to the study design (Table 1). $5x10^4$ MNCs suspended in 50 μL 10% FCS in IMDM were added to wells. Viability of the MNCs were tested with trypan blue (Sigma, St Louis, MO, USA) and viable cells were more than 90%. Duplicated culture flasks were incubated at 37^0C in a humidified atmosphere of 5% CO_2 for 14 days. ECs were counted on day 14 by inverted microscope, and morphologically well-hemoglobinized colonies consisting of more than 50 cells were regarded as BFU-E.

Table 1. Plan and concentrations of the cytokines in the culture mediums: GM-CSF 10 ng/ml, G-CSF 10 ng/ml, IL-3 10 ng/ml, SCF 50 ng/ml, IGF-I 100 ng/ml and Epo 2 U/ml

	Epo	*SCF*	*IGF-I*
Wells with IL-3 GM-CSF G-CSF	-	-	-
	+	-	-
	-	+	-
	-	-	+
	+	+	+
Wells without IL-3 GM-CSF G-CSF	-	-	-
	+	-	-
	-	+	-
	-	-	+
	+	+	+

Table 2. Effect of imatinib mesylate (IM) on the EC growth in the presence of Epo, SCF and IGF-I

Cytokine Free	*IM -*	*IM +*	*% inhibition*
PV1	34	27	20
PV2	0	0	-
PV3	0	0	-
Epo	*IM -*	*IM +*	*% inhibition*
PV1	57	31	45
PV2	0	0	-
PV3	4	0	100
SCF	*IM -*	*IM +*	*% inhibition*
PV1	49	33	32
PV2	0	0	-
PV3	0	1	-
IGF-I	*IM -*	*IM +*	*% inhibition*
PV1	56	29	48
PV2	0	0	-
PV3	0	0	-
Epo+SCF+IGF-I	*IM -*	*IM +*	*% inhibition*
PV1	82	42	48
PV2	26	0	100
PV3	40	1	97.5

Cytokines and imatinib mesylate:

GM-CSF (Sandoz Pharma AG, Basel, Austria) 10 ng/ml, G-CSF (Roche, Basel, Switzerland) 10 ng/ml, IL-3 (Sigma, St Louis, Missouri, USA) 10 ng/ml, Epo (Roche Diagnostics, Mannheim, Germany) 2 U/ml, SCF (Sigma, St Louis, Missouri, USA) 50 ng/ml, and IGF-I (Sigma, St Louis, Missouri, USA) 100 ng/ml were added to culture wells as described in Table 1. Penicillin-streptomycin (Biological Industries, Kibbutz Beit Haemek, Israel) 100 U/ml and amphotericin B (Biological Industries, Kibbutz Beit Haemek, Israel) 4 µg/ml were added to all culture mediums, respectively. The quantities of cytokines were determined according to the generally accepted optimal dose for BFU-E growth as shown by the previous studies [21,23-32].

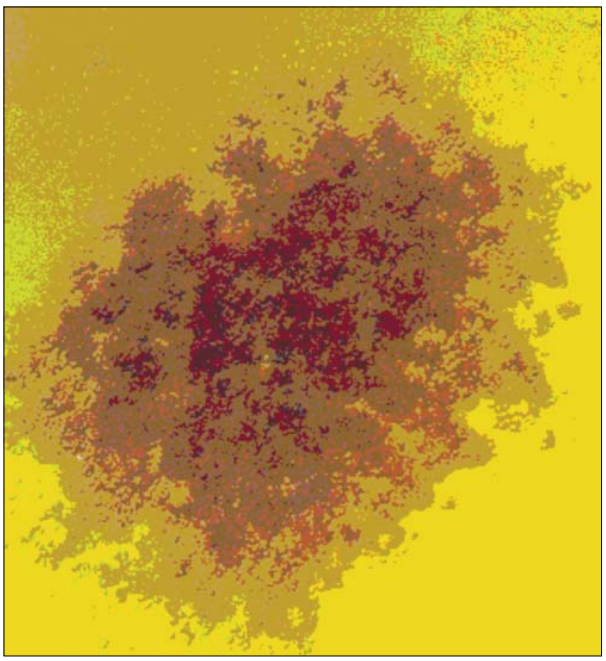

Figure 1. BFU-E in the presence of Epo, SCF, IGF-I, IL-3, G-CSF and GM-CSF.

Cultures were studied either in the presence or absence of 10 µM imatinib mesylate. Imatinib mesylate was a kind gift from Novartis Pharmaceutical (Basel, Switzerland).

Statistical analyses:

Results are given as percent stimulation or inhibition with respect to the differences between the colony counts of culture mediums.

RESULTS

Patient 1: Spontaneous ECs formation in the absence of cytokines was observed. The presence of IL-3, G-CSF and GM-CSF (standard cytokine combination) led to 35% more colony growth (34 versus 46 ECs). Imatinib mesylate inhibited colony growth in both of the conditions. However, inhibition was more prominent in the presence of standard cytokines (20% versus 43%). Without standard cytokine combination, Epo, SCF or IGF-I produced similar EC growth (57, 49 and 56 in order). Inhibitory effect caused by imatinib mesylate on EC growth in the presence of these cytokines was similar (45%, 32% and 48%, respectively) (Table 2). The most striking EC growth was

Table 3. Effect of imatinib mesylate (IM) on the EC growth in the presence of Epo, SCF and IGF-I together with IL-3, G-CSF and GM-CSF (*Standard Cytokine Combination, SCC).

*SCC**	*IM -*	*IM +*	*% inhibition*
PV1	46	26	43
PV2	8	5	37.5
PV3	14	4	71
SCC+Epo	*IM -*	*IM +*	*% inhibition*
PV1	60	58	3
PV2	5	0	100
PV3	14	5	64
SCC+SCF	*IM -*	*IM +*	*% inhibition*
PV1	61	52	14.5
PV2	18	0	100
PV3	22	0	100
SCC+IGF-I	*IM -*	*IM +*	*% inhibition*
PV1	59	52	12
PV2	5	2	60
PV3	10	6	40
SCC+Epo+SCF+IGF-I	*IM -*	*IM +*	*% inhibition*
PV1	125	74	40
PV2	159	5	96.8
PV3	110	8	92.7
Control 1	32	0	100
Control 2	64	10	84
Control 3	65	0	100
Control 4	33	5	84.8

obtained by combination of all cytokines (standard cytokines with Epo, SCF and IGF-I) (Figure 1), and imatinib mesylate inhibited the effect of this combination by 40%. Combination of standard cytokines with Epo, SCF or IGF-I produced similar colony growth. The inhibition patterns of combinations were also similar (Table 3).

Patients 2 and 3: Without standard cytokine combination, EC formation was observed in patient 3 with Epo (4 colonies). Aside from this result, both patients were able to form ECs only in the presence of Epo, SCF and IGF-I together. However, the inhibition of imatinib mesylate on EC formation was striking (Table 2). With standard cytokine combination, with or without Epo, SCF or IGF-I, these patients were able to form ECs. In the presence of all cytokines, EC growth was very prominent as observed in patient 1. Effect of imatinib mesylate on the EC growth was more evident in patients 2 and 3 than patient 1 (Table 3).

Control Group: EC formation was observed only in the presence of all cytokines. However, the number of ECs could not reach that of patients with PV. Inhibition of imatinib mesylate on EC growth was evident.

DISCUSSION

The increase in red cell mass in PV is the clinically predominant feature and this increased red cell mass may be due to either increased proliferation and/or reduced apoptosis. Many studies have been published concerning the pathogenesis of PV. However, the pathogenesis of the disorder has not been established yet, and the molecular mechanism for erythrocytosis in PV is still unknown.

Hematopoietic progenitors of patients with PV displayed two types of colony formation: the first was exogenous cytokine-independent and was hypersensitive to current cytokines, and the second displayed hypersensitivity to current exogenous cytokines, but was exogenous cytokine-dependent. Although Epo, SCF and IGF-I combination provided a colony stimulator effect, addition of IL-3, G-CSF and GM-CSF to this combination led to a more striking EC growth. In the second type, the inhibitory effect of imatinib mesylate on colony formation was more prominent.

It has been shown by Oehler *et al.* [21] that imatinib mesylate could inhibit autonomous BFU-E growth in a dose-dependent manner and this effect was lesser in the presence of IL-3, GM-CSF and Epo. Heinrich *et al.* [20] pointed out that treatment of M-07e cells with STI 571 inhibited Steel factor (SLF)-dependent, but not GM-CSF–dependent, proliferation. Although PV cells and M-07e cells have different characteristics and we did not study the absolute effect of GM-CSF, we have to remark that, in our study, the inhibitory effect of imatinib mesylate was striking in the presence of all cytokines including GM-CSF.

These observations suggest that PV may not be a homogeneous disorder. A group of patients may have abnormalities in signal transduction pathways resulting in cytokine independence. The others may have hypersensitivity to cytokines/growth factors or some patients may have alterations in the antiapoptotic mechanisms or a combination of these mechanisms.

There is no direct evidence yet that imatinib mesylate could inhibit the effects of Epo on erythropoiesis. Although it has not been studied in PV, the effect of imatinib mesylate on IGF-I is not clear [33,34]. In our study, addition of IGF-

I resulted in slightly more colony growth in the exogenous cytokine-independent type and imatinib mesylate overcame this effect.

In brief, considering the results of former studies together with this study, it can be argued that imatinib mesylate is effective in PV on the intersecting signal transduction mechanisms in which SCF and its receptor have a part. BFU-E growth of healthy donors was observed only in the presence of all cytokines and this colony formation was inhibited by imatinib mesylate. Development of anemia and other cytopenias during imatinib treatment might be associated with this observation. Further studies are warranted to determine the potential efficacy of STI 571 in the treatment of PV.

References

1. Prcal JF, Axelrad AA. Letter: Bone-marrow responses in polycythemia vera. N Engl J Med. 1974;290:1382. No abstract available.
2. Biljanovic-Paunovic L, Ruvidic R, Pavlovic-Kentera V. Endogenous BFU-E in peripheral blood in diagnosis of polycythemia vera. Eur J Haematol 1990;45:262-6.
3. de Wolf JT, Beentjes JA, Esselink MT, Smit JW, Halie RM, Clark SC, Vellenga E. In polycythemia vera human interleukin 3 and granulocyte-macrophage colony-stimulating factor enhance erythroid colony growth in the absence of erythropoietin. Exp Hematol 1989;17:981-3.
4. Dai CH, Krantz SB, Green WF, Gilbert HS. Polycythaemia vera. III. Burst-forming units-erythroid (BFU-E) response to stem cell factor and c-kit receptor expression. Br J Haematol 1994;86:12-21.
5. Correa PN, Eskinazi D, Axelrad AA. Circulating erythroid progenitors in polycythemia vera are hypersensitive to insulin-like growth factor-I in vitro: studies in an improved serum-free medium. Blood 1994;83:99-112.
6. Wickrema A, Chen F, Namin F, Yi T, Ahmad S, Uddin S, Chen YH, Feldman L, Stock W, Hoffman R, Platanias LC. Defective expression of the SHP-1 phosphatase in polycythemia vera. Exp Hematol 1999;27:1124-32.
7. Dai CH, Krantz SB, Sawyer ST. Polycythemia vera. V. Enhanced proliferation and phosphorylation due to vanadate are diminished in polycythemia vera erythroid progenitor cells: a possible defect of phosphatase activity in polycythemia vera. Blood 1997;89:3574-81.
8. Dai CH, Krantz SB, Means RT, Horn ST, Gilbert HS. Polycythemia vera blood burst-forming units-erythroid are hypersensitive to interleukin-3. J Clin Invest 1991;87:391-6.
9. Okajima Y, Matsumura I, Nishiura T, Hashimoto K, Yoshida H, Ishikawa J, Wakao H, Yoshimura A, Kanakura Y, Tomiyama Y, Matsuzawa Y. Insulin-like growth factor-I augments erythropoietin-induced proliferation through enhanced tyrosine phosphorylation of STAT5. J Biol Chem 1998;273:22877-83.
10. Panzenböck B, Bartunek M, Mapara MY, Zenke M. Growth and differentiation of human stem cell factor/erythropoietin dependent erythroid progenitor cells in vitro. Blood 1998;92: 3658-68.
11. Michl P, Spoettl G, Engelhardt D, Weber MM. Alterations of the insulin-like growth factor system in patients with polycythemia vera. Mol Cell Endocrinol 2001;181:189-97.
12. Means RT, Krantz SB, Sawyer ST, Gilbert HS. Erythropoietin receptors in polycythemia vera. J Clin Invest 1989;84:1340-4.
13. Dai CH, Krantz SB, Dessypris EN, Means RT, Horn ST, Gilbert HS. Polycythemia vera II. Hypersensitivity of bone marrow erythroid, granulocyte-macrophage and megakaryocyte progenitor cells to interleukin-3 and granulocyte-macrophage colony stimulating factor. Blood 1992;80:891-9.
14. Martin FH, Suggs SV, Langley KE, Lu HS, Ting J, Okino KH, Morris CF, McNiece IK, Jacobsen FW, Mendiaz EA, Birkett NC, Smith KA, Johnson MJ, Parker VP, Flores JC, Patel AC, Fisher EF, Erjavec HO, Herrera CJ, Wypych J, Sachdev RK, Pope JA, Leslie I, Wen D, Lin C-H, Cuppies RL, Zsebo KM. Primary structure and functional expression of rat and human stem cell factor. Cell 1990;63:203-11.
15. Matsui Y, Zsebo KM, Hogan BLM. Embryonic expression of a haematopoietic growth factor encoded by the S1 locus and ligand for c-kit. Nature 1990;347:667-9.
16. Zsebo KM, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, Hsu R-Y, Birkett NC, Okino KH, Murdock DC, Jacobsen FW, Langley KE, Smith KA, Takeishi T, Cattanach BM, Gali SJ, Suggs SV. Stem cell factor is encoded at the S1 locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 1990;63:213-24.
17. Lowry PA, Zsebo KM, Deacon DH, Eichman CE, Quesenberry PJ. Effects of rrSCF on multiple cytokine responsive HPP CFC generated from SCA+Lin -murine hematopoietic progenitors. Exp Hematol 1991;19:994-6.

18. Migliaccio G, Migliaccio AR, Valinsky J, Langley K, Zsebo K, Visser JW, Adamson JW. Stem cell factor induces proliferation and differentiation of highly enriched murine hematopoietic cells. Proc Natl Acad Sci U S A 1991;88:7420-4.
19. Dai CH, Krantz SB, Zsebo KM. Human burst-forming units-erythroid need direct interaction with stem cell factor for further development. Blood 1991;78:2493-7.
20. Heinrich MC, Griffith DJ, Druker BJ, Wait CL, Ott KA, Zigler AJ. Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood 2000; 96:925-32.
21. Oehler L, Jaeger E, Eser A, Sillaber C, Gisslinger H, Geissler K. Imatinib mesylate inhibits autonomous erythropoiesis in patients with polycythemia vera in vitro. Blood 2003; 102:2240-2.
22. Tey SK, Cobcroft R, Grimmett K, Marlton P, Gill D, Mills A. A simplified endogenous erythroid colony assay for the investigation of polycythemia. Clin Lab Haem 2004;26:115-21.
23. Frostad S, Bjerknes R, Abrahamsen JF, Olweus J, Bruserud O. Insulin-like growth factor-1 (IGF-1) has a costimulatory effect on proliferation of committed progenitors derived from human umbilical cord $CD34^+$ cells. Stem Cells 1998;16:334-42.
24. Bohmer RM. IL-3-dependent early erythropoiesis is stimulated by autocrine transforming growth factor beta. Stem Cells 2004;22:216-24.
25. Majka M, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ. Numerous growth factors, cytokines, and chemokines are secreted by human CD341 cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. Blood 2001;97:3075-85.
26. Mrug M, Stopka T, Julian BA, Prchal JF, Prchal JT. Angiotensin II stimulates proliferation of normal early erythroid progenitors. J Clin Invest 1997;100:2310-4.
27. Larghero J, Gervais N, Cassinat B, Rain JD, Schlageter MH, Padua RA, Chomienne C, Rousselot P. Farnesyltransferase inhibitor tipifarnib (R115777) preferentially inhibits in vitro autonomous erythropoiesis of polycythemia vera patient cells. Blood 2005;105:3743-5.
28. Geissler K, Ohler L, Fodinger M, Kabrna E, Kollars M, Skoupy S, Lechner K. Interleukin-10 inhibits erythropoietin-independent growth of erythroid bursts in patients with polycythemia vera. Blood 1998;92:1967-72.
29. Tsantes AE, Tassiopoulos ST, Papadhimitriou SI, Bonovas S, Poulakis N, Vlachou A, Filioussi K, Loukopoulos D. Theophylline treatment may adversely affect the anoxia-induced erythropoietic response without suppressing erythropoietin production. Eur J Clin Pharmacol 2003;59:379-83.
30. Dai C, Chung IJ, Krantz SB. Increased erythropoiesis in polycythemia vera is associated with increased erythroid progenitor proliferation and increased phosphorylation of Akt/PKB. Exp Hematol 2005;33:152-8.
31. Naito M, Kawashima A, Akiba T, Takanashi M, Nihei H. Effects of an angiotensin II receptor antagonist and angiotensin-converting enzyme inhibitors on burst forming units-erythroid in chronic hemodialysis patients. Am J Nephrol 2003;23:287-93.
32. Ugo V, Marzac C, Teyssandier I, Larbret F, Lecluse Y, Debili N, Vainchenker W, Casadevall N. Multiple signaling pathways are involved in erythropoietin-independent differentiation of erythroid progenitors in polycythemia vera. Exp Hematol. 2004 Feb;32:179-87.
33. Li J, Kleeff J, Guo J, Fischer L, Giese N, Buchler MW, Friess H. Effects of STI571 (gleevec) on pancreatic cancer cell growth. Mol Cancer 2003;2:32-41.
34. Myllarniemi M, Frosen J, Calderon Ramirez LG, Buchdunger E, Lemstrom K, Hayry P. Selective tyrosine kinase inhibitor for the platelet-derived growth factor receptor in vitro inhibits smooth muscle cell proliferation after reinjury of arterial intima in vivo. Cardiovasc Drugs Ther 1999;13:159-68.