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YM-201627: An orally active antitumor agent with selective inhibition of vascular endothelial cell proliferation

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Abstract

We developed an oral administration-compatible, small molecular weight antitumor agent, YM-201627 by screening for the inhibition of the proliferation of VEGF-stimulated HUVECs. YM-201627 selectively inhibited the proliferation of various endothelial cell lines induced by VEGF, bFGF, and FBS (at IC_{50} s of 0.0039–0.12 μM), that would not be expected to have any direct antiproliferative effect on other cell types. YM-201627 inhibited angiogenesis in vitro at a concentration of 0.01 μM. In the in vivo studies, it inhibited microvessel formation induced by human melanoma A375 cells suspended in Matrigel (86% with twice-daily doses of 30 mg/kg). Moreover, once-daily oral dosing of YM-201627 to mice bearing A375 xenografts elicited significant antitumor activity (73% with daily doses of 10 mg/kg). These results suggest that YM-201627 is a selective growth inhibitor of endothelial cells, which may be useful for treatment of solid tumors.

Keywords: YM-201627; Angiogenesis; Endothelial cells; Vascular endothelial growth factor (VEGF)

1. Introduction

The occurrence of cancers has been markedly increased during the last decade. Although intensive research during the past few years has led to considerable progress in the ability to diagnose and

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treat cancer diseases at an early stage, the prognoses for patients are still not satisfactory. Recently, it has been well recognized that angiogenesis is essential for solid tumor growth and metastasis [1,2]. Angiogenesis is a complex process of forming new blood vessels from the preexisting vessels that required in many physiological and pathological conditions [3,4]. The protrusion of endothelial cells allows local degradation of the basement membrane of the parent vessel, and then endothelial cells migrate outward in tandem to form a capillary sprout. The cells then proliferate, followed by lumen formation with subsequent branching. In general, vascular proliferation occurs only during embryonic development and is a very slow process in the adult, with few exceptions (e.g. wound healing and in the female reproductive system). In contrast, many pathological conditions (e.g. cancer, atherosclerosis, and diabetic retinopathy) are characterized by persistent, unregulated angiogenesis [5]. Therefore, experimental and clinical investigators continue to seek to identify medicinal agents capable of inhibiting the process of angiogenesis. One approach seeks to identify compounds that can retard the vascular endothelial growth factor (VEGF) signaling cascade in the vascular endothelial cells. Tumor VEGF expression has been clinically associated with disease progression in a range of solid malignancies [6–10]. This correlation is largely attributed to its ability to induce tumor angiogenesis by stimulating endothelial cell mitogenesis [11] and chemotaxis [12], which increases endothelial cell-associated protease activity [13–15]. It is expected that tumor growth was likely repressed by continual abrogation of the VEGF pathway in tumor endothelium. Although bevacizumab, a recombinant humanized monoclonal antibody to VEGF with efficacy against colorectal and other malignancies, has already been approved for patients [16], no orally active or small molecular antitumor agents with selective inhibition of vascular endothelial cell proliferation has yet been approved.

We have aimed to produce a therapy that may be administrated orally. YM-201627, a 2-arylimidazo[2,1-*b*][1,3]benzothiazole derivative, was identified through screening for inhibition of VEGF-stimulated HUVEC proliferation. In this study, we investigated its activities in vitro and in vivo, and suggested possibilities for a novel, orally active antitumor agent that blocks endothelial cell growth.

2. Materials and methods

2.1. Agents and cell lines

YM-201627, an *N*,*N*-Diethyl-2-(3-imidazo[2,1-*b*][1,3]benzothiazol-2-ylphenoxy)ethanamine dihydrochloride (Fig. 1), and SU5416, a KDR inhibitor [17], were synthesized at Yamanouchi Pharmaceutical Co., Ltd. Five normal human endothelial cells (HUVEC, HPAEC, HAEC, HMVEC-d Neo, and HDMVEC-d Ad) were obtained from Clonetics (San Diego, CA, USA). All these endothelial cells were cultured on gelatin-coated plates with endothelial growth medium-2 (EGM Bullet kit). Three fibroblast cell lines (NHLF, NIH/3T3, and Balb/3T3) and two human cancer cell lines (A375 and HT1080) were obtained from American Type Culture Collection (Rockville, MD, USA). These cell lines were cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA) and 1% antibiotic-antimycotic (Gibco BRL). All cell lines were cultured at 37 °C in a humidified chamber containing 95% air and 5% CO_2.

2.2. Inhibition of cell proliferation

All normal cells were initially plated on gelatin-coated 96-well plates (1×10^4 cells/well) for 24 h, and then transferred into Medium supplemented with 0.1% FBS. After 24 h, the cells were dosed with YM-201627 for 2 h and stimulated by human recombinant VEGF (10 ng/mL, R&D Systems, Minneapolis, MN, USA), human recombinant basic fibroblast growth factor (bFGF) (10 ng/mL, R&D Systems) or FBS (2%) for 18 h. The cultures were pulsed with 5 μCi/well of [^{3}H] thymidine (Amersham Bioscience, Piscataway, NJ,

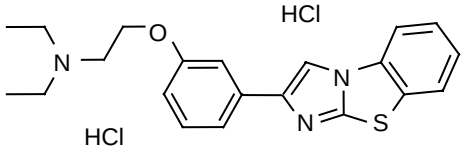

Fig. 1. Chemical structure of YM-201627.