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Research paper

Augmentation of myelopoiesis in a murine host bearing a T cell lymphoma following in vivo administration of proton pump inhibitor pantoprazole

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ABSTRACT

Proton pump inhibitors (PPI) are being proposed as potent antitumor agents, owing to their ability to specifically induce tumor cell death by reversing H^+ ion homeostasis. As tumor growth induces myelosuppression in tumor-bearing hosts, it remains unclear if PPI can also modulate tumor-induced myelosuppression. Thus, we studied the effect of in vivo administration of pantoprazole (PPZ), a PPI, on myelopoiesis in a murine model of a transplantable T cell lymphoma, designated as Dalton's lymphoma (DL). Intraperitoneal administration of PPZ to tumor-bearing mice resulted in an enhanced bone marrow cellularity, inhibited induction of apoptosis and augmented bone marrow cell (BMC) survival. BMC of PPZ-administered tumor-bearing mice showed elevated number of F4/80 positive cells, augmented colony forming ability and differentiation in bone marrow-derived macrophages (BMDM) with higher expression of F4/80 and CD11c markers. This study also presents evidences to indicate that PPZ-dependent augmentation of myelopoiesis in the tumor-bearing host is dependent on an enhanced expression of M-CSF and receptors for M-CSF & GM-CSF in BMC, along with a modulation in the expression of cell survival regulatory molecules PUMA, Bcl2, p53 and caspase-activated DNase (CAD). BMDM obtained from PPZ-administered tumor-bearing mice also showed an augmented expression of TLR-2, tumoricidal activity, production of NO and monokines: IL-1, IL-6 & TNF-α. The study discusses the possible mechanisms underlying PPZ-dependent augmentation of myelopoiesis. Taken together, the present study proposes that a PPZ-dependent alleviation of tumor-induced myelosuppression could contribute to an augmented myelopoiesis.

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1. Introduction

Tumor growth has been demonstrated to be accompanied by immuno- and myelo-suppression [1–3]. Nevertheless, chemo- and radio-therapeutic applications against malignancies also trigger myelosuppression [4,5], contributing to failure of cancer immunotherapy [6,7]. Consequently, it is imperative to restore hematopoiesis and antitumor immune responses in a tumor-bearing host.

Neoplastic cells express a unique repertoire of tumor-specific proton pumps (V-H^+ATPase), which augment tumor cell survival [8,9]. Thus, one of the recent antineoplastic therapeutic approaches targets proton pumps in tumor cells by proton pump inhibitors (PPI) [10–14]. Fortunately, there is little evidence of toxicity of various PPI on normal cells [14,15]. Earlier reports have indicated variable actions of PPI on bone marrow homeostasis in some non-malignant pathologies [16–18]. However, it is unclear if administration of PPI to a tumor-bearing host can provide additional therapeutic benefits by alleviating tumor-associated myelo- and immuno-suppression.

PPI commonly used for antineoplastic therapy are derivatives of benzimidazole. Pantoprazole (PPZ) (5-(Difluoromethoxy)-2-(((3,4-dimethoxy-2-pyridinyl)methyl) sulfinyl)-1H-benzimidazole) is a member of this class of PPI, which is distinct in its pharmacological and biological actions compared to other PPI of the same class owing to its unique binding affinity to proton pumps and a lower speed of reversibility [19–21]. In view of these observations, in the present investigation we selected PPZ for studying its action on hematopoiesis of a tumor-bearing host.

Abbreviations: PPI, proton pump inhibitor; PPZ, pantoprazole; DL, Dalton's lymphoma; BMC, bone marrow cells; BMDM, bone-marrow derived macrophages; L929CM, L929-conditioned medium; M-CSF, macrophage-colony stimulating factor; M-CSFR, M-CSF receptor; GM-CSFR, granulocyte macrophage colony stimulating factor receptor; PUMA, p53 up-regulated mediator of apoptosis; CAD, caspase-activated DNase; CFU, colony forming unit; CFU-M, colony forming unit-macrophage; CFU-GM, colony forming unit-granulocyte-macrophage; CFU-G, colony forming unit-granulocyte.

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Murine models of spontaneous tumors have been shown to mimic human malignancies most closely and thus utilized for investigating host-tumor interactions and development of anti-neoplastic strategies [22–24]. Further, considering the fact that hematological malignancies are one of the most complicated cancers for treatment [25,26], using a murine model of a transplantable T cell lymphoma of spontaneous origin, designated as Dalton's lymphoma (DL), several aspects of host-tumor relationship have been investigated [1,27–37]. Moreover, DL growth was associated with manifestation of myelosuppression accompanied by induction of apoptosis in BMC [1,27]. Further it was demonstrated that administration of PPZ to DL-bearing mice resulted in tumor growth retardation owing to inhibition of tumor cell survival along with an augmented induction of apoptosis [38] and reversal of tumor-induced suppression of tumor-associated macrophages [39]. However, it remained unclear if PPZ administration could also modulate myelopoiesis in tumor-bearing host. In view of the above-mentioned observations, in the present investigation, we studied the effect of in vivo administration of PPZ to DL-bearing mice on survival and myeloid differentiation of BMC. To the best of our knowledge, this study reports for the first time that PPZ exerts myelopoietic effect in a tumor-bearing host.

2. Materials and methods

2.1. Mice and tumor system

Pathogen-free inbred adult male mice of BALB/c (H-2^d) strain were used at 8-12 weeks of age. The mice received food and water *ad libitum* and were treated with utmost human care with approval of the institutional animal ethical committee of the Banaras Hindu University in the animal rooms of the School of Biotechnology. For all purposes mice were killed by cervical dislocation. Dalton's lymphoma (DL) is maintained in ascitic form by serial transplantation in BALB/c mice or in an *in vitro* cell culture system by serial passage. Irrespective of whether the DL cells were obtained from the *in vitro* culture system maintained as suspension cultures or from the ascitic fluid they exhibited similar phenotypic features. Serial passage of DL in mice was carried out by transplanting 1×10^5 DL cells mouse^{-1}, in 0.5 ml phosphate buffered saline (PBS).

2.2. Reagents

All reagents used were of tissue culture or analytical grade. Tissue culture medium RPMI 1640 was purchased from Hyclone (USA), supplemented with 20 μg/ml gentamycin, 100 μg/ml streptomycin, 100 IU penicillin purchased from Himedia (India) and 10 % fetal calf serum from Hyclone (USA), henceforth, referred to as complete medium. Injectable PPZ (Altana pharma, Germany) was resuspended in PBS immediately before use. Antibodies against Bcl2, p53, CAD, IL-1, IL-6, IL-10, TNF-α, TLR-2, TGF-β & β-actin and fluorochrome conjugated antibodies against F4/80, CD11c and their isotype controls were obtained from Imgenex (USA), BD Pharmingen (USA), eBioscience (USA) and Chemicon (UK). Secondary antibodies conjugated to alkaline phosphatase were obtained from Bangalore Genie (India). Primers for RT-PCR were purchased from Hysel, India. BCIP/NBT was purchased from Amresco (USA). TUNEL assay kit was purchased from Invitrogen (USA). Annexin-V assay kit was purchased from Imgenex (USA).

2.3. Protocol for PPZ administration to tumor-bearing mice

PPZ administration to tumor-bearing mice was carried out as per protocol standardized in our laboratory for optimum tumor growth retardation. Tumor cells (1×10^5cells) were transplanted to mice in groups of ten each followed by intraperitoneal administration of PPZ 48 h after tumor transplantation at a dose of 4 mg/kg body weight in 0.2 ml PBS on every 48 h interval till day 15 as optimized earlier [38]. Control mice were administered with same volume of PBS without PPZ.

2.4. Bone marrow cell (BMC) preparation

BMC were obtained from the femurs of DL-bearing mice following a method described earlier [29]. Briefly, the mice were killed by cervical dislocation and the BMC were obtained from the femoral shafts by flushing with serum-free medium and agitating gently to prepare a single cell suspension which was washed twice with serum free medium by centrifugation at $200 \times g$ at 4 °C. Viability of BMC was estimated using the standard trypan blue dye exclusion test as described previously [31]. A 10 μl sample of cell suspension was mixed with an equal volume of 0.4% trypan blue in PBS and the cells were counted using a hemocytometer. Cells that did not exclude the trypan blue were considered nonviable. The BMC population was analyzed for flow cytometry of macrophage marker F4/80 using easyCyte HT flow cytometer (Guava, USA) using standard procedure as per manufacturer's instruction.

2.5. Cell survival assay

Cell survival was determined by standard MTT assay according to a method described earlier [35] with slight modifications. MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] (5 mg/ml in PBS) was added to each well (50 μl/well) of the culture plate containing 200 μl medium and incubated at 37 °C for 4 h. The medium was then carefully removed, without disturbing the dark blue formazan crystals. Fifty μl DMSO was added to each well and mixed thoroughly to dissolve the formazan crystals. Plates were then read on a microplate reader (Labsystems, Finland) at a wavelength of 540 nm. Readings are presented as optical density (O.D.) at 540 nm.

2.6. Preparation of L929 conditioned medium

L929-cell conditioned medium (L929CM) was used as a source of macrophage-colony stimulating factor (M-CSF) [40]. L929CM was prepared according to a method described earlier [29]. L929 cells, obtained from National Centre for Cell Science, Pune, India, were incubated in RPMI-1640 supplemented with 10% FCS for 5–7 days. Cell-free supernatant was then harvested from the confluent monolayer of L929 cells, passed through 0.22 μm membrane filter and kept at −20 °C until use.

2.7. Morphological evaluation of apoptotic cells by Wright–Giemsa staining

Apoptotic cell population was enumerated by a method described earlier [27]. Cell suspension was smeared on a slide, air-dried, fixed in methanol, stained with Wright–Giemsa staining solution, mounted in glycerine and analyzed under light microscope (Carl Zeiss, Germany) at 400× magnification. Apoptotic cells were identified on the basis of morphological features that included contracted cell bodies, condensed, uniformly circumscribed and densely stained chromatin, and membrane bound apoptotic bodies containing one or more nuclear fragments. The percentage of apoptotic cells was determined by counting more than 300 cells in at least three separate randomly selected microscopic fields.

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