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PS-341 (Bortezomib) inhibits proliferation and induces apoptosis of megakaryoblastic MO7-e cells

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Abstract

PS-341 (Bortezomib) is a dipeptide boronic acid proteasome inhibitor with antitumor activity that induces apoptosis in different human cancer cell lines.

We investigated effects of PS-341 (Bortezomib) on cell proliferation, cell cycle progression, induction of apoptosis and differentiation in a megakaryoblastic (MO7-e) cell line.

PS-341 was able to retain NF-κB in the cytoplasm and inhibit cell growth ($IC_{50} = 22.5$ nM), in a dose/time-dependent way. This anti-proliferative activity resulted to be lineage-specific, because other leukemic cell lines (KG1a, K562/R7, HL60/DNR) were unaffected by the PS-341 treatment.

Moreover, PS-341 in MO7-e induced a significant pro-apoptotic effect from 10 nM concentration (40% versus 12% in the control, $p < 0.05$).

On the other hand, at lower concentration (5 nM), Bortezomib blocked cell cycle in the G2 phase. Finally, this compound was able to down-regulate WT1 expression.

No significant effects on cell differentiation were found.

Because a spontaneous NF-κB activation has been reported in megakaryocytes from patients affected by myeloproliferative disorders, Bortezomib would so be an attractive therapeutic tool for these malignancies, including essential thrombocythemia or idiopathic myelofibrosis.

Preliminary data show an inhibiting activity of Bortezomib in the megakaryocytic colonies formation.

Finally, also down-regulation of the WT1 gene Bortezomib-driven could be relevant, because of the role that this gene would play in the pathogenesis of acute and chronic myeloproliferative disorders.

Keywords: PS-341; Bortezomib; Megakaryoblastic leukemia; WT1; NF-κB; Apoptosis

1. Introduction

The ubiquitin-proteasome is the most important intracellular pathway for protein degradation; it consists of a multicatalytic structure able to recognize protein substrates marked with an ubiquitin chain. Thus, the 26S proteasome plays a fundamental role in degrading key regulatory

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proteins that govern cell cycle, transcription factor activation, apoptosis and cell trafficking [1].

Regulatory proteins degraded by this system include p53, cyclins, cyclin-dependent kinase inhibitors p27 and p21, and the nuclear factor (NF)-κB.

NF-κB is important for cell survival and it is activated in response to cell stress, including cytotoxic agents, radiations, DNA damage. NF-κB also regulates the expression of genes involved in apoptosis, such as Bcl2 and Bcl-xL, cell cycle progression, inflammation, angiogenesis (including IL6, IL8, VEGF) [2].

NF-κB is normally bound in the cytosol to inhibitor κB-α (IKBα); degradation of IKBα is required for NF-κB translocation into the nucleus and activation of target genes. NF-κB is constitutively activated in several solid tumors and hematological malignancies [3,4], including myeloproliferative chronic disorders [5].

Among these, idiopathic myelofibrosis (IMF) is a hematological disorder characterized by fibrosis, hypercellularity, excessive deposits of extracellular matrix proteins and neoangiogenesis in the bone marrow [6].

The mechanisms of fibrosis remain undefined, but several evidences show that it would be a reactive process secondary to dysfunctions caused by the clonal hematopoietic stem cell [7], with consequent up-regulation of extracellular matrix proteins and cytokines, such as TGF-β, bFGF, PDGF, TPO [8,9].

In particular, TGF-β1 levels are increased in the bone marrow and peripheral blood of patients with IMF where this cytokine appears to be very relevant.

About this topic, Rameshwar et al. reported that activation of monocytes in patients with IMF is accompanied by increased induction of IL1 and TGF-β that would be mediated by NF-κB [10].

Initial adhesion of IMF monocytes would induce the nuclear translocation of NF-κB that was required for induction of IL1 and TGF-β.

Nevertheless, NF-κB is not relevant only for monocytes, but also for megakaryocytes, that in the IMF are clonal, morphologically abnormal and certainly involved in the triggering fibrosis through production of numerous cytokines, including TGF-β1 [11].

Recently, Komura et al. showed a spontaneous NF-κB activation in megakaryocytes from patient with IMF [5], probably mediated by the FKBP51 gene.

FKBP51 is an immunophilin, member of a multigene family containing several proteins able to bind FK506; FKBP51 could induce NF-κB activation by at least two ways: (1) the activation of the JAK2/STAT5 pathway; (2) the inhibition of calcineurin, with consequent degradation of IKBα.

Komura et al. showed that over expression of FKBP51 in IMF megakaryocytes and consequent NF-κB activation was involved in the TGFβ1 secretion, thus highlighting the importance of NF-κB activation in the fibrosis development.

On this basis, inhibitors of the ubiquitin-proteasome pathway could be tested in the IMF.

Today, the proteasome inhibitor PS-341 (Bortezomib), a modified dipeptidyl-boronic acid that binds selectively and reversibly to the proteasome, is a very promising molecule for cancer therapy [12]. It blocks the degradation of IKB, thereby keeping NF-κB in its inactivated state.

By inducing degradation of part of IL6 receptor, triggering activation of JNK, with consequent release of cytochrome *c* and activation of downstream caspases [13], by inducing generation of reactive oxygenation species (ROS) [14] and by inhibiting DNA repair [15], Bortezomib resulted effective as anti-proliferative agent in many tumour cell lines and neoplasias (non-small cell lung cancer, breast carcinoma, Hodgkin's disease, mantle cell lymphoma, multiple myeloma) [16–18].

On these bases, it would be useful to determine whether Bortezomib could be effective in the IMF.

To address this issue, we evaluated the in vitro cytotoxic activity of this proteasome inhibitor on a megakaryoblastic, TPO- and GM-CSF-dependent cell line (MO7-e), that our group previously reported to be a good model for idiopathic myelofibrosis [19].

In order to test if these effects would be lineage-specific, different acute leukemia cell lines were incubated with PS-341 and proliferative activity has been evaluated.

Moreover, interesting data from literature report that members of the NF-κB/Rel family are important for activating expression of WT1 [20], the Wilm's tumor suppressor gene that has been reported being up-regulated in relapsing acute leukemia, chronic myeloid leukemia in blast crisis and advanced cases of myelodysplastic syndromes [21].

Levels of WT1 mRNA were thus measured before and after treatment with PS-341 in the MO7-e cell line.

Finally, megakaryocyte CFU appearance in bone marrow samples from five patients affected by IMF cultured with or without Bortezomib was also assayed.

Our results show that Bortezomib plays a significant antiproliferative activity on MO7-e and that this effect would be promising in the myelofibrosis in vivo model.

2. Methods

2.1. Chemicals

PS-341 (Bortezomib) was supplied by Millenium Pharmaceuticals Inc. (Cambridge, MA, USA). Bortezomib was dissolved in phosphate-buffered saline (PBS) as a 2 mM stock solution and diluted to the required concentrations with serum-free culture medium.

2.2. Cell cultures

Megakaryoblastic MO7-e cell line (DSMZ: German Collection of Micro-organisms and cell cultures) was grown in RPMI-1640 medium (Gibco-Life Technologies, USA), supplemented with 10% fetal bovine serum (FBS), 2 mmol/l