Journal of Surgical Research **147,** 194–199 (2008)
doi:10.1016/j.jss.2008.02.005

A Pilot Study of Perillyl Alcohol in Pancreatic Cancer[1]

Jesus M. Matos, M.D.,* C. Max Schmidt, M.D., Ph.D., M.B.A.,*,¶,[2] Howard J. Thomas, M.D.,*,¶
Oscar W. Cummings, M.D.,§,¶ Eric A. Wiebke, M.D.,*,¶ James A. Madura, M.D.,*,¶
Loehrer J. Patrick Sr., M.D.,‡,¶ and P. L. Crowell, Ph.D.†,¶

*Department of Surgery, †Department of Biology, ‡Department of Medicine, and §Department of Pathology, ¶Indiana University Cancer Center, Indiana University School of Medicine at Indiana University/Purdue University at Indianapolis, Indianapolis, Indiana

Submitted for publication January 7, 2008

Background. **Chemotherapy has been largely unsuccessful in pancreatic cancer. Measurement of cell-specific biological endpoints may clarify the evaluation of a newer generation of compounds. Perillyl alcohol has shown chemotherapeutic activity in preclinical systems through enhancing apoptosis.**

Aims. **To pilot a new trial template for testing novel agents in pancreatic cancer and to assess the biological activity of perillyl alcohol in patients with resectable pancreatic cancer.**

Methods. **Apoptosis was quantified with ApopTag *in situ*, Bak staining, and light microscopy. Tumor size, serum CA 19–9 level, and survival were also measured.**

Results. **Eight patients enrolled. Toxicity was mild and perillyl alcohol was generally well tolerated. Tumor size and CA 19–9 level were unchanged with perillyl alcohol treatment. Survival time was longer in patients who received full perillyl alcohol treatment (288 ± 32 days) compared to those who did not (204 ± 96 days), but this result did not achieve statistical significance ($P = 0.2$). There was a trend toward greater apoptosis in patients receiving perillyl alcohol compared to fresh operative controls; there was also a suggestion of greater apoptosis in tumor compared to normal pancreatic tissue in the same patient.**

Conclusions. **Incorporation of cell-specific biological endpoints is challenging but feasible and should be used in clinical studies of pancreatic cancer treatment. Our pilot study suggests that perillyl alcohol may indeed have effects on biological endpoints. This study will serve as a useful template for examining cell-specific biological endpoints in the testing of future agents that are thought to induce apoptosis in pancreatic cancer.**

Key Words: **pancreatic cancer; perillyl alcohol; apoptosis; Bak.**

INTRODUCTION

Adenocarcinoma of the exocrine pancreas is the fourth leading cause of cancer-related death in the United States [1]. Despite available treatment modalities, less than 5% of patients survive 5 years. There are over 30,000 patients with a new diagnosis of pancreatic cancer each year in the United States and virtually the same number of deaths [1].

Chemotherapy has been largely unsuccessful in pancreatic cancer. Novel chemotherapies and novel approaches to chemotherapeutic trials are needed. Endpoints of classical response rates are insufficient to identify promising chemotherapeutic approaches to this disease. Alternatively, biological markers may determine their efficacy at the tissue level. For example, the measurement of cell-specific endpoints, such as apoptosis, may clarify the evaluation of a newer generation of compounds that can be used in combination with traditional anti-neoplastic agents.

Perillyl alcohol is a monoterpene that has shown both chemopreventative and chemotherapeutic activity in several preclinical cancer models. Monoterpene chemotherapeutic activity has been demonstrated preclinically in both rat mammary tumors and associated liver metastases [2, 3] as well as pancreatic tumors of both hamster and human cell origin [4, 5]. The chemotherapeutic activity of monoterpenes may occur through at least two mechanisms of action. First, the monoterpenes can inhibit the isoprenylation of small guanine nucleotide regulatory proteins (G-proteins) including the ras proto-oncogene *in vitro*. In the case of

[1] Jesus M. Matos and C. Max Schmidt contributed equally to the preparation of this manuscript.

[2] To whom correspondence and reprint requests should be addressed at Biochemistry/Molecular Biology, 1044 W Walnut St, R4-039, Indianapolis, IN 46202. E-mail: maxschmi@iupui.edu.

0022-4804/08 $34.00

p21 ras, this results in a loss of normal membrane association and, as a result, its ability to transform cells [6]. Perillyl alcohol, in particular, is a hydroxylated metabolite of limonene and has been shown to be even more effective than the parent drug in this respect [2, 3, 7]. The second mechanism of chemotherapeutic action involves the enhancement of apoptosis. This has been demonstrated in rat liver [8] and mammary [9] tumors, and in both human and hamster pancreatic carcinoma cell lines [4].

Stayrook *et al.* has studied pancreatic ductal carcinoma cell lines in an attempt to identify the mechanism of perillyl alcohol antitumor activity [4]. A greater reduction in cell growth was identified in transformed hamster pancreatic ductal epithelial cell lines *in vitro* compared to untransformed cell lines after perillyl alcohol exposure. Subsequent assays showed no decreased in the level of (^{3}H)-thymidine incorporation in the transformed cell lines, suggesting that decreased cell proliferation did not account for this differential growth rate. Statistically significant induction of apoptosis was found in the transformed hamster cell line as well as three human pancreatic carcinoma cell lines using Oncor's ApopTag immunofluorescent labeling. Expression of Bcl-2-related suppressors and promoters of apoptosis were also evaluated by Western blot. Bak, an apoptosis-promoting protein, was induced in transformed hamster cell lines and in all three human pancreatic cancer cell lines evaluated [4].

Given this mechanism of action in pancreatic carcinoma cell lines, namely, apoptosis as demonstrated by the induction of Bak and direct assessment using ApopTag immunofluorescent labeling, these endpoints provide an excellent model in which to directly assess the *in vivo* antitumor activity of perillyl alcohol in humans. Data from phase I studies demonstrate that perillyl alcohol has been well tolerated with the exception of nausea, bloating, and eructation described as tolerable by patients. Doses up to 2.4 g/m^2 TID have been delivered without reaching the maximum tolerated dose. However, a dose of 1200 mg/m^2 four times a day has been recommended for phase II trials [10–19]. Grade IV granulocytopenia has been observed in heavily pretreated patients, but it resolved with discontinuation of therapy. Activity level of this drug has been limited to prostate specific antigen and carcinoembryonic antigen-125 responses in prostate and ovarian carcinoma.

In this pilot study, we proposed to treat patients with pancreatic cancer preoperatively with perillyl alcohol. The trial was conducted to assess the biological response of human pancreatic carcinoma to perillyl alcohol and to provide a template for the testing of future drugs that are thought to induce apoptosis in pancreatic cancer. The activity of perillyl alcohol was determined by measurement of apoptosis and Bak assays in these patients' tumor specimens. These parameters were compared to "normal" adjacent pancreas as well as archived paraffin-embedded and fresh frozen pancreatic cancer tissue from control patients who did not undergo treatment with perillyl alcohol. Tumor size, tumor marker (serum Ca 19-9 levels), and survival were also followed as measures of treatment response.

METHODS

Patient Population

The patients in this trial received their medical and surgical care at the Indiana University Hospital in Indianapolis, Indiana.

Study

The primary objective of this study was to assess the biological activity of perillyl alcohol in patients with potentially resectable pancreatic adenocarcinoma. The secondary objectives were, first, to characterize the acute toxicity of perillyl alcohol in patients with pancreatic cancer; second, to evaluate the antitumor activity of perillyl alcohol in patients with pancreatic cancer; and third, to provide a trial template for examining cell specific biological endpoints in the testing of future agents which may induce apoptosis in pancreatic cancer.

To be eligible for the study, a histological or cytologic diagnosis of pancreatic adenocarcinoma was required prior to initiation of treatment. All patients enrolled were amenable to surgery with curative intent based upon preoperative radiographic staging. Performance status of patients needed to be 0–2 on the Eastern Cooperative Oncology Group scale. Patients had to be at least 18 years of age and have adequate bone marrow reserve, defined by white blood cell count of $\geq$3500/mm^3, platelet count $\geq$100,000/mm^3, and Hgb $\geq$9.5 g/dL. Exclusion criteria included evidence of metastatic disease, pregnancy (or breastfeeding), serious concomitant systemic disorders, active infection, liver or renal dysfunction. Patients with other malignancies or prior chemotherapy or radiotherapy for pancreatic cancer were also excluded. Patients taking any investigational agent within the month prior to enrolling, or on anticoagulants, cholesterol-lowering agents, high-dose vitamins, or antioxidants were not eligible unless they were able to be discontinued at least 72 h prior to initiating treatment.

The treatment protocol involved giving patients perillyl alcohol capsules at a dose of 1200 mg/m^2 p.o. four times daily with dosing intervals greater than 4 hours apart (i.e., 7 a.m., 12 p.m., 5 p.m., 10 p.m.). The treatment began 15 days prior to their scheduled pancreatic resection. The patients took their final dose on the evening prior to resection. There was no continuation of therapy postoperatively.

All operations were performed at Indiana University Hospital under the direction of a surgical member of the GI Oncology Multidisciplinary Program at the Indiana University Cancer Center. Surgical resection was performed for curative intent using either pancreaticoduodenectomy, distal pancreatectomy, or total pancreatectomy, depending upon the extent of tumor involvement. At the time of specimen removal from the operative field, the attending surgeon transected the specimen and obtained, in conjunction with the pathologist, a sample of both tumor and normal pancreatic tissue. These tissues were immediately preserved for histological analysis, ApopTag immunofluorescent labeling, and immunohistochemical analysis of Bak expression in 5-μm paraffin tissue sections.

Patients enrolled in the study underwent a thorough pretreatment evaluation including history and physical examination, laboratory profile including complete blood count, differential and platelet count, prothrombin time and partial thromboplastin time, serum electrolytes, and liver function tests. A chest X-ray, as well as other