Phenoxodiol: Isoflavone Analog with Antineoplastic Activity

Toni K. Choueiri, MD, Robert Wesolowski, MD, and Tarek M. Mekhail, MD

Corresponding author
Toni K. Choueiri, MD
Department of Hematology and Medical Oncology, Taussig Cancer Center, The Cleveland Clinic Foundation, 9500 Euclid Avenue, R-35, Cleveland, OH 44195, USA.
E-mail: choueit@ccf.org

Introduction

Flavonoids are naturally occurring plant hormones known to have several functional properties including the regulation of plant cell cycle kinetics and death. These plant hormones show similar effects in animals and therefore may have an antimitotic and antiapoptotic effect [1].

Observations of lower incidence of cancer with dietary isoflavone intake [2•,3•] prompted the National Cancer Institute to undertake clinical evaluation of isoflavonoids, such as genistein and flavopiridol, as antineoplastic agents. In vitro studies of isoflavonoids demonstrated an antitumor effect against various types of human and animal cancer cell lines, including melanoma [4], leukemia [5], breast cancer [6], gastrointestinal cancers [7], prostate cancer [8], and many other solid tumors [9]. Naturally occurring plant isoflavonoids act via many different pathways, including signal transduction modulation [1], interruption of cell cycle [10], antioxidant activity [11], induction of apoptosis [12], inhibition of angiogenesis [13,14], and decrease in cancer cell adhesion [15].

One of the first isoflavones tested in clinical studies was flavopiridol. It attracted much interest recently because of its activity against specific cellular targets. Flavopiridol is a cell cycle targeted kinase (cdk) inhibitor [16] but appears to have a number of other mechanisms of action, including the induction of apoptosis [17]. Early phase II studies suggest minimal activity in gastric cancer [18] and non–small-cell lung cancer [19] and modest activity in mantle cell lymphoma [20] and chronic lymphocytic leukemia [21]. A phase I trial of the combination of flavopiridol with paclitaxel or with irinotecan seems promising [22]. Although response rates were generally less than 10% in all these studies, 50% to 70% of patients achieved disease stabilization in two trials [19,20].

Genistein is a pan-inhibitor of tyrosine kinases in human tumor cells and exerts modest anticancer activity against a wide range of human and animal cancer cell lines [23,24]. Steric alteration of the genistein molecule to create phenoxodiol has yielded a synthetic isoflavonoid with 1,2-diphenylpropane-ring structure that has considerably greater anticancer activity, an improved bioavailability and metabolic profile, and substantially different pharmacology (Investigator’s brochure, phenoxodiol injection; Novogen Limited, Sydney, Australia). This review presents the background bench work with phenoxodiol trying to clarify the exact mechanism of action behind its antineoplastic activity. Early phase I and II trials, many published in abstract format, are also reviewed, with focus on the toxicity and preliminary efficacy of this agent.

Phenoxodiol Potential Mechanisms of Action: In Vitro and Animal Studies

In vitro studies

Phenoxodiol mechanisms of action have recently been investigated but remain only partially understood. Initial work from Constantinou and Husband [25] showed that phenoxodiol appears to selectively inhibit topoisomerase (topo) II in a dose-dependent manner by stabilizing the topo II–mediated cleavable complex. The topo II inhibi-
tory effects of phenoxodiol were comparable with those of other antitumor agents, such as etoposide, and were stronger than those of genistein.

Early evidence of the antiapoptotic mechanism of phenoxodiol and the possible reversal of chemoresistance in cancer cell lines was reported by Kamsteeg et al. [26•]. In this study, chemotherapy-resistant human ovarian cancer cells, isolated from ascitic fluids of ovarian cancer patients, underwent apoptosis following phenoxodiol treatment. FLICE inhibitory protein (FLIP) is an important antagonist of the proapoptotic Fas pathway competing with caspases for binding into the death-inducing signaling complex (DISC). Phenoxodiol was found in this study to induce FLIP downregulation and therefore activation of the caspases, leading to tumor cell death [26•].

Similar work from Sapi et al. [27] has shown that phenoxodiol restores sensitivity in docetaxel-resistant epithelial ovarian cancer cells by downregulating the expression of X-linked inhibitor of apoptosis (XIAP), one of the most potent antiapoptotic proteins. Phenoxodiol, by interfering with XIAP activity, functions as a chemosensitizer to docetaxel and could provide a more effective treatment for refractory ovarian cancer.

Straszewski-Chavez et al. [28] also showed that phenoxodiol is an apoptosis inducer. First-trimester human trophoblast cells underwent apoptosis, as evidenced by the decrease in cell viability in the phenoxodiol-treated cells. This mechanism appeared again to be secondary to an increased degradation of XIAP and subsequent caspase-dependent apoptosis.

More recently, Aguero et al. [29] showed that phenoxodiol may have another possible mechanism of action different from caspase-dependent apoptosis. In this study, phenoxodiol promoted G (1)-S arrest by the specific loss in cdk-2 activity due to p53-independent p21 (WAF1) induction. This novel property of phenoxodiol may have major clinical implications, as the majority of human malignancies have aberrations in cell cycle progression regulation. Emerging data suggest that the mechanism of action of phenoxodiol may be via the sphingomyelin pathway. This pathway involves the reversible catabolism of sphingomyelin, the principal component of the plasma membrane sequentially into ceramide, sphingosine, and then sphingosine-1-phosphate (S-1-P). S-1-P levels are elevated in cancer cells in response to cisplatin resistance [30]. By downregulating the production of S-1-P, phenoxodiol may increase sensitivity to many chemotherapeutic agents, including cisplatin.

Animal studies
Phenoxodiol was selected for further testing in female Sprague-Dawley rats injected with dimethylbenz-a- anthracene (DMBA) to induce mammary carcinogenesis. Phenoxodiol was given at a 50- and 75-mg/kg diet. Phenoxodiol significantly reduced tumor incidence rate at both doses (P<0.05). Tumor latency was increased from 70.4 days in the control group to 92.9 (P=0.04) days and 97.8 (P=0.03) days in the groups that were fed 50 and 75 mg/kg of phenoxodiol, respectively. Compared with the control animals, which were fed basal diet, tumor multiplicity was reduced by 42% (P=0.04) in the group that was fed 50 mg/kg of phenoxodiol and by 49% (P=0.01) in the group that was fed 75 mg/kg of phenoxodiol [31]. These data suggest that phenoxodiol is an effective chemopreventive agent against DMBA-induced mammary carcinogenesis.

Other studies showed that phenoxodiol administered orally once daily for 18 days to mice bearing xenografts of human prostate cancer cells (androgen-dependent or independent tumors) resulted in 80% reduction in tumor growth (Investigator’s brochure, phenoxodiol injection; Novogen Limited).

These preclinical studies demonstrate a variety of potential mechanisms for the action of phenoxodiol. The reversal of chemoresistance to drugs such as cisplatin and docetaxel is of particular interest and could provide a more effective treatment for chemotherapy-refractory cancers.

Clinical Studies of Phenoxodiol in Humans
Clinical trials with phenoxodiol are predominantly phase I and early phase II studies, and many trials are ongoing

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CI—continuous infusion; IV—intravenous; OR—objective response; SD—stable disease.