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Biochemical and Biophysical Research Communications 338 (2005) 158–160
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PBT-3, a hepoxilin stable analog, causes long term inhibition of growth of K562 solid tumours in vivo ☆

Xiang Li [a], Na Qiao [a], Denis Reynaud [a], Mohamed Abdelhaleem [a], Cecil R. Pace-Asciak [a,b,*]

[a] *Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8*
[b] *Department of Pharmacology, Faculty of Medicine, University of Toronto, Canada M5S 1A8*

Received 29 June 2005
Available online 10 August 2005

Abstract

We demonstrate herein that daily administration of PBT-3 for 8 days to NU/NU mice bearing solid tumours derived from the s.c. administration of the leukemic cell line K562 results in inhibition of growth of the tumours in vivo, and this inhibition lasts for 60 days after stopping treatment with PBT-3 before recovery of tumour growth is re-established. Similar findings were observed when the mice were treated with Gleevec (STI-571). These results provide new evidence that PBT-3 is effective in controlling solid tumour growth in vivo and suggest that the PBT family may be useful in the development of new drugs in cancer therapy.

Keywords: Hepoxilin stable analogs; PBT; Solid tumours; K562; NU/NU mice; Apoptosis; Novel therapeutics

The leukemia cell line, K562, is a well-established in vitro cell model of chronic myeloid leukemia [1]. It expresses the Bcr-Abl fusion protein that renders the cell resistant to apoptosis [2]. Bcr-Abl has increased tyrosine kinase (TK) activity required for malignant transformation and apoptosis resistance [2,3]. Gleevec, a well-known chemotherapeutic agent in CML, inhibits the TK activity of Bcr-Abl, inhibits proliferation of Bcr-Abl positive cells, and induces apoptosis both in vitro and in vivo [4,5]. However, resistance to Gleevec has been noted [6,7]. We have previously shown that PBT-3 [8], a stable analog of the hepoxilins [9] (see Scheme 1)—products derived from the 12-lipoxygenase pathway of the arachidonic acid cascade [10,11], inhibits K562 cell proliferation in vitro and induces apoptosis [12]. We have also shown that PBT-3 is biologically active in vivo in controlling the growth of K562 solid tumours derived from the s.c. transplantation in NU/NU mice during an 8 day daily treatment in vivo [12,13]. This study was therefore carried out to determine at what stage after cessation of PBT-3 treatment would tumour growth be re-established.

Materials and methods

Materials. PBT-3 methyl ester was prepared in our laboratory by Dr. Peter Demin [8]. It was purified to >99% purity by RP-HPLC and verified by mass spectrometry. The K562 cell line was obtained from ATCC (Manassas, VA, USA). Cells were grown in RPMI 1640 medium (HyClone) supplemented with 10% fetal calf serum (FCS), 100 μg/ml of antibiotics (penicillin and streptomycin) at 37 °C in 5% CO_2. Cell viability was assessed by Trypan blue dye exclusion (>95%).

Animals. Six-week-old female mice (Crl: NU/NU-nuBr) ≈20 g in weight were purchased from Charles River Laboratories (St. Constant, Quebec, Canada) and housed in the animal facility of the Research

☆ *Abbreviations:* Me, methyl ester; PBT-3, 10-hydroxy-11,12-cyclopropyl-eicosa-5*Z*,8*Z*,14*Z*-trienoic acid; s.c., subcutaneous; i.t., intratumour; i.v., intravenous.

* Corresponding author. Fax: +1 416 813 5086.
E-mail address: pace@sickkids.ca (C.R. Pace-Asciak).

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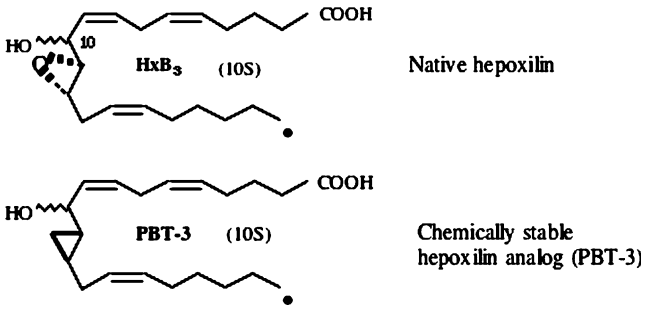
doi:10.1016/j.bbrc.2005.07.180



Scheme 1. Structures of PBT-3 and the corresponding parent hepoxilin, HxB3.

Institute, The Hospital for Sick Children. The mice had free access to chow and water. Animals were injected s.c. in the left flank with 1×10^7 K562 cells/animal and the cells were allowed to grow into solid tumours during the next 2 weeks. At this stage, the tumours reached a volume of 80–100 mm^3. Animals were then randomly assigned into groups and each animal was treated with vehicle (control group, 100 μl saline containing 7 μl ethanol), PBT-3 (30 or 10 μg twice daily in 100 μl vehicle), and STI-571 (10 or 3 ng twice daily in 100 μl vehicle). Drug and vehicle administration was carried out via the i.t. route in the morning and i.v. route in the afternoon. Treatment was continued daily for 8 days. Animals whose tumours reached a volume of 2500–3000 mm^3 had to be sacrificed according to our Animal Care Committee protocol [1]. Beyond the 8 day treatment, animals were monitored every 3 days for tumour growth with volume measurement and when the tumours reached the 2500 mm^3 size, animals were sacrificed. Tumour volume was measured with calipers using the formula: $V = S^2$ (mm) $\times$ L (mm)/2, where S and L are shortest and longest diameters of the tumour, respectively [14].

Results and discussion

Fig. 1 shows the tumour volume for each animal in the various groups at two doses: (A) STI-571 (Gleevec) at 10 ng and PBT-3 at 30 μg/animal or (B) at 3 ng and 10 μg, respectively, during the initial 8-day treatment and the period following cessation of the treatment up to 75 days. As shown in Fig. 1A, the animals treated with PBT-3 or STI-571 showed almost complete inhibition of growth during the 8-day treatment period, while in the vehicle-treated control group, all animals had to be sacrificed because of a rapid rate of tumour growth, the tumours having reached the designated endpoint (2500 mm^3 or greater). The subsequent period after cessation of drug treatment in the treated groups was most interesting. In all PBT-3-treated animals, tumour growth remained inhibited until the 60th day from the start of treatment, i.e., 52 days after the last exposure to PBT-3. In the Gleevec group (STI-571), tumours began growing several days earlier, with the exception of one animal whose tumour began growing at day 20. When the dose of both drugs was reduced by 1/3 (Fig. 1B), there was little difference between the drug-treated and control groups indicating that the threshold dose for PBT-3 was between 10 and 30 μg/mouse (400–1200 μg/kg/day for 8 days) and 3–10 ng for Gleevec (120–400 ng/kg/day for 8 days). Previous results have shown that the tumours treated

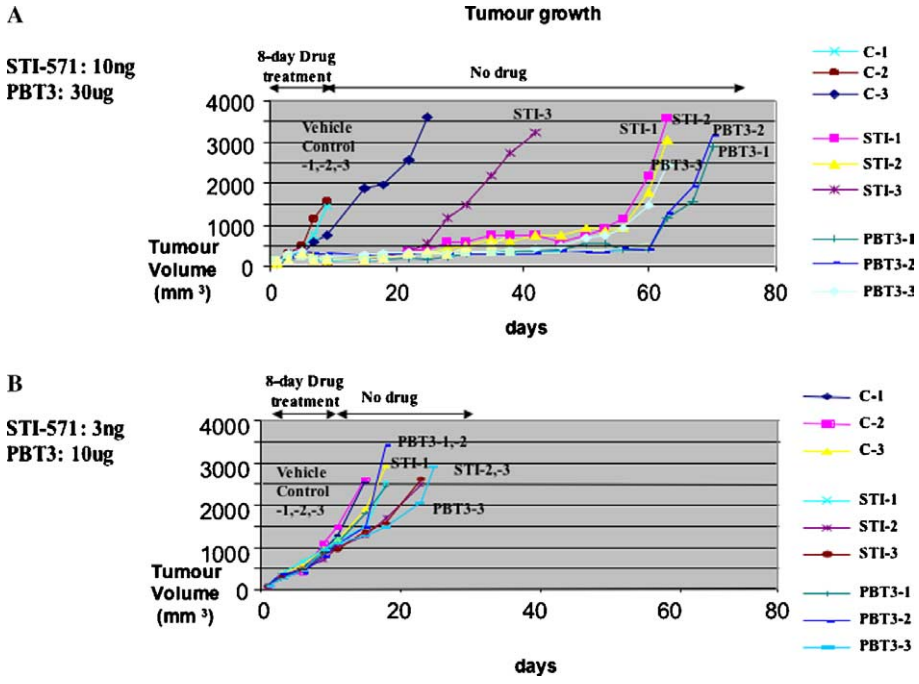


Fig. 1. (A,B) Growth rates of tumours derived from s.c. transplantation of K562 cells in female NU/NU mice. Administration of drugs (PBT-3 or STI-571 (Gleevec) was carried out twice daily for 8 days to groups of mice whose tumours had grown to a volume of 80–100 mm^3. Administration of drugs or vehicle was made in 100 μl volume intra-tumour (am) and intravenous (pm). On the eighth day, drug administration was terminated and the animals were monitored every 3 days for tumour volume until the tumours reached the designated endpoint size (>2500 mm^3). Note the long time taken for tumour growth inhibition to wear off in the PBT-3 group (A). Data from nine individual mice are shown for each panel.

in vivo with PBT-3 as well as STI-571 showed apoptosis with DNA fragmentation and a positive TUNEL assay [13].

The present results demonstrate that PBT-3 controls growth of solid tumours in NU/NU mice and that the effect of PBT treatment lasts for many weeks after treatment is discontinued. These results demonstrate that PBT-3 is bioavailable in vivo and its effects on the tumours are long lasting. It would be of interest to determine whether a second treatment of the tumours with PBT-3 around the time when the tumours begin to grow again (day 60) would inhibit tumour growth once again.

Acknowledgment

This work was supported by a grant from the Ontario Cancer Research Network.

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