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Sensitivity of chronic myeloid leukemia hemopoietic progenitors to PTT-119 in combination with human recombinant interferon alpha and gamma

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Summary. PTT-119, a new synthetic alkylating compound, has shown a marked "in vitro" inhibitory effect on chronic myeloid leukemia (CML) granulo-monocytic precursors (CFU-GM) at doses greater than 5 μg/ml. Based on previous experiences of synergistic associations between alkylating drugs and biological modifiers, we tested the effects of low doses of PTT-119 (from 0.1 to 1 μg/ml) in concert with alpha, gamma, or alpha + gamma interferons and compared to IFNs alone, in order to investigate an alternative choice for treatment of CML patients in chronic phase. Our results showed a significantly higher CFU-GM cloning inhibition after addition of 100 or 1,000 U/ml of alpha IFN to 0.1 μg/ml PTT-119 (from 39.6% ± 26.6 SD to 80.7% ± 10 SD and 91.5% ± 8 SD, respectively), while gamma IFN resulted in only a slight increase in colony growth inhibition when compared to the drug used alone. The association of alpha plus gamma IFN coupled with PTT-119 treatment did not significantly improve the results observed after exposure of leukemic progenitors to PTT-119 and alpha IFN alone. We conclude that a combined treatment with PTT-119 and IFN is probably worth testing both for purging methods before autologous bone marrow transplantation and for in vivo administration in chronic myeloid leukemia.

Key words: PTT-119 – Chronic myeloid leukemia – Alpha interferon – Gamma interferon – Autologous bone marrow transplantation

Introduction

PTT-119, a new synthetic alkylating compound, has been shown to possess a marked antineoplastic activity in several "in vivo" and "in vitro" experimental systems [4, 15, 16, 17]; encouraging clinical results have been reported as second or third line therapy of lymphoproliferative malignancies [6, 12].

In a prior study we showed that PTT-119 has a remarkable inhibitory effect on the growth of colonies from granulocyte-macrophage progenitors (CFU-GM) of patients with Ph1+ chronic myeloid leukemia (CML), albeit at doses that highly impare the growth of normal CFU-GM [5]. The "in vitro" growth of Ph1+ CFU-GM is also significantly inhibited by human recombinant alpha interferon (IFN), alone and in association with gamma IFN [2, 10, 11, 14]. Both IFNs are also clinically effective for treatment of Ph1+ CML, where they induce a consistent proportion of karyotypic conversions [10].

In light of these results, we investigated the effects of the exposure of Ph1+ CFU-GM to PTT-119 in combination with alpha, gamma, and alpha + gamma IFN.

Materials and methods

CML cells. Leukemic cells were obtained from peripheral blood or bone marrow of 10 patients with Ph1+ CML in chronic phase prior to any IFN treatment and at least 1 month after the administration of any antineoplastic drug. The samples were anticoagulated with preservative-free heparin; the mononuclear fraction was collected after sedimentation on Fycoll-Hypaque (Lymphoprep, Nyeegard, Norway). Residual T lymphocytes were removed from peripheral blood-derived samples by E rosette sedimentation as previously described [13] to less than 1% of the nucleated cells.

Chemotherapeutic compound. PTT-119 [p-fluoro-phenyl-L-alanyl-3-m-bis-(2-chloroethyl) amino-phenylalanyl-methionine ethylester-hydrochloride] was provided by PROTER SPA research division Opera, Milan, Italy. The drug was prepared and diluted as described elsewhere [4]. Briefly, the drug was first dissolved in N,N dimethyl-acetamide, absolute ethanol and propylene glycol: the solution was diluted with 50% acqueous propylene glycol to 1 mg/ml and final dilutions were performed in culture medium.

Interferons. Recombinant human interferon gamma (1×10^7 U/mg) was supplied by Boehringer Ingelheim. Recombinant human interferon alpha 2a (3×10^6 U/mg) was supplied by Hoffman La Roche. Alpha and gamma IFNs were diluted with Iscove's Modified Dulbecco's Medium (IMDM) (Gibco Europe, Paysley, UK) to reach the final concentrations.

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Cell cultures. 1×10^5 CML cells were plated in 35 mm Petri dishes in 1 ml of 0.9% methylcellulose and IMDM added with 1% bovine serum albumin (Sigma Chemicals Co., St. Louis, USA), 10% Foetal Calf Serum (Gibco) 100 U GM-CSF as source of colony stimulating activity and 5×10^{-2} M 2-Mercaptoethanol. Parallel platings were made in each experiment finding a linear relationship between the number of cells (ranging from 0.25×10^5 to 5×10^5 cells) and the number of formed colonies. PTT-119 was added to the culture medium at concentrations of 0.1, 0.5, and 1 μg/ml alone and in combination with alpha, gamma, and alpha + gamma IFN at concentrations of 100 and 1,000 U/ml. The appropriate concentrations of the vehicle for PTT-119 were given to the control dishes. A subset of experiments was made adding to the culture medium alpha, gamma, alpha + gamma IFNs at concentrations of 100 and 1,000 U/ml. For each experiment a short-term incubation (120 min) was performed, adding to the culture medium alpha IFN and PTT at the same doses. After incubation and two washings, cells were plated and incubated exactly as described for continuous exposure. In vitro PTT concentrations were based on a range corresponding to plasma concentrations achievable in patients [8]; IFN concentrations were based on extensive previous experimental work [14]. The dishes were incubated at 37°C in a fully humidified 5% CO_2 atmosphere. Colonies (more than 50 cells) were scored at day 14 with an inverted microscope. Colony counts were given as the mean of triplicate counts.

Statistical analysis. Analysis of variance for repeated measurements was performed for multiple comparisons of the percentage of surviving colonies (compared to controls) from cells treated with PTT-119 + IFN at different doses with the percentage of surviving colonies from cells treated with PTT-119 alone. In presence of significant values of "f", the Least Significant Difference test was applied and p values less than 0.05 were rejected.

Results

In Table 1 we report the effects of the addition of alpha IFN to PTT-119 at different concentrations. The cytotoxic activity of the lowest dose of PTT-119 was significantly increased by the addition of alpha IFN ($P<0.025$ and $P<0.01$ with 100 and 1,000 U/ml respectively). At higher drug concentrations the association of alpha IFN did not cause a significant CML CFU-GM growth inhibition when compared to PTT-119 alone, with only one exception: 0.5 μg/ml PTT-119 plus 1,000 U/ml alpha IFN.

The effect of the association of PTT-119 and gamma IFN is reported in Table 2. No significant difference was seen comparing the combination with the alkylating agent alone. The association of alpha plus gamma IFN coupled with PTT-119 showed a marked cytotoxic effect (Table 3), but this did not statistically differ from the inhibition obtained after the exposure of CML CFU-GM to PTT-119 combined with alpha IFN alone. The effects of IFNs alone and in association are shown in Table 4. The exposure of CML CFU-GM to PTT plus alpha IFNs for a short period (120 min) confirmed the data obtained with continuous exposure even with slightly reduced cytotoxic activity (see Table 5).

Table 1. Inhibition of CML CFU-GM growth in cultures treated with increasing concentrations of PTT-119 and of alpha IFN. Colonies were scored at day 14 (mean of triplicate counts)

Treatment	Colony forming inhibition[a]	*P*
PTT-119 (0.1 μg/ml)	39.6 ± 26.6	
+ Alpha IFN 100 U/ml	80.7 ± 10	< 0.025
+ Alpha IFN 1,000 U/ml	91.5 ± 8	< 0.01
PTT-119 (0.5 μg/ml)	70.2 ± 19.3	
+ Alpha IFN 100 U/ml	87.1 ± 11.8	NS
+ Alpha IFN 1,000 U/ml	95.4 ± 5.5	< 0.05
PTT-119 (1 μg/ml)	81.2 ± 17.3	
+ Alpha IFN 100 U/ml	91.6 ± 15.1	NS
+ Alpha IFN 1,000 U/ml	95.7 ± 8.6	NS

[a] The numbers are expressed as mean ± SD of percentage of colony growth inhibition in comparison with control cultures. Absolute number is 184 ± 21 SD×10^5 cells plated (set at 100%; mean of 10 experiments)

Table 2. Inhibition of CML CFU-GM growth in cultures treated with increasing concentrations of PTT-119 and of gamma IFN[a]

Treatment	Colony forming inhibition[a]	*P*
PTT-119 (0.1 μg/ml)	39.6 ± 26.6	
+ Gamma IFN 100 U/ml	54.0 ± 27.1	NS
+ Gamma IFN 1,000 U/ml	66.4 ± 28	NS
PTT-119 (0.5 μg/ml)	70.2 ± 19.3	
+ Gamma IFN 100 U/ml	76.9 ± 18.6	NS
+ Gamma IFN 1,000 U/ml	80.3 ± 20.5	NS
PTT-119 (1 μg/ml)	81.2 ± 17.3	
+ Gamma IFN 100 U/ml	87.1 ± 14.3	NS
+ Gamma IFN 1,000 U/ml	87.7 ± 14.6	NS

[a] See Table 1 for technical details

Table 3. Inhibition of CML CFU-GM growth in cultures treated with increasing concentrations of PTT-119 and alpha plus gamma IFN[a]

Treatment	Colony forming inhibition[a]	*P*
PTT-119 (0.1 μg/ml)	39.6 ± 26.6	
+ Alpha plus gamma IFN 200[b] U/ml	82.6 ± 15	< 0.025
+ Alpha plus gamma IFN 2,000[b] U/ml	93.8 ± 10	< 0.005
PTT-119 (0.5 μg/ml)	70.2 ± 19.3	
+ Alpha plus gamma IFN 200[b] U/ml	86.7 ± 17	NS
+ Alpha plus gamma IFN 2,000[b] U/ml	95.8 ± 6.1	< 0.05
PTT-119 (1 μg/ml)	81.2 ± 17.3	
+ Alpha plus gamma IFN 200[b] U/ml	91.2 ± 16.4	NS
+ Alpha plus gamma IFN 2,000[b] U/ml	97.1 ± 6.6	NS

[a] See Table 1 for technical details

[b] 200 = 100 alpha + 100 gamma IFN units; 2,000 = 1,000 alpha + 1,000 gamma IFN units

Table 4. Inhibition of CML CFU-GM growth in cultures treated with IFN alone

Treatment	Colony forming inhibition[a]
Alpha IFN 100 U/ml	29 ± 18
Alpha IFN 1,000 U/ml	40 ± 23
Gamma IFN 100 U/ml	18 ± 16
Gamma IFN 1,000 U/ml	36 ± 15
Alpha + gamma IFN 200[b] U/ml	50 ± 19
Alpha + gamma IFN 2,000[b] U/ml	84 ± 16

[a] See Table 1 for technical details
[b] See Table 3

Table 5. Inhibition of CML CFU-GM growth in cultures treated for 120 minutes with increasing concentrations of PTT-119 and alpha IFN[a]

Treatment	Colony forming inhibition[a]
PTT-119 (0.1 μg/ml)	15 ± 10.6
+ Alpha IFN 100 U/ml	40.1 ± 13
+ Alpha IFN 1,000 U/ml	51.3 ± 11
PTT-119 (0.5 μg/ml)	22.1 ± 12
+ Alpha IFN 100 U/ml	69.2 ± 15
+ Alpha IFN 1,000 U/ml	70.3 ± 12
PT-119 (1 μg/ml)	31.1 + 16.6
+ Alpha IFN 100 U/ml	69.6 + 12
+ Alpha IFN 1,000 U/ml	72.1 + 14

[a] See Table 1 for technical details. Numbers exposed as means ± SD of percentage of colony growth inhibitors in comparison with control cultures. Absolute number is $207 \pm 18 \times 10^5$ cells plated (set at 100%; mean of 10 experiments)

Discussion

Recombinant alpha IFN is currently undergoing experimental and clinical evaluation regarding its effects on CML [2, 10, 11, 14]. Preliminary "in vitro" and "in vivo" data show a definite activity of alpha IFN, resulting in partial or complete karyotypic conversion or in control of the disease [9, 10]. However, a considerable subset of patients is not controlled by alpha IFN as single therapy. As a consequence, IFN therapy must be associated or substituted by other drugs affecting CML proliferation (e.g. hydroxyurea, alkylating agents). The rationale of these associations is based on several experimental results and on a few "in vivo" data. Extensive experimental evidence suggests synergistic activity of the association of alpha + gamma IFN, with a few "in vivo" confirmations [2, 14]. Furthermore, the cytotoxic effects of bleomycin, cisplatin, 5 fluorouracil, doxorubicin and Co 60 gamma rays were enhanced by concomitant application of beta IFN [3, 7]. Similar results were obtained by combining new anthracycline analogues with alpha IFN [1]. In addition, a few clinical cases received benefit from the association of alpha IFN and hydroxyurea.

PTT-119, which demonstrated defined "in vitro" effects on CML cell growth, could have a role, in association with IFNs, in the treatment of this disease. We therefore studied the "in vitro" growth of fresh CML cells from 10 patients after addition of PTT-119 alone or associated with alpha, gamma and alpha + gamma IFN. A significant increase in the inhibitory activity of the alkylating compound was seen after addition of alpha IFN, at doses that, if used alone, produce a very slight inhibition of CML cell growth (29% and 40% with 100 and 1,000 U/ml of alpha IFN, 18 and 36% with 100 and 1,000 U/ml of gamma IFN) [14]. The effect of the association was evident at concentrations of PTT-119 (0.1 μg/ml) well below the levels achievable in man [8]. These observations suggest that the combination of the alkylating tripeptide PTT-119 and alpha IFN could have a role both in vivo, in the management of patients affected by CML in chronic phase where they are scarcely responsive to IFNs alone, and in vitro, for marrow purging of CML before autologous bone marrow transplantation.

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