A novel anti-pancreatic cancer agent, LY293111

Xian-Zhong Ding[a,c], Marks S Talamonti[a], Richard H. Bell Jr[a] and Thomas E. Adrian[a–c]

Arachidonic acid is metabolized by two major pathways, cyclooxygenases and lipoxygenases. The metabolites catalyzed by these enzymes are important mediators of acute and chronic inflammation. Both enzymes and their metabolites are well recognized to be involved in cancer development and progress. It is well documented that inhibition of cyclooxygenase 2 (COX-2) activity decreases cancer incidence and inhibits tumor growth. It has also been reported that 5-lipoxygenase is involved in cancer cell survival and proliferation. 5-Lipoxygenase metabolites including both 5-HETE and leukotriene (LT) B_4 directly mediate cancer cell growth. Although 5-HETE receptors are still elusive, two LTB_4 receptor subtypes (BLT_1 and BLT_2) have been characterized. Both 5-lipoxygenase and LTB_4 receptors are upregulated in both pancreatic cancer and early pancreatic cancer lesions; hence, these proteins are potential targets for cancer treatment and prevention. Recent studies have shown that an orally stable leukotriene (LT) B_4 receptor antagonist, LY293111, has a potent anti-pancreatic cancer effect. LY293111 inhibits pancreatic cancer growth, induces tumor cell apoptosis both *in vitro* and *in vivo*, and enhances the anti-pancreatic cancer effect of gemcitabine. LY293111 exhibits its anti-cancer effects through LTB_4 receptors and peroxisome-proliferator activated receptor-γ. A phase I clinical trial indicated that LY293111 is well tolerated by patients with no significant side-effects. LY293111 may be a valuable drug for treatment of pancreatic cancer, especially in combination with gemcitabine. A double-blinded, placebo-controlled phase II clinical trial with LY293111 is currently underway. This review summarizes the current research status of LY293111 as an anti-cancer agent with a focus on pancreatic cancer. *Anti-Cancer Drugs* 16:467–473

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Departments of [a]Surgery, [b]Pathology and [c]Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.

Correspondence to T. E. Adrian, Department of Surgery, Northwestern University, Tarry Building, 4-711, 303 East Chicago Avenue, Chicago, IL 60611, USA. Tel: +1 312 503-3489; fax: +1 312 503-3491; e-mail: tadrian@northwestern.edu

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death (after lung, breast and colon) in the US and the incidence of this disease has not declined. Patients diagnosed with pancreatic cancer have to face a disease with an extremely poor prognosis because of late diagnosis and lack of effective therapies [1–5]. Mortality almost equals incidence and most pancreatic cancer patients die within 6 months after being diagnosed [2–5]. Potentially curative surgery can only be performed in about 20% of these patients, because of metastatic spread or involvement of major blood vessels [2–5]. Even in this selected group, the 5-year survival rate is less than 20%, because of early tumor recurrence or metastatic tumor progression [2–5]. Gemcitabine is widely used for standard therapy in pancreatic cancer patients in adjuvant and palliative treatment protocols without significant extension of lifespan [5,6]. Therefore, new therapeutic strategies are urgently required for pancreatic cancer patients. This review summarizes the recent development of a novel potential anti-pancreatic cancer agent, LY293111, which is an orally stable leukotriene (LT) B_4 receptor antagonist and also a peroxisome-proliferator activated receptor-γ (PPAR-γ) agonist.

LT and LT receptors

5-Lipoxygenase is one of a family of lipoxygenase enzymes that metabolize arachidonic acid to hydroperoxy-eicosatetraenoic acids (HPETEs) [7,8]. Leukotrienes are a family of lipid mediators that are derived from arachidonic acid by the action of 5-lipoxygenase, which mediate acute and chronic inflammation [7,8]. In concert with 5-lipoxygenase activating protein (FLAP), 5-lipoxygenase catalyses the insertion of an oxygen moiety in the arachidonic acid backbone, and converts arachidonic acid first to 5-HPETE and then to LTA_4 [7,8]. LTA_4 is then converted to either LTB_4 or LTC_4 by the action of LTA_4 hydrolase or LTC_4 synthase, respectively [7,8]. LTC_4 is transported out of cells and then metabolized to LTD_4 and LTE_4. LTB_4 was the first LT isolated [7–10]. It is a potent chemotactic agent for neutrophils, eosinophils and monocytes [7–10]. LTB_4 enhances the adhesion of neutrophils to the vascular endothelium and enhances their migration across the endothelial wall [7–10].

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Studies over more than a decade have revealed that LTB_4 produces its biological effects by binding to its specific receptors, called BLT and including two subtypes, BLT_1 and BLT_2 [9–10]. In 1997, Yokomizo *et al.* cloned the first LTB_4 receptor, BLT_1, which is a putative seven transmembrane domain protein receptor with 352 amino acids [11]. BLT_1 mRNA is predominantly expressed in leukocytes, granulocytes, macrophages and eosinophils, and its expression could be induced in activated macrophages [11,12]. Interestingly, during the analysis of transcriptional regulation of the human BLT_1 gene, a putative open reading frame for a novel GPCR with structural similarity to BLT_1 was identified and later defined as BLT_2 [13]. Compared with BLT_1, BLT_2 has a low affinity for LTB_4 [11–13]. Using the open reading frame probe of BLT_2, Kamohara *et al.* and Yokomizo *et al.* reported that the highest expression of BLT_2 is found in spleen, leukocytes and ovary [13,14]. However, there is still controversy regarding the expression profile of BLT_2 in different tissues.

BLT receptor antagonists as anti-inflammatory agents

Multiple approaches have been attempted to suppress LT activity in order to block inflammation and treat asthma. The most promising one would be to directly inhibit 5-lipoxygenase activity, therefore blocking secretion of all LTs. The most widely studied clinical inhibitor of 5-lipoxygenase is zileuton, which is a hydroxyurea compound with chelating activity to lipoxygenase [15–17]. Zileuton inhibits the active site of 5-lipoxygenase at concentrations that do not inhibit cyclooxygenase, 12-lipoxygenase or 15-lipoxygenase [15–18]. Another avenue to inhibit LT formation is to block FLAP activity, thus preventing cytoplasmic to membrane translocation and activation of 5-lipoxygenase [7,8,17]. MK-0591 is a widely used 5-lipoxygenase-activating protein inhibitor for biomedical research [7,8,17]. Even though it strongly inhibits 5-lipoxygenase activity and blocks LT generation, its use in the clinic is limited by marked side-effects. The final pharmacological approach to block LT activity is to selectively block the actions of LTB_4 or the sulfidopeptide LTs using specific receptor antagonists.

In an attempt to identify novel anti-inflammatory drugs, synthetic LTB_4 receptor antagonists were developed in several laboratories a decade or so ago. One synthetic compound, SC-41930 (7-[3-(4-acetyl-3-methoxy-2-propylphenoxy)propoxy]3,4-dihydro-8-propyl-2*H*-1-benzopyran-2-carboxylic acid) was shown to be a LTB_4 antagonist in 1989 [18–20]. Another compound, ONO-4057 (5-[2-(2-carboxyethyl)-3-[6-(4-methoxyphenyl)-5E-hexenyl]oxyphenoxy]valeric acid), was reported to be an orally active LTB_4 antagonist [21,22]. Both SC-41930 and ONO-4057 displace LTB_4 binding to LTB_4 receptors in neutrophils, inhibit the LTB_4-induced rise in cytosolic free calcium and inhibit neutrophil aggregation, chemotaxis or degranulation induced by LTB_4 [18–22].

During the same period, researchers at the Lilly Research Laboratories reported two classes of chemical antagonists for LTB_4 receptors, benzophenone dicarboxylic acids and hydroxyacetophenones. LY223982 [(*E*)-5-(3-carboxybenzoyl-2-[[6-(4-methoxyphenyl)-5-hexenyl]oxy] benzenepropanoic acid] was the most potent antagonist among the benzophenone dicarboxylic acid derivatives; while LY255283 (1-[5-ethyl-2-hydroxy-4-[[6-methyl-6-(1*H*-tetrazol-5-yl)heptyl]oxy]phenyl]ethanone) was the most potent one from the hydroxyacetophenone derivatives [23,24]. Both of these compounds potently block LTB_4 binding to its receptors within the nanomolar range and inhibit various biological functions stimulated by LTB_4 *in vitro* [23,24]. Unfortunately, *in vivo* experiments discouraged a potential clinical use of these two compounds, because of poor oral bioavailability [25]. In 1995, investigators at the Lilly Research Laboratories reported LY293111, a novel derivative of LY255283, to be an orally stable, more potent LTB_4 receptor antagonist [26,27]. Compared with other LTB_4 receptor antagonists, LY293111 is superior at blocking the cellular functions induced by LTB_4 [26,28].

Inflammation, cyclooxygenases, lipoxygenases and cancer

The epidemiological data show a clear and strong association between chronic inflammatory conditions and cancer development, even though the conditions causing inflammation may vary [29–34]. It can be due to chronic infection caused by a virus, bacteria or parasite, or it may be due to a non-infective physical or chemical irritant [29–34]. For example, chronic infection with the bacterium *Helicobacter pylori* causes atrophic gastritis, which can lead to dysplasia and adenocarcinoma [35]. Hepatitis B and C viruses account for more than 80% of cases of hepatocellular carcinoma worldwide [32]. The inflammatory bowel diseases, ulcerative colitis and Crohn's disease, predispose to the development of cancers of the large bowel and/or terminal ileum, although a causative infections agent has never been conclusively identified [36]. For non-infectious inflammation, chronic reflux of gastric acid and bile into the distal esophagus causes chemical injury, and in the long term can lead to Barrett's esophagus and eventually to esophageal adenocarcinoma [33]. Thus, it is apparent that chronic inflammation is a common underlying theme in the development of many different malignancies.

Although the mechanisms for the association between inflammation and cancer are not fully understood, growth factors, cytokines and chemokines released into the inflammatory environment are associated with tumor development and progression [30,34]. High

concentrations of free radicals and nitric oxide can induce DNA damage and promote cancer development [30,34]. Over the past decade, much attention has been paid to the role of cyclooxygenases in cancer development, specifically its inducible isoform, cyclooxygenase 2 (COX-2) [37–39]. COX-2 is active within both inflamed and malignant tissues [37–39]. The expression of COX-2 and COX-2 metabolites increases during the multistage progression of tumors [37–39]. By metabolizing arachidonic acid to prostaglandins, COX-2 induces cellular resistance to apoptosis, modulation of cellular adhesion and motility, promotion of angiogenesis, and immunosuppression [40–45]. Epidemiological data has implicated COX-2 in the pathogenesis of a number of epithelial malignancies, especially colorectal cancer [46,47]. Inhibition of the enzyme with COX inhibitors is associated with a dramatic reduction in the incidence, morbidity and mortality of colorectal cancer [46–49].

Another arachidonate metabolic enzyme, 5-lipoxygenase, has also been implicated as an important regulator for cancer cell proliferation and survival in addition to the inflammatory mediator role of its metabolites [50,51]. 5-Lipoxygenase is upregulated in human pancreatic, breast and prostate cancer [52–56]. Inhibition of 5-lipoxygense activity by 5-lipoxygenase inhibitors, FLAP inhibitors or anti-sense oligonucleotides targeted to 5-LOX inhibits cancer cell proliferation and induces apoptosis [57–61]. The molecular mechanism for 5-lipoxygenase-mediated cancer cell proliferation appears to be mediated, at least in part, through its metabolites [62,63]. We have reported that 5-HETE directly stimulates proliferation, and activates mitogen-activated protein kinases (MAPK), tyrosine kinases and AKT pathways in pancreatic cells [63]. Since the most important 5-lipoxygenase metabolites are LTs, we recently examined secretion of LTB_4 by and expression of LTB_4 receptors on pancreatic cancer cells. Our data indicate that LTB_4 is secreted from human pancreatic cancer cells, while LTB_4 receptors (both BLT_1 and BLT_2) are expressed in these cells [53,64,65]. By immunocytochemical staining, we showed that both BLT_1 and BLT_2 are expressed and upregulated in pancreatic cancer tissues, contrasting with a lack of expression in normal pancreatic ducts [53,65]. Moreover, BLT_2 receptors are upregulated in early pancreatic cancer lesions [pancreatic intraepithelial neoplasias (PanINs)], including PanIN1b, PanIN2 and PanIN3, suggesting that 5-lipoxygenase, LTB_4 and its receptors are involved in early pancreatic cancer development [65].

In vitro studies have demonstrated that LTB_4 directly stimulates pancreatic cancer cell proliferation [64]. LTB_4 also enhances both MEK/ERK and phosphatidylinositol-3-kinase/AKT activities by stimulating phosphorylation of these enzymes in pancreatic cancer cells [66]. The LTB_4 receptor antagonist, LY293111 blocks LTB_4-mediated kinase phosphorylation [66]. In contrast, LTD_4 does not affect pancreatic cancer growth, and a LTD_4 receptor antagonist has no effect on pancreatic cancer cell proliferation and apoptosis [64]. From the combined experimental data, it is clear that 5-lipoxygenase, LTB_4 and its receptors play important roles in regulating pancreatic cancer cell proliferation, resistance to apoptosis and pancreatic cancer progress, even possible at the early stage of pancreatic cancer.

The anti-cancer effects of LY293111

LY293111 inhibits cancer growth both *in vitro* and *in vivo*

In vitro studies showed that LY293111 inhibits cell proliferation in a concentration- and time-dependent manner in multiple human cancer cell lines, including pancreatic, colonic and breast cancer [64,67]. The effective concentration for inducing cancer cell growth inhibition is less than 1 μM *in vitro*. Within 6 h of treatment, LY293111 at a concentration of 500 nM significantly inhibits DNA synthesis in multiple human pancreatic cancer cells [64]. To confirm the involvement of LTB_4 receptors in mediating the effect of LY293111 on human pancreatic cancer cell proliferation, another selective LTB_4 receptor antagonist, U75302, was used in comparison with a selective LTD_4 antagonist, LY171883 [64,66]. U75302 inhibits the proliferation of pancreatic cancer cells; but it is less potent than LY293111, as expected from the lower receptor affinity of this drug [64,66]. In contrast, the selective LTD_4 antagonist LY171883 had no significant effect on pancreatic cancer cell growth [64]. LY293111 markedly slows down the growth of s.c. xenografts of human pancreatic cancer in athymic mice at a dose of 250 mg/kg/day [64]. To confirm the anti-pancreatic cancer effect of LY293111, pancreatic cancer cells with stable expression of enhanced green fluorescent protein (GFP) were orthotopically implanted into the duodenal lobe of the pancreas of athymic mice. Our data show that LY293111 significantly inhibits the growth of the orthotopically implanted pancreatic cancer in concert with blocking metastatic spread to the liver and other organs [68].

LY293111 induces cancer cell apoptosis

In parallel with growth inhibition, LY293111 induces cellular apoptosis in multiple cancer cell lines tested. Induction of apoptosis by LY293111 was supported by the following evidence [64]. (i) LY293111 induced dramatic morphological changes in human pancreatic cancer cells following a short period of treatment. The treated cells became rounded, exhibited membrane blebbing, chromatin condensation and nuclear fragmentation, and finally detached from the microplate, suggesting progressive apoptosis. (ii) LY293111 induced DNA fragmentation. This was confirmed by the terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) assay. For example, treatment of both MiaPaCa-2 and AsPC-1 pancreatic cancer cells with

250nM LY293111 for 24h significantly increased TUNEL-positive staining cells by more than 10-fold. LY293111 also dramatically increased the number of TUNEL-positive cells in pancreatic tumors harvested from the s.c. transplant experiments in athymic mice. (iii) LY293111 induced poly ADP-ribose polymerase (PARP) cleavage and caspase-3 cleavage. (iv) LY293111 induced cancer cell apoptosis through the mitochondrial pathway, because the drug induced caspase-9, but not caspase-8, cleavage. Direct evidence for mitochondria as a target for LY293111 comes from the fact that it induced cytochrome *c* release into the cytoplasm. Following LY293111 treatment, concentrations of the anti-apoptotic proteins, Mcl-1 and Bcl-2, decreased in pancreatic cancer cells, while levels of the pro-apoptotic protein, Bax, increased [69]. This is likely to account for the cytochrome *c* release from mitochondria, and subsequent activation of caspase-9 and the downstream caspase cascade for apoptosis.

LY293111 blocks LTB_4-induced cell proliferation and kinase phosphorylation

To elucidate the molecular mechanism for LY293111-induced growth inhibition, specifically the possible role of LTB_4 receptors in the anti-cancer effect of LY293111, we examined whether LY293111 blocks the biological effect of LTB_4 on human cancer cells. Treatment of pancreatic cancer cells with LTB_4 induced concentration- and time-dependent cell proliferation in multiple human cancer cells [66]. LTB_4 directly induced MEK/ERK phosphorylation time dependently. Stimulation of cancer cell proliferation by LTB_4 is mediated by MEK/ERK since MEK inhibitors, PD098029 and U0126, prevented LTB_4-induced MEK/ERK phosphorylation [66]. Pretreatment of pancreatic cancer cells with LY293111 blocks LTB_4-induced MEK/ERK phosphorylation, suggesting that LY293111 effectively blocks biological activities of LTB_4 on pancreatic cancer cells. Moreover, LY293111 reverses LTB_4-induced cancer cell proliferation [64,66,69]. When taken together, these data allow us to conclude that LY293111 blocks pancreatic cancer cell proliferation through inhibiting the biological actions of LTB_4, as a LTB_4 receptor antagonist.

LY293111 is a PPAR-γ agonist

PPARs are nuclear hormone receptors, initially described as molecular targets for chemicals which induce peroxisomal proliferation [70,71]. The most extensively studied PPAR is PPAR-γ. PPAR-γ is a ligand-activated transcription factor, and a key regulator of adipogenic differentiation and glucose homeostasis [70,71]. Multiple studies have shown a role of PPAR-γ in tumor growth inhibition [72–74]. Immunocytochemistry revealed that PPAR-γ is upregulated in human pancreatic cancer. Interestingly, PPAR-γ agonists inhibit cancer cell proliferation and induce pancreatic cancer cell apoptosis [75–77]. *In vivo* xenograft studies have demonstrated that PPAR-γ ligands are anti-tumorigenic, due to anti-proliferative, pro-differentiation and anti-angiogenic effects [72–74,77]. In animal models, PPAR-γ ligands have preventive effects against chemical carcinogenesis [78]. This finding was initially based on structural analysis and was supported by functional studies. LY293111 was shown to have PPAR-γ agonist activity, as evidenced by its ability to induce adipogenic differentiation *in vitro* [79]. Normalization of circulating glucose levels by LY293111 in the ZDF rat diabetes model further suggests that LY293111 is an anti-diabetic, PPAR-γ agonist [79]. Further studies suggested that the anti-cancer effect of LY293111 might be mediated, at least in part, by PPAR-γ [79]. PPAR-γ-negative cancer cell are less responsive to LY293111-induced growth inhibition [79,80]. Our recent studies have shown that the anti-proliferative effects of LY293111 in pancreatic cancer could be partially reversed by the PPAR-γ antagonist GW9662 *in vitro*. It is possible that anti-cancer effects of LY293111 might also be partially mediated by other unknown mechanisms. However, based on the current data, both LTB_4 receptor and PPAR-γ are involved in the anti-tumor activity of LY293111.

LY293111 enhances the anti-cancer effect of gemcitabine

Gemcitabine is widely used as a standard therapy in pancreatic cancer patients in adjuvant and palliative treatment protocols. Even though survival is only prolonged for about 1 month with gemcitabine, clinical data suggest that it improves quality of life for pancreatic cancer patients. Recently, we examined anti-pancreatic cancer effects of LY293111 in combination with the clinical anti-pancreatic cancer agent gemcitabine in an orthotopic pancreatic cancer model [68]. The orthotopic pancreatic cancer model is superior to the s.c. cancer model because it is less likely to modify the biological characteristic of pancreatic cancer cells, providing a favorable growth environment for them. It also allows easy monitoring of hepatic and lymph node metastasis with GFP stable expressing cells. In this model, animals without any treatment following implantation of GFP-expressing S2O13 pancreatic cancer cells developed end-stage disease with invasive cancer obstructing the duodenum and bile duct [68]. The animals develop liver, lung and lymph node metastases, and eventually peritoneal carcinomatosis with malignant ascites and cachexia [68]. Both gemcitabine and LY293111 alone significantly inhibited tumor growth and reduced the incidence of liver metastasis. However, combined treatment of gemcitabine with LY293111 was significantly more effective than either treatment alone in blocking tumor growth [68]. Combined treatment also significantly relieved tumor-induced cachexia and maintained stable body weights compared with either drug alone [68]. Furthermore, LY293111 and gemcitabine significantly decreased the incidence of duodenal obstruction and metastasis [68]. These experimental results show that

combined therapy of gemcitabine and LY293111 potently inhibits the growth and metastases of the very rapidly growing and aggressive pancreatic adenocarcinoma, and suggest that it might be a valuable way for treatment of pancreatic cancer patients.

LY293111 is orally stable and well tolerated by patients in phase I clinical trial

A phase I clinical trial was carried out to examine the tolerability and pharmacokinetics of LY293111 administered orally twice daily in dosages between 200 and 800mg twice a day. The most common side-effects observed following oral administration were diarrhea, abdominal pain and nausea. Among the patients in the trial, two patients, one with progressive chondrosarcoma and the other with melanoma, had stable disease during LY293111 treatment [81]. Based on the phase I clinical data, it can be concluded that LY293111 can be safely administered orally. The toxicities observed were easy to manage [81]. A double-blinded, placebo-controlled phase II clinical trial of LY293111 in combination with gemcitabine is underway, involving 19 sites in six countries [82].

Future studies

In order to move on to the clinic for treatment of pancreatic cancer or other malignant diseases, phase II clinical trials are necessary to indicate anti-cancer efficacy. It is also necessary to elucidate whether LY293111 could effectively enhance the anti-cancer effects of other clinically approved chemotherapeutic agents and which of these agents is most suitable for combined therapy with LY293111. Finally, the molecular mechanisms for the anti-cancer effect of LY293111 are not fully understood. Apparently, both LTB_4 receptors and PPAR-γ are involved in LY293111-mediated growth inhibition of pancreatic cancer cells. Which of the two targets plays a dominant role is still controversial and this needs to be resolved. Whether or not other pathways are targeted by LY293111 should also be investigated.

Conclusion

More studies are needed before LY293111 could be brought to the clinic for treatment of cancer patients. However, preclinical and phase I clinical data support that LY293111 is a safe and potent anti-pancreatic cancer agent LY293111 inhibits cancer cell proliferation and induces apoptosis. LY293111 is also effective in blocking pancreatic tumor growth and metastasis *in vivo*. A phase I clinical trial showed that oral treatment with LY293111 is tolerable with manageable side-effects. LY293111 might become a valuable anti-pancreatic cancer agent if the on-going clinical trials are successful.

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