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LY293111 Improves Efficacy of Gemcitabine Therapy on Pancreatic Cancer in a Fluorescent Orthotopic Model in Athymic Mice[1]

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Abstract

Pancreatic cancer has an abysmal prognosis because of late diagnosis and lack of effective therapeutics. New drugs are desperately needed. The present study determined the effect of the LTB_4 receptor antagonist, LY293111, on tumor growth and metastases in a fluorescent orthotopic model of pancreatic cancer. Pancreatic cancer cells (S2-013) with stable expression of enhanced green fluorescent protein were implanted into the duodenal pancreatic lobe of athymic mice. Animals were allocated to four groups (eight mice per group): control (no treatment); LY293111; gemcitabine; and LY293111 + gemcitabine. Monitoring of the surgical procedure and follow-up examinations at 2, 3, and 4 weeks after implantation to monitor tumor growth and metastases were performed using a fluorescence microscope and the reversible skin-flap technique. A staging and scoring system was developed to evaluate tumor progression, based on the TNM classification. Control animals developed end-stage disease with invasive cancer, metastases, and cachexia. Tumor growth and incidence of metastases were significantly reduced in all treated mice. However, combined treatment with LY293111 and gemcitabine was most effective. LY293111 is a novel therapeutic agent for pancreatic cancer, which improves the efficacy of gemcitabine. It is well tolerated and can be administered orally and, therefore, provides a new hope for patients suffering from pancreatic adenocarcinoma.

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Introduction

Patients diagnosed with pancreatic cancer have to face a disease with an abysmal prognosis and little hope for cure because effective therapies are not available. Pancreatic cancer is the fourth leading cause of cancer death in both men (after lung, prostate, and colon cancers) and women (after lung, breast, and colon cancers) in the United States, and the incidence of this disease has not declined. Indeed, it has increased in Japanese and African Americans over the last decades [1–3]. Mortality almost equals incidence and most patients die within 6 months after being diagnosed with this disease [1,4]. Potentially curative surgery can only be performed in about 20% of these patients because of metastatic spread or involvement of major blood vessels [4,5]. However, even in this selective group, the 5-year survival rate is only approximately 20% because of early tumor recurrence or metastatic tumor progression [6]. Gemcitabine is widely used as a standard therapy in pancreatic cancer patients in neoadjuvant, adjuvant, and palliative treatment protocols. However, besides improving quality of life, survival is only prolonged for about 1 month [7,8]. Therefore, new therapeutic strategies are urgently required for pancreatic cancer patients.

[2-Propyl-3-[3-[2-ethyl-4-(4-fluorophenyl)-5-hydroxyphenoxy]-propoxy]phenoxy]-benzoic acid (LY293111) is a leukotriene B_4 (LTB_4) receptor antagonist, which showed marked growth inhibition of human pancreatic cancer cells *in vitro* and in subcutaneous xenograft models, inducing apoptosis and S-phase arrest [9]. Recently, it has been shown that LTB_4 receptors are overexpressed in human pancreatic cancer cells and tissues [10]. Moreover, LTB_4 stimulates the growth of human pancreatic cancer cells by inducing ERK1/2 phosphorylation, which can be inhibited by LY293111 [11,12]. LTB_4 is a final product of the arachidonic acid–metabolizing 5-lipoxygenase (5-LOX) pathway and is well-known as a biologic mediator in several chronic inflammatory diseases as asthma, psoriasis, rheumatoid arthritis, and inflammatory bowel disease [13,14]. As in other cancers, cyclooxygenase-2 (COX-2) plays a role in the growth and spread

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of pancreatic cancers [15]. However, the 5-LOX pathway seems to play an even more important role in pancreatic cancer growth and development [15,16]. The LTB_4 antagonist activity of LY293111 was evaluated previously in clinical testing for inflammatory conditions [17–20]. Although it was found to be safe and well-tolerated, the development of the drug for inflammatory conditions was discontinued [21].

However, to bring new treatments from the laboratory into the clinic, adequate *in vivo* studies are required. The subcutaneous xenograft model for pancreatic cancer is limited because the tumor is growing in an unusual environment (subcutaneous) without high concentrations of important growth factors, such as insulin. There are also differences in tumor biology and morphology in this xenotopic site [22,23]. Therefore, in the current study, we used an orthotopic tumor model in athymic mice to determine the effectiveness of LY293111 alone and in combination with gemcitabine *in vivo*. This model employs S2-013 cells with stable transfection of green fluorescent protein (GFP). Because the tumors grow in their natural tissue environment, they develop metastases and mimic the clinical course of pancreatic cancer with obstruction of the duodenum and bile duct, and induction of cachexia. Moreover, the S2-013 human pancreatic cancer cell line was chosen among several others because of its metastatic potential and high aggressiveness to challenge the effectiveness of LY293111. The fluorescent model enables dynamic monitoring of tumor growth and metastases under different therapeutic strategies [24,25].

Materials and Methods

Cell Lines and Cell Cultures

S2-013, a subclone of SUIT-2, is a well-differentiated cell line derived from a liver metastasis of human pancreatic adenocarcinoma [26–28]. These cells were stably transfected with enhanced GFP and provided by Dr. M.A. Hollingsworth (Eppley Cancer Institute, Omaha, NE). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Cellgro; Mediatech, Inc., Herndon, VA). S2-013 tumor cells were harvested from 90% confluent cultures grown in T75 flasks (Corning, Inc., Corning, NY). The tumor cells were trypsinized, then the cell number was counted using a Guava Personal Cytometer (Guava Technologies, Inc., Hayward, CA) and resuspended in DMEM.

Animals and Surgical Orthotopic Tumor Cell Implantation (SOI)

Thirty-two 6- to 8-week–old female athymic nude (nu/nu) mice purchased from the National Cancer Institute were used in this study. The mice were housed in a two-way barrier facility in microisolator cages on static racks, fed with autoclaved laboratory rodent food pellets and acclimatized to the facility for 1 week before SOI. Animal weight was recorded everyday. Their use in this study was approved by the Institutional Animals Care and Use Committee and all procedures were conducted in accordance with the regulations and standards of the National Institutes of Health.

Mice were anesthesized intraperitoneally with 0.05 ml of a mixture of 0.4 ml of ketamine (Fort Dodge Animal Health, Fort Dodge, IO), 0.1 ml of xylazine (Phoenix Scientific, Inc., St. Joseph, MO), and 0.5 ml of NaCl. The abdomen was sterilized with alcohol pads and a 0.5-cm midline incision was performed. The abdominal wall was wrapped with wet gauze. After pulling the stomach on the surface, the pancreas was then carefully exposed and tumor cells (5×10^5 in 10 μl of DMEM) were injected into the duodenal lobe using a monoject 200 27-gauge×1/2 in. polypropylene hub hypodermic needle (Kendall, Mansfield, MA) and a 50-μl glass syringe (Hamilton Company, Reno, NV). The needle was carefully withdrawn and the injection sealed with a dry cotton tip. The successful injection was confirmed using a stereo fluorescence microscope. After the stomach and pancreas were returned to the peritoneal cavity, the incision was closed in two layers with vicryl-coated Rapide sutures 4-0 (Ethicon, Inc., Somerville, NJ). Once the mice were ambulatory, they were placed in the animal barrier facility. The mice were kept in a sterile environment throughout the procedure.

Therapy

One day after SOI, the mice were randomized into four groups: Group I (Control) received daily oral doses of the vehicle, DMSO (1/2 μl/g per day); Group II (LY293111) received daily oral doses of LY293111 (250 mg/kg per day, dissolved in DMSO and administered 1/2 μl/g per day); Group III (Gemcitabine) received daily oral dosages of DMSO (1/2 μl/g per day) and intraperitoneal injections of gemcitabine (60 mg/kg per dose dissolved in PBS and administered 2 μl/g per day) on days 4, 7, 10, and 13 after SOI; and Group IV (LY293111 + gemcitabine) received daily oral dosages of LY293111 (250 mg/kg per day) and intraperitoneal injections of gemcitabine (60 mg/kg per dose) on days 4, 7, 10, and 13 after SOI. LY293111 and gemcitabine were provided by Eli Lilly (Indianapolis, IN). Eight mice were assigned to each group and treated for 4 weeks, at which time the experiment was terminated and animals were euthanized.

Surgical Follow-Up Procedures

The mice underwent follow-up procedures 2 weeks after SOI. Mice were anesthesized with the same mixture of ketamine, xylazine, and NaCl. A horizontal arc-shaped incision was made through the skin and connective tissue was bluntly separated from the peritoneum with a curved scissors to free the skin flap (reversible skin flap) [29]. Digital pictures of tumors were taken with and without fluorescence, and staging of peritoneal tumors, pancreatic tumors, lymph node metastases, liver metastases, and ascites was performed and recorded according to our TMPN scoring system (Table 1, Figure 1). The scores from each category were multiplied with each other because patterns in medicine follow multiplicative, rather than additive, rules. The incision was closed with vicryl-coated Rapide 4-0 sutures. Follow-up surgeries were performed in the second, third, and fourth weeks after SOI.