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LPA receptor agonists and antagonists (WO2010051053)

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Summary

Lysophosphatidic acid (LPA) is a bioactive lipid involved in signaling pathways that result in cell survival, proliferation, migration, and invasion. These cellular responses are a critical element of both normal development as well as pathophysiology. In particular, dysregulated LPA production and function have been linked with cancer and cardiovascular disease. RxBio, Inc., have generated several series of LPA analogs with varied agonist/antagonist function at the LPA₁₋₃ G protein-coupled receptor targets of LPA signaling. These analogs are simplified relative to LPA, through deletion of the glycerol moiety linking the LPA phosphate and fatty acid groups. One of the example compounds was shown to protect intestinal crypt cells from radiation-induced apoptosis in mice when whole-body irradiation occurred two hours after oral dosing.

Keywords

lysophosphatidic acid; radioprotection; chemoprotection; cancer; LPA₂ receptor

1. Introduction

Lysophosphatidic acids (LPA) are a family of phosphorylated monoacylglycerols, that differ primarily in the identity of the fatty acyl group, as well as in the position of that fatty acyl group (sn-1 versus sn-2 acyl group position on the glycerol backbone). LPA stimulates varied cellular responses through activation of both intracellular and extracellular targets as recently reviewed (Figure 1).[1] The intracellular targets include the well-characterized nuclear receptor, peroxisome proliferator activated receptor (PPAR) γ as well as actin-binding proteins such as gelsolin, villin, formin, and adseverin. The extracellular targets include G protein-coupled receptors in the endothelial differentiation gene (EDG) family (LPA₁₋₃) as well as former orphan receptors, most of which associate with the purinergic receptor family based on sequence analysis (LPA₄₋₅, GPR35, GPR87, P2Y10). LPA also serves as a feedback inhibitor of autotaxin (ATX), a circulating enzyme that converts lysophosphatidyl cholines (LPC) into LPA.[2]

Cytoprotective effects of LPA have been reported in numerous cell types and contexts.[3,4] Recent work demonstrates that LPA₂ can be a critical mediator of the antiapoptotic signals of LPA,[5] involving both G protein pathways and interactions of motifs in the C-terminal tail of LPA₂ with PDZ and LIM-domain proteins.[6] LPA₁ receptor involvement in neuronal cell survival was demonstrated both through assays in cultured cells and by reduced Schwann cell number in adult LPA₁ receptor homozygous knockout mice relative to WT animals, and supported claimed use of LPA in promoting survival of neuronal cells in

Declaration of Interest

A Parrill has co-authored a paper with the inventor in the past, when he was a graduate student. She declares no other conflict of interest. This paper was supported by NIH grant RO1 HL 084007.

culture.[7] Claims of cytoprotection have also previously been made in the context of neuronal cells protection from ischemia-reperfusion injury using LPA receptor agonists in a rat common carotid artery blockage model.[8] These studies and others have definitively shown that LPA agonists show both chemoprotective and cytoprotective effects, related to the best-substantiated therapeutic claims of WO2010051053.[9]

Linkages between LPA receptors and cancer initiation, progression, and metastasis are well-established and have been extensively reviewed.[10–13] LPA promotes cell proliferation, [10–14] enhances motility and invasiveness, stimulates angiogenesis, [10–13,15] and induces production of cytokines that promote inflammation. Overexpression of receptors responsive to LPA as well as ATX, which produces LPA, have been demonstrated to occur in highly invasive tumors. Thus a scientifically sound framework to support claims of LPA receptor antagonists as anticancer agents is in place.

2. Chemistry

RxBio, Inc. claims a series of phosphate, thiophosphate, and boranophosphate esters as well as individual alpha fluorinated phosphonate and di-acidic species (Figure 2) as examples of agonists and antagonists of the lysophosphatidic acid (LPA) receptors (WO 2010/51053). These compounds are both structurally simplified relative to LPA, which contains a glycerol linker between the phosphate headgroup and the fatty acid tail, as well as exhibiting common bioisosteric replacements. Simplified LPA species lacking the glycerol linker were reported to show LPA receptor activities as early as 2003.[16,17] The thiophosphate headgroup was first reported in a selective LPA₃ agonist in 2003.[18] Alpha-halogenated phosphonates have also been previously reported by other research groups (earliest report in 2005) as displaying LPA receptor activity.[19] The cyclopropyl analogs are related to a natural product identified from myxoamebae in 1992.[20] In contrast, the boranophosphoric acid, dithiophosphoric acid, and dicarboxylic acid example compounds present truly novel chemical features for LPA receptor ligands, although these are not particularly inventive modifications.

3. Biology

Example compounds were evaluated using a series of biological assays ranging from *in vitro* assays of receptors recombinantly expressed in mammalian cells to *in vivo* assays of radioprotection in mice. The primary assay for specific agonist or antagonist activity using the LPA_{1–3} receptors expressed in rat hepatoma cells (RH7777 cell line) by monitoring their influence on calcium release from intracellular stores. This assay demonstrated that unsaturated analogs generally show greater potency than saturated analogs, that the LPA_{1–2} receptors are more sensitive to double bond position along the hydrophobic chain than the LPA₃ receptor. In contrast to differences in the hydrophobic tail, which predominantly impact potency rather than activity profile, headgroup changes drastically influenced both receptor selectivity and agonism versus antagonism. These studies represent incomplete characterization of example compound activity at the cell-surface GPCR that have been confirmed to show LPA-mediated responses (Figure 1). Activity at the peroxisome proliferator activated receptor (PPAR) γ was assessed *in vitro* using an acyl-coenzyme A oxidase-luciferase reporter gene construct in the green monkey kidney cell line (CV-1), but results from this assay were not reported. Based on these cell-based assays, oleoylthiophosphate (LPA₂ agonist with EC₅₀ = 244 nM) was further evaluated as a radioprotectant, both in cultured intestinal epithelial cells (IEC-6 cell line) and in mice. Cellular radioprotection was monitored using a DNA fragmentation assay on pre-treated cells irradiated with a 20 Gray single γ -radiation dose from a ¹³⁷Cs source. *In vivo* radioprotection was monitored by counting surviving intestinal crypt cells after irradiating

pre-treated mice with either a 12 or 15 Gray single γ -radiation dose from a ^{137}Cs source. Mice treated 2 hours prior to irradiation with either LPA or oleoylthiophosphate showed greater numbers of surviving intestinal crypt cells per segment. The use of LPA agonists as pre-treatments for protection against radiation-induced crypt cell death is promising as a means to ameliorate the negative side-effects of radiation therapy. However, it should be noted that much of the same biological data provided in this patent application were previously provided as part of a US patent with a priority filing date in 2003 (US 7,217,704). [21] Pharmacokinetic studies of oleoyl thiophosphate in monkeys indicates a relatively long half-life and good absorbance after oral dosing,[22] provided added support for the therapeutic value of this particular compound.

4. Expert Opinion

The claimed compounds present common bioisosteric replacements for the phosphate headgroup of LPA, such as thiophosphates and alpha-halophosphates, as well as a common bioisosteric replacements at sites of unsaturation in the hydrophobic tail of LPA, namely cyclopropyl functionality. The example compounds are somewhat simplified relative to LPA, in that they lack the glycerol moiety that links the LPA phosphate and fatty acid components. Nevertheless, modifications such as these, methods for synthesis of related compounds, and the resulting LPA receptor consequences appeared in the literature prior to the US priority date of this application, March 2008.[16–20,23–29] The most innovative example compounds for modulation of LPA function are those with boranophosphate and di-acidic headgroups.

The primary biological screens described in the application focus on the EDG-family LPA receptors, LPA_{1-3} , and on the single known nuclear receptor, $\text{PPAR}\gamma$. This focus reflects the well-known LPA targets as of the priority date, but clearly does not reflect the current spectrum of reported LPA receptors (Figure 1). In particular, the activity of the claimed compounds at the non-EDG GPCR targets of LPA as well as against the actin-binding protein targets of intracellular LPA action have not been addressed. It is quite likely that specific therapeutic outcomes will require selective activation or inhibition of a carefully-optimized subset of the many LPA targets.

Stimulation of LPA receptors promotes cell survival in numerous cell types, including neuronal cells and the crypt cells in the intestine. This pro-survival response provided the rationale for the investigation of LPA agonists as potential chemoprotective and radioprotective agents. Such an application has particular relevance to prevent the diarrhea and intestinal side-effects that occur during radiation treatments for cancer. One example molecule, oleoyl thiophosphate, from WO2010051053 was definitively demonstrated to enhance the survival of intestinal crypt cells when whole-body irradiation of mice was performed two hours after oral administration. Subsequent pharmacokinetic studies in monkeys demonstrate good absorption of this compound after oral dosing and a relatively long half-life. These subsequent studies provide further evidence of potential therapeutic benefit in primates. *In vivo* experiments are not reported for the remaining example molecules. The therapeutic claims for other indications, including cancer treatment, wound treatment, organ preservation, and treatment of dermatological conditions, are also not supported by direct studies on the example compounds. Ample evidence in the literature supports LPA receptor involvement in signaling pathways relevant to these claims, but further *in vivo* investigations of the claimed compounds for these specific indications will be necessary to definitively support their clinical applications.

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Figure 1.
Biological targets of LPA.

Figure 2. Chemical compounds claimed as LPA receptor agonists and/or antagonists, both by general formula (top) and specific examples presented.