Pharmacokinetic and Pharmacodynamic Characterization of an Oral Lysophosphatidic Acid Type 1 Receptor-Selective Antagonist


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ABSTRACT

Lysophosphatidic acid (LPA) is a bioactive phospholipid that signals through a family of at least six G protein-coupled receptors designated LPA1–6. LPA type 1 receptor (LPA1) exhibits widespread tissue distribution and regulates a variety of physiological and pathological cellular functions. Here, we evaluated the in vitro pharmacology, pharmacokinetic, and pharmacodynamic properties of the LPA1-selective antagonist AM095 (sodium, 4-[3-methyl-4-[(R)-1-phenyl-ethoxycarbonylamino]-isoxazol-5-yl]-biphenyl-4-yl)-acetate) and assessed the effects of AM095 in rodent models of lung and kidney fibrosis and dermal wound healing. In vitro, AM095 was a potent LPA1 receptor antagonist because it inhibited GTPγS binding to Chinese hamster ovary (CHO) cell membranes overexpressing recombinant human or mouse LPA1 with IC50 values of 0.98 and 0.73 μM, respectively, and exhibited no LPA1 agonism. In functional assays, AM095 inhibited LPA-driven chemotaxis of CHO cells overexpressing mouse LPA1, (IC50 = 778 nM) and human A2058 melanoma cells (IC50 = 233 nM). In vivo, we demonstrated that AM095: 1) had high oral bioavailability and a moderate half-life and was well tolerated at the doses tested in rats and dogs after oral and intravenous dosing, 2) dose-dependently reduced LPA-stimulated histamine release, 3) attenuated bleomycin-induced increases in collagen, protein, and inflammatory cell infiltration in bronchoalveolar lavage fluid, and 4) decreased kidney fibrosis in a mouse unilateral ureteral obstruction model. Despite its antifibrotic activity, AM095 had no effect on normal wound healing after incisional and excisional wounding in rats. These data demonstrate that AM095 is an LPA1 receptor antagonist with good oral exposure and antifibrotic activity in rodent models.

Introduction

Lysophosphatidic acid (LPA) is a bioactive lysophospholipid that signals through a family of six known G protein-coupled receptors, designated LPA1–6 (Yanagida et al., 2007; Choi et al., 2010). As its name indicates, LPA1 was the first LPA receptor to be identified, and LPA1 expression has since been detected in a variety of tissues (Anliker and Chun, 2004). Via coupling to the downstream G proteins, Gq11 and G12/13, LPA1 regulates a wide range of cellular functions, including proliferation, migration, survival, and differentiation (Moolenaar, 1999; Contos et al., 2000). Gene deletion studies in mice have shown that LPA1 plays a prominent physiological role in embryonic development (Yang et al., 2002). However, increasing in vitro and in vivo evidence also demonstrates a pathophysiological role for LPA1 in lung and kidney fibrosis (Pradere et al., 2007; Tager et al., 2008; Swaney et al., 2010), neuropathic pain (Inoue et al., 2004), and some cancers (Shida et al., 2003; Boucharaba et al., 2006).

In patients with idiopathic pulmonary fibrosis, LPA levels are elevated in bronchoalveolar lavage (BALF), and LPA1 is highly expressed on lung fibroblasts isolated from idiopathic pulmonary fibrosis BALF (Tager et al., 2008). In animals, pulmonary vascular leakage and fibrosis were also attenuated in LPA1 knockout mice after bleomycin lung injury (Tager et al., 2008). We recently demonstrated that an LPA1 antagonist reduced lung injury, vascular leakage, inflammation, and fibrosis and decreased BALF concentrations of profibrotic and proinflammatory mediators in the mouse bleomycin-induced model of lung fibrosis (Tager et al., 2008).
whereas AM966 has only 9-fold greater selectivity for LPA1. The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.). The hLPA1/CHO and mLPA1/CHO stamelanoma cells were obtained from the American Type Culture Collection (Manassas, VA.).

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AM955 (sodium, 4-[3-methyl-4-(R)-1-phenyl-ethoxycarbonylmino)-isoxazol-5-yl]-biphenyl-4-yl)-acetate) is a selective, orally bioavailable LPA1 antagonist that is currently in preclinical development for lung and skin fibrosis. Based on mouse calcium flux assays, AM955 has similar LPA1 potency to that of our recently published LPA1 antagonist, (4'-{4-[2-chloro-phenyl]-ethoxycarbonyl-amino}-3-methyl-isoxazol-5-yl}-biphenyl-4-yl)-acetic acid (AM966) (Swaney et al., 2010). However, AM955 is more than 200-fold more selective for LPA1 compared with LPA3, whereas AM966 has only 9-fold greater selectivity for LPA1. The selectivity profile of AM955 and antifibrotic activity in a mouse skin fibrosis model have been described by Castelino et al. (2010). Here, we characterize the in vitro pharmacology and in vivo pharmacokinetic and pharmacodynamic properties of AM955 and demonstrate its efficacy in rodent models of LPA-stimulated histamine release, bleomycin-induced lung fibrosis, and UUO-driven kidney injury. Studies were also conducted to address the toxicological implications of antagonizing LPA1 in the setting of normal wound healing.

Materials and Methods

Drugs

AM955 is a novel LPA1 receptor antagonist developed by Amira Pharmaceuticals (Fig. 1). AM966 was synthesized as described previously (Swaney et al., 2010), and Debio-0719 (formerly Swaney et al., 2010). Moreover, LPA stimulated pancreatic cell migration and increased proliferation of malignant pleural mesothelioma cells, and these effects were inhibited by an LPA1/3 antagonist (Yamada et al., 2008; Komachi et al., 2009). It was recently shown that LPA released from tumor cells acts as a paracrine stimulator of cell differentiation, tumor growth, and angiogenesis via an LPA1-dependent signaling mechanism (Jeon et al., 2010). Therefore, we propose that LPA1 antagonism may be efficacious in treating a variety of fibrotic, inflammatory, and proliferative diseases.

Fig. 1. Structure of AM095.
were purchased from Charles River Laboratories, Inc. (Wilmington, MA). Female CD-1 mice (Harlan; 25–35 g) were used for histamine release assays. For wound healing studies male rats (250–300 g; noncatheterized) were purchased from Harlan. For bleomycin and UUO studies, female C57BL/6 mice (Harlan; 20–25 g) were used. Rats (one per cage) and mice (four per cage) were given free access to food and water and allowed to acclimate for at least 7 days before each study. Animals were housed in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal protocols were approved by the Amira Pharmaceuticals Institutional Animal Care and Use Committee. In all studies, animals were fasted 15 to 24 h before dosing. For rats, AM095 was administered intravenously at a dose of 2 mg/kg in 0.9% saline given as a 1 mL bolus injection into the jugular vein. To determine oral exposure, AM095 was administered as a solution in 0.5% methylcellulose via an oral gavage at a dose of 10 mg/kg in a volume of 3 mL/kg. Blood samples (approximately 300 μL of total blood) were taken from each rat via the jugular vein catheter at times up to 24 h postdose (10–11 samples per animal) in potassium EDTA tubes. After each sampling, the catheter was flushed with an equivalent volume of saline. Plasma samples were prepared by centrifugation of whole blood, were stored frozen (−80°C) before analysis. AM095 was dosed intravenously at 2 mg/kg and orally at 5 mg/kg to male beagle dogs (n = 3). Plasma samples were collected and analyzed for AM095 concentration by liquid chromatography/mass spectrometry as described previously (Swaney et al., 2010).

Histamine Release. For time-course and dose-response studies, mice received intravenous LPA challenge (3–300 μg/mouse) in 0.1% fatty acid-free bovine serum albumin) and were then sacrificed at the indicated time points to determine plasma histamine concentrations. For the antagonist studies, mice received AM095 (1–30 mg/kg p.o.) in a volume of 10 mL/kg 2 h before the intravenous LPA (300 μg/mouse) challenge. Histamine concentrations in the plasma were determined by enzyme immunoassay (VWR Scientific, West Chester, PA). Drug concentrations in plasma were determined by mass spectrometry. The EC50 was determined by nonlinear regression of plotting the plasma concentrations versus percentage of inhibition of histamine.

Bleomycin Model. Studies were conducted as described previously (Swaney et al., 2010).

Ureteral Obstruction Model. Mice underwent UUO or sham surgery to the left kidney. In brief, a longitudinal, upper left incision was performed to expose the left kidney. The renal artery was located and a 60 silk thread was passed between the artery and the ureter. The thread was looped around the ureter and knotted three times insuring full ligation of ureter. The kidney was returned to abdomen, the abdominal muscle was sutured, and the skin was closed with staples. The contralateral (right) kidney served as an uninjured control. AM095 (30 mg/kg) or vehicle (water) was given 1 to 4 h before UUO and then b.i.d. thereafter by oral gavage. After 8 days, mice were euthanized using inhaled CO2, and the kidneys were harvested and cut in half for histopathological and biochemical analysis of fibrosis. To assess fibrosis, half of the kidney was fixed in 10% neutral buffered formalin and stained using Masson’s trichrome. The other half of the kidney was frozen at −80°C for subsequent biochemical analysis of collagen content.

Collagen Analysis. Soluble collagen concentrations in BALF and kidney tissue were assessed using the Sircol soluble collagen assay kit (Biocolor Ltd., Carrickfergus, UK) according to manufacturer’s recommendations. For BALF, 50 μL was analyzed according to the manufacturer’s recommendations. Data are expressed as micrograms of collagen per milliliter of BALF. For tissue collagen concentrations, kidney tissue was thawed and 1 mL of RIPA lysis buffer (490 mL of phosphate-buffered saline, 5 mL of Nonidet P40, 2.5 g of deoxycholic acid, and 5 mL of 10% SDS containing protease inhibitor) was added to each sample. Tissue was homogenized for 1 min using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY). The homogenate was sonicated at 40 W for 45 to 60 s and centrifuged at 10,000g for 10 min to remove insoluble debris. Kidney collagen concentrations were expressed as microgram of collagen per milligram of protein.

Rat Dermal Wound Healing. Animals were weighed and dosed orally with AM095 (30 mg/kg) or vehicle (water) 1 h before wounding and then daily dosing for 14 days. Based on AM095 drug exposure rates (see Fig. 3A), once daily was used. A single incisional wound and a single excisional wound were made on the back of each animal while under isoflurane anesthesia. For incisional wounds, a surgical scalpel was used to create a 1-cm-long, full-thickness incision in the dorsal skin of the animal. The incision was placed parallel to the midline along the dorsal skin of the animal. For excisional wounds, an 8-mm, full-thickness skin biopsy punch was made on the back of each animal opposite the site of the incision. Animals were recovered from the anesthesia and housed individually during the recovery period. The wounds were allowed to heal without further treatment other than test compound or vehicle delivered orally as noted above. Immediately after wounding (day 0) and at specific days afterward a photograph was taken of each wound while animals were under isoflurane anesthesia. Images were digitally analyzed to measure wound healing. At the end of the study (day 14) animals were euthanized with CO2 inhalation. Wound closure was determined for each subject on days 1, 3, 7, and 14 for rats relative to day 0.

Statistical Analyses. All values are expressed as the means ± S.E.M. Statistical analysis was performed by use of Student’s t test for two-group analysis or one-way ANOVA for multiple group comparisons followed by Newman-Keuls post hoc comparisons (Prism; GraphPad Software Inc., San Diego, CA). Statistical significance was set at p < 0.05.

Results

In Vitro

GTPγS Binding and Chemotaxis. AM095 was recently described as being a potent and selective LPA1 receptor antagonist as determined by whole-cell, calcium flux-based assays (Castelino et al., 2010). We further profiled AM095 using both a GTPγS binding assay and a cellular chemotaxis assay. AM095 inhibited GTPγS binding to human and mouse LPA1 in a dose-dependent manner, resulting in average IC50 values of 0.98 ± 0.33 and 0.73 ± 0.19 μM, respectively (Fig. 2A). As shown in Fig. 2B, the IC50 of AM095 in the human LPA1 GTPγS binding assay was comparable with that of our previously published compound AM966 (IC50 = 0.98 ± 0.17 μM) and the Debio-0719 compound (IC50 = 0.60 ± 0.04 μM) (Swaney et al., 2010). AM095 was tested for agonist activity by incubating increasing concentrations of AM095 (0.001–30 μM) with membranes expressing human LPA1 and measuring GTPγS binding (Fig. 2C). AM095 showed no agonist activity at the LPA1 receptor at any concentration tested, whereas increasing concentrations of the endogenous ligand, LPA, showed dose-dependent stimulation of GTPγS binding with an average EC50 of 38 nM. To examine the nature of the antagonism at the LPA1 receptor, AM095 was tested for its ability to antagonize LPA-stimulated GTPγS binding over a range of LPA concentrations. Increasing concentrations of AM095 caused a rightward shift in the LPA concentration response curves, lowered the Bmax and increased the IC50. Based on the lowering of the Bmax in the LPA concentration response curves, AM095 functions as an insurmountable antagonist at the LPA1 receptor (Fig. 2D). To assess the potency of AM095 in an intact cell functional assay, we measured the effects of AM095 on LPA-stimulated chemotaxis using both recombinant and native
cells. AM095 inhibited LPA₁-driven chemotaxis of both human A2058 melanoma cells (IC₅₀ = 0.23 ± 0.10 μM; n = 7) and mouse LPA₁/CHO cells (IC₅₀ = 0.78 ± 0.07 μM; n = 3).

**In Vivo**

**Pharmacokinetics.** The plasma concentration time curves for AM095 in male Sprague-Dawley rats and male beagle dogs after both intravenous and oral administration are shown in Fig. 3. After oral (10 mg/kg) dosing in rats, AM095 plasma concentrations peaked at 2 h with a Cₘₐₓ of 41 μM, thereafter decreasing to 10 nM by 24 h. After intravenous (2 mg/kg) dosing, a Cₘₐₓ of 12 μM was observed within 15 min, which also decreased to approximately 10 nM by 24 h, yielding a t½ of 1.79 h (Fig. 3A). In dogs, a single oral dose of 5 mg/kg yielded a peak plasma concentration of 21 μM within 15 min of dosing, which then decreased to 10 nM by 24 h. In contrast, an intravenous dose of 2 mg/kg resulted in a Cₘₐₓ of 11 μM within 15 min and decreased to 15 nM by 8 h, yielding a t½ of 1.5 h (Fig. 3B). The oral exposure was high for both rats and dogs with a oral bioavailability of 100. The detailed pharmacokinetic parameters for AM095 are shown in Table 1.

**AM095 Inhibits LPA-Stimulated Histamine Release in Mice.** Previous studies have shown that histamine release is increased after intravenous LPA administration and this effect is inhibited by a mixed LPA₁/3 antagonist (Hashimoto et al., 2006). Here, we examined the specific role of LPA₁ in histamine release and used the histamine assay as an in vivo pharmacodynamic readout to confirm the LPA₁-selective action of AM095. As shown in Fig. 4A, LPA stimulated histamine release in a dose-dependent manner, resulting in a nearly 14-fold stimulation at the highest concentration tested (300 μg/mouse). AM095 dose-dependently inhibited histamine release with an ED₅₀ of 8.3 mg/kg and a maximal reduction of 80% at a dose of 30 mg/kg (Fig. 4B). By plotting the percentage inhibition of histamine release versus AM095 plasma concentrations for each individual animal and assuming a maximum response of 80% we generated an EC₅₀ of ~1.5 μM (Fig. 4E). This value is in good agreement with the mouse GTP₇ binding and mLPA₁/CHO cell chemotaxis IC₅₀ values of ~0.8 μM.

**AM095 Reduces Lung Collagen, Vascular Leakage, and Inflammation after Bleomycin-Induced Lung Injury.** We recently demonstrated that AM966, an LPA₁ receptor antagonist, inhibits fibrosis, inflammation, and vascular leakage at multiple time points after bleomycin-induced lung injury in mice (Swaney et al., 2010). However, based on the improved mouse LPA₁ selectivity of AM095 compared with
AM095, we repeated the 7-day bleomycin studies using AM095. We found that AM095 significantly \( p < 0.05 \) decreased BALF collagen and protein at doses of 30 and 60 mg/kg, resulting in an ED\(_{50}\) of approximately 10 mg/kg for both endpoints (Table 2). Furthermore, significant reductions in both macrophage and lymphocyte infiltration were observed in response to AM095 treatment. These findings confirm that the antifibrotic activity in the lung is related to LPA\(_1\) antagonism and further validate the use of a selective LPA\(_1\) receptor antagonist in this setting.

**AM095 Reduces Kidney Fibrosis in a Mouse UUO Model.** To determine whether inhibition of fibrosis by AM095 would generalize to other tissues, mice underwent UUO and kidney fibrosis was measured after treatment with AM095 (30 mg/kg) for 8 days. Trichrome staining revealed increases in fibrotic tissue in obstructed (Fig. 5Ab) compared with unobstructed (Fig. 5Aa) kidneys of vehicle-treated mice. Fibrosis was decreased in AM095-treated mice (Fig. 5Ad) compared with obstructed mice receiving vehicle treatment (Fig. 5Ab). No significant changes in collagen deposition were seen in mice receiving AM095 alone (Fig. 5Ac). These findings were confirmed after biochemical measurement of total collagen in kidney tissue (Fig. 5B).

**AM095 Does Not Affect Normal Wound Healing.** LPA is recognized as a modulator of dermal wound healing (Demeyer et al., 2000; Balazs et al., 2001), and LPA receptor-mediated regulation of fibroblast function is believed to play a role in wound repair (Watterson et al., 2007). Therefore, we investigated the effects of AM095 in rat models of full-thickness incisional and excisional dermal wounding. As shown in Fig. 6, once-daily oral administration of AM095 (30 mg/kg) did not affect the extent or rate of wound closure after either incisional or excisional wounding in rats.

**Discussion**

LPA is a pleiotropic lysophospholipid that has gained increasing recognition for its ability to regulate both physiologic-
As a primary in vitro screen, AM095 was evaluated for its ability to inhibit LPA₁ activation by its endogenous ligand, LPA. Inhibition of LPA₁ activation was measured as a decrease in the LPA-stimulated association of GTP with the receptor/G-protein complex. This assay has previously been used to assess the potency of nonselective LPA receptor antagonists (Ohta et al., 2003). The average IC₅₀ values for the AM095-mediated inhibition of LPA-stimulated GTP·Y binding to membranes expressing human or mouse LPA₁ were 0.98 and 0.73 μM, respectively. It is noteworthy that AM095 did not demonstrate any agonist effects on LPA₁ at concentrations as high as 30 μM. There was a significant level of basal GTP·Y binding in the absence of exogenous LPA, and this basal level of activity was also inhibited by AM095. At this point, it is unclear whether the basal activity reflects the presence of endogenous LPA in the membrane preparation or is LPA-independent. Studies to address this question are ongoing. It is noteworthy that the nature of antagonism of AM095 differs from the LPA mimetic, (S)-phosphoric acid mono-[3-(4-benzyloxy-phenyl)-2-octadec-9-enylamino-propyl] ester (VPC12249), in that LPA-stimulated GTP·Y binding was insurmountable in the presence of AM095 (Heise et al., 2001). It is noteworthy that we also observed insurmountable antagonism with Ki16425 in the GTP·Y assay and insurmountable antagonism with AM095 and Ki16425 in an LPA₁-mediated calcium flux assay (data not shown). There are several proposed mechanisms for insurmountable antagonism that include (but are not limited to) allosteric binding, slow dissociation of antagonist-receptor complexes, irreversible binding, or antagonist-mediated conformational changes in the receptors rendering them refractory to agonist stimulation. We are currently performing experiments to further understand the nature of this insurmountable antagonism.

Studies have shown that LPA stimulates the migration or chemotaxis of multiple human cancer cell lines through an LPA₁ receptor-dependent mechanism and that this process critically depends on G₁-coupled Rac1 activation (Hama et al., 2004). Moreover, G₁ coupling mediates LPA-stimulated chemotaxis of CHO cells recombinantly expressing the human LPA₁ receptor (Sugimoto et al., 2006). Therefore, as a secondary functional readout, we measured the ability of AM095 to inhibit LPA-driven cell chemotaxis. AM095 inhibited chemotaxis of human A2058 melanoma cells and mouse LPA₁/CHO cells, providing further confirmation of the in vitro potency and the functional antagonism of LPA₁ by AM095. This is of particular importance in light of previous findings that showed that an LPA₁/3 antagonist (Ki16425) blocked migration of various cancer cells in vitro (Hama et al., 2004) and increased LPA₁ activation and expression potentiated the metastasis of cancer cells to bone (Boucharaba et al., 2004, 2006).

The pharmacokinetic profile of AM095 was assessed in male Sprague-Dawley rats and male beagle dogs (see Fig. 3 and Table 2). AM095 was well absorbed, had a moderate half-life, and was well tolerated after oral dosing in both species. We observed some variation in the time to maximum plasma concentration between species after oral dosing. However, the time to peak plasma concentration can vary because of fasted/fed conditions, stress, transit time, and other factors and was not directly investigated in these studies.

As a pharmacodynamic readout of LPA₁ antagonism, we examined the ability of AM095 to inhibit histamine release in mice after an intravenous administration of LPA. Hashimoto et al. (2006) have previously shown that LPA-stimulated histamine release in mice depends on LPA₁ and/or LPA₃.
AM095 inhibited histamine release in a dose-dependent manner, resulting in an 82% reduction at the 30 mg/kg (b.i.d.) dose. The lack of a 100% reduction by AM095 probably reflects the contribution of other LPA receptors (e.g., LPA3) to the LPA-induced release of histamine. In summary, these findings demonstrate that LPA-stimulated histamine release is mediated primarily through LPA1 and, in vivo, 30 mg/kg AM095 shows maximal functional antagonism of LPA1.

We recently established that an LPA1 antagonist (AM966) decreases pulmonary vascular leakage, inflammation, and fibrosis at multiple time points in a mouse bleomycin model (Swaney et al., 2010). However, AM966 has only 9-fold greater selectivity for LPA1 versus LPA3 in mice, whereas AM095 is over 200-fold more selective for LPA1 (Castelino et al., 2010; Swaney et al., 2010). For this reason, we investigated the effects of AM095 on the development of lung fibrosis in a 7-day bleomycin model. By repeating the 7-day bleomycin studies using AM095 we were able to confirm that the reduction in vascular leakage, inflammation, and fibrosis observed in the AM966 studies (Swaney et al., 2010) was, indeed, related to antagonism of LPA1 and not LPA3. Consistent with our previous work, AM095 (1–60 mg/kg) dose-dependently decreased BALF collagen and protein concentrations and reduced inflammatory cell infiltration in the lung after bleomycin insult. To explore the efficacy of AM095 to inhibit fibrosis in multiple tissues, we conducted subsequent studies in the UUO kidney fibrosis model. Pradère et al. (2007) showed previously that LPA1 was a mediator of kidney fibrosis in mice. The antifibrotic effects of AM095 in the kidney were consistent with those observed in the lung, confirming the efficacy of AM095 to reduce fibrosis in multiple organs.

A common toxicological concern with antifibrotic agents is whether, via their mode of action, patients will exhibit a delay in normal wound healing or develop nonhealing wounds as a result of their antifibrotic therapy. Studies have shown that topical LPA promotes dermal wound healing (Demoyer et al., 2000; Balazs et al., 2001) and LPA is recognized for its ability to modulate fibroblast function during tissue repair (Watterson et al., 2007). To address concerns regarding the potentially negative effects of AM095 on normal wound healing, we conducted incisional and excisional wounding studies in rats. It is noteworthy that therapeutic doses of AM095 had no effect on wound healing rate or the magnitude of wound closure in rats. Therefore, it seems that
perturbing the basal state of LPA1 activation does not affect the normal wound healing process.

In summary, AM095 is a potent, orally bioavailable LPA1 receptor antagonist with favorable pharmacokinetic properties in rodents and dogs and good pharmacokinetic and pharmacodynamic properties and demonstrated efficacy in two rodent models of tissue fibrosis. Based on the potential role of LPA1 in cancer, neuropathic pain, and tissue fibrosis, AM095 has exciting potential for treating a range of clinical diseases/disorders.

Authorship Contributions

**Participated in research design:** Swaney, Stebbins, and Lorrain.

**Conducted experiments:** Swaney, Chapman, Correa, Stebbins, Broadhead, Bain, Santini, Darlington, Ziff, and Lorrain.

**Contributed new reagents or analytic tools:** Parr, Roop, and Seiders.

**Performed data analysis:** Swaney, Stebbins, Bain, King, Baceei, Lee, and Lorrain.

**Wrote or contributed to the writing of the manuscript:** Swaney, Prasit, Hutchinson, Evans, Bain, and Lorrain.

References


