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Proton pump inhibitor Lansoprazole is a nuclear Liver X Receptor agonist

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Abstract

The liver X receptors (LXR α and LXR β) are transcription factors that control the expression of genes primarily involved in cholesterol metabolism. In brain, in addition to normal neuronal function, cholesterol metabolism is important for APP proteolytic cleavage, secretase activities, A β aggregation and clearance. Particularly significant in this respect is the LXR mediated transcriptional control of *APOE*, which is the only proven risk factor for late onset Alzheimer's disease. Using a transactivation reporter assay for screening pharmacologically active compounds and off patent drugs we identified the Proton Pump Inhibitor Lansoprazole as an LXR agonist. In secondary screens and counter-screening assays, it was confirmed that Lansoprazole directly activates LXR, increases the expression of LXR target genes in brain-derived human cell lines, and increases Abca1 and Apo-E protein levels in primary astrocytes derived from wild type but not LXR α/β double knockout mice. Other PPIs activate LXR as well, but the efficiency of activation depends on their structural similarities to Lansoprazole. The identification of widely used, drug with LXR agonist-like activity opens the possibility for systematic preclinical testing in at least two diseases – Alzheimer's disease and atherosclerosis.

Keywords

Liver X Receptors; Proton Pump Inhibitors; Gene expression; LXR knockout mice; Primary astrocytes; ABCA1; APOE

1. Introduction

Analysis of the human genome sequence has revealed a total of 48 nuclear receptors that comprise the largest superfamily of evolutionarily conserved transcription factors – receptors for all major steroid hormones, thyroid hormone, vitamin D, and retinoic acid, as well as the “orphan receptors” whose ligands remain unknown [1]. Within the superfamily nuclear Liver X Receptors (LXR)¹ belong to a class of metabolite-activated transcription factors that form obligate heterodimers with the retinoid X receptor (RXR). In the absence of ligand, most RXR

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¹Abbreviations: LXR - liver X receptor; PPI - proton pump inhibitor; ABCA1 - ATP binding cassette transporter A1; CompL - Lansoprazole; A β - amyloid β ; RXR – retinoid X receptor; IFN – interferon; NSAID – non-steroidal antiinflammatory drug.

heterodimers are bound to DNA in association with co-repressors, histone deacetylases and chromatin-modifying factors to maintain active repression of target genes. Ligand binding initiates a conformational change in the receptor, exchange of co-repressors for co-activators, and initiation of target gene transcription. LXR/RXR heterodimers are examples of receptors that can both activate and repress gene expression in a signal and gene-specific manner. The LXR/RXR complex, which can be activated by ligands of either partner, binds to LXR responsive elements (LXREs) consisting of DR4 (direct nucleotide repeat 4) [2]. In the absence of ligands, LXRs bind to the cognate LXRE in complex with co-repressors such as silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and nuclear receptor co-repressors (NCoR). Under these conditions the transcriptional activity of the receptor is suppressed. In the presence of ligands, the corresponding LXR undergoes a conformational change that induces release of co-repressors recruitment of specific coactivators and subsequent transcription of target genes [3,4].

Two isotypes of LXR have been identified - LXR α and LXR β . While LXR α is expressed at its highest levels in liver and at lower rates in intestine, lung, macrophages, adipose tissue, adrenal glands and kidney; LXR β is expressed ubiquitously in all tissues examined [1]. Although the two isotypes are very similar in structure with a high degree of homology between their ligand binding domains, their nuclear retention, localization and function display some differences. LXR target genes are involved in cholesterol metabolism, lipoprotein remodeling and lipogenesis. Systemic activation of LXRs thus initiates a series of tissue-specific transcriptional programs that regulate whole - body cholesterol content. Pharmacological activation of these receptors *in vivo* results in increased HDL levels and net cholesterol loss. This finding led to identification of LXR agonists as potent antiatherogenic agents in rodent models of atherosclerosis [2].

During the last several years the search for new LXR agonists has been reinforced by the discovery that LXR and their primary responsive genes, ABCA1 in particular, have a role in amyloid beta (A β) aggregation, deposition and clearance from brain [5,6,7,8,9,10]. Importantly, APOE gene (and its $\epsilon 4$ allele), the inheritance of which is the only proven risk factor for Late Onset AD, is transcriptionally controlled by LXR.

This article describes the identification and *in vitro* characterization of the Proton Pump Inhibitor (PPI) Lansoprazole as an LXR agonist. It provides data on its receptor type specificity and effects in mouse primary and human derived established cell lines that substantiate further testing of its therapeutic efficiency in *in vivo* models of Alzheimer's disease and atherosclerosis.

2. Materials and Methods

2.1. Chemicals and Reagents

Lansoprazole, Omeprazole and Esomeprazole were from Sigma (St Louis, MO), Pantoprazole from LKT Laboratories (St Paul, MN) and T0901317 from Cayman Chemical (Ann Arbor, MI). A library of commercially available compounds – small molecules and off-patent drugs was provided by the Drug Discovery Institute, University of Pittsburgh. All other reagents and materials for cell culture and general use (if not specified) were from Invitrogen (Carlsbad, CA) and Fisher Scientific (Pittsburgh, PA).

2.2. Transactivation reporter assay

pCMX - hLXR α and *pCMX - hLXR β* are expression constructs that contain CMV promoter and the full length canonical sequence of the corresponding receptor, including DNA and Ligand Binding Domains. The reporter plasmid *TK-LXREx3-Luc* drives the expression of Firefly

luciferase by thymidine kinase promoter with 3 consecutively inserted consensus LXR response elements (gcttTGGTCActcaAGTTCAagtta) from rat *Cyp7a* gene. The plasmids were obtained from D. Mangelsdorf (University of Texas, SWMC, Dallas, TX) and have been used to initially characterize the structural requirements for LXR ligand binding [11]. Expression of Renilla luciferase by *pRL-SV40* (Promega, Madison, WI) provides an internal control value for normalization of Firefly luciferase reporter gene expression. *pRL-SV40* vector contains SV40 enhancer/promoter region, SV40 origin of replication with transient, episomal replication in cells expressing SV40 large T antigen and thus, a strong constitutive expression of Renilla luciferase.

Cos-7 cells (ATCC, Manassas, VA) were chosen for the assay to facilitate the simultaneous transfection and expression of three different minigenes under the control of different minimal promoters which helps to avoid cross activation. The cells allow electroporation at 75–80% confluence within 24 hrs of plating.

Electroporation was carried out in Amaxa Nucleofector (Lonza, Cottonwood, AZ,) using a kit of reagents optimized for *Cos-7* cells and according to the manufacturer's protocol. Transfection efficiency was tested by the percent of cells transfected with pMAX-GFP (Lonza) and examined under fluorescent microscopy at different times post-transfection. In all preliminary experiments the transfection efficiency was higher than 90%. Library compounds were applied 18–20 hrs after the transfection and plates were processed for luminescence measurement 16–20 hrs later. The optimized conditions of the assay allowed for running a single test, from plating the cells until luminescence reading, in less than 40 hrs.

Dual-Glo Luciferase system (Promega) – a homogeneous reagent assay that enables fast and simple quantitation of a stable luminescent signal from two reporter genes in a single sample was used to identify LXR agonist-like compounds.

2.3. Human cell lines and primary mouse cells

In addition to *Cos-7* cells the following cell lines were used in this study: U-118, U-87, CCF, H4 (all obtained from ATCC). Cells were maintained in DMEM/F12 supplemented with 10% Bovine Growth Serum (BGS) and standard concentrations of L-Glutamine and Pen/Strep. For treatment, cells were plated in 24 well plates and after 24 hrs treated with the corresponding compound in DMEM/F12 supplemented with 2% delipidated serum. Depending on the assay and endpoint, 24 or 48 hrs later the cells were lysed for further assays.

Primary mouse mixed glial cells were derived from P1 newborn C57BL/6J, $LXR\alpha^{-/-}$, $LXR\beta^{-/-}$ or $LXR\alpha/\beta$ -ko mice and processed as previously [12]. Briefly, cortices and hippocampi were dissociated with trypsin-EDTA for 10 min at room temperature and cultivated in DMEM/F12 medium supplemented with 10% BGS, L-Glutamine and antibiotics. Cells were plated on poly-D-lysine coated T75 Costar flasks and after 3 weeks replated in 24 well plates for further treatment as described for established cell lines. The mixed glial cultures were treated for 24 or 48 hrs and then lysed for further assays.

2.4. RNA isolation and real time quantitative PCR

RNA was purified using RNeasy spin columns (Qiagen) according to the manufacturer's protocol. For TaqMan based RT-QPCR first-strand cDNA was synthesized using Sprint RT Complete Random Hexamer strips (Clontech) with 300 ng of total RNA. Gene-specific primers and probes for mAbca1 (Mm00442646), hABCA1 (hs010591-18), hAPOE (Hs00171168), mApoE (Mm0043-7573), and 18s (Hs99999901) were obtained from Applied Biosystems. Real-time PCR was carried out using standard PCR-conditions on ABI 7500 Real-Time PCR

System. Amplification plots were analyzed by Comparative Δ Ct method with 18s rRNA as housekeeping genes.

2.5. Western blotting

Cells were washed, scraped in PBS and lysed in 10 mM Tris-HCl, pH 7.3, 1 mM MgCl₂, 0.25% SDS, 1% Triton X-100 in the presence of protease inhibitors (10 μ g/ml leupeptin, 10 μ g/ml aprotinin, and 10 μ g/ml 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). For western blot analysis, extracts containing 20–50 μ g of total protein were reduced with 2-mercaptoethanol in NuPAGE loading buffer without boiling, electrophoresed on 10% NuPAGE Trisglycine gels (Novex, San Diego), and transferred to nitrocellulose membranes. For detection of ABCA1 we used a mouse primary antibody raised against human ABCA1 protein. For detection of Apo-E the membranes were processed with a goat primary antibody raised against human Apo-E. Membranes were then incubated with a goat anti-mouse IgG or donkey anti-goat conjugated to horseradish peroxidase for ABCA1 and APOE respectively and processed for visualization by enhanced chemiluminescence ECL Plus (Amersham Biosciences). Western blotting with anti-Actin antibody (Santa Cruz) was used for normalization. The relative intensities of the bands were quantified by densitometry.

2.6. Statistical analysis

All experiments were routinely performed in triplicate and repeated at least twice. The differences were evaluated by *t*-test or one way Anova (GrapPad) Prism depending on the number of variables and considered significant where $p < 0.05$.

3. Results

3.1. Identification of Lansoprazole as an LXR activator in a reporter transactivation assay

To identify LXR activators/agonists we used a cell based luciferase transactivation assay and screened a library of ~860 commercially available compounds – small molecules and off-patent drugs. The assay was performed using Cos7 cells transfected with a Firefly luciferase reporter gene containing 3 copies of LXR response element, an expression plasmid for LXR α (pCMX-hLXR α) or LXR β (pCMX-hLXR β) each of them expressing the full length canonical sequence of the corresponding LXR receptor including DNA and Ligand binding domains, and a Renilla luciferase reporter for normalization.

To validate that Lansoprazole can activate LXR the transactivation assay with Cos-7 cells was repeated using both LXR α and LXR β expression plasmids and treatment concentrations ranging from 0.1 μ M to 50 μ M. Figure 1A shows the Firefly luciferase activity in response to increasing concentrations of Lansoprazole (Cpd L) in cells transfected with LXR α and demonstrates that 5 and 10 μ M had the maximum response of 2-fold increase over the vehicle treatment. This activity was equal to 80% of EC50 response to T0 treatment using the same plasmid combinations. Figure 1B demonstrates the increase in luciferase activity over the vehicle treatment in response to increasing concentrations of Lansoprazole in cells transfected with plasmid expressing the ligand binding domain of LXR β . A maximum response of 3-fold over vehicle was observed at 50 μ M of Lansoprazole. At 10 μ M the compound activated LXR β to 120% of the T0 EC50 response. From these transactivation assays it was concluded that Lansoprazole can activate LXRs and is a candidate for further testing in cell lines to examine its ability to act as an LXR agonist in vitro on endogenous levels of LXRs.

3.2. Proton pump inhibitors upregulate ABCA1 expression in human H4 cells

To examine if Lansoprazole mediated activation of LXR depends on its chemical structure or is a result of its recognized biological effects (e.g. covalent binding to and inhibition of gastric

H,K-ATPase) we tested 3 other compounds which belong to the same group of PPIs – Omeprazole, Pantoprazole and Esomeprazole, all strong PPIs and formally considered derivatives of the parent compound Omeprazole.

To examine if they upregulate LXR-target gene expression, H4 cells were treated with each of the compounds and ABCA1 mRNA expression level was analyzed by RT-QPCR. As shown on Figure 2, Lansoprazole had the strongest effect and Pantoprazole was similarly active. Omeprazole upregulated ABCA1 mRNA expression to 1.5 fold ($p < 0.05$) and Esomeprazole was inactive. Thus, the activity of PPIs depends on their structure, as close structural analogs express similar activity, and at least one very potent PPI in clinical use does not upregulate ABCA1 transcription.

3.3. Lansoprazole upregulates ABCA1 transcription in Human brain derived cell lines in a concentration dependent manner

To examine if the effect of Lansoprazole is dose – dependent, cells from four different glial-derived human cell lines were treated with increasing concentrations for 24 hrs. These cell lines are with known *APOE* genotype/allele presentation (Figure 3) and have been frequently used by other groups in LXR activation assays [13,14]. The reference compound T0 and vehicle were positive and negative controls respectively. A concentration-dependent response determined by measuring ABCA1 mRNA expression was detected in cells from all cell lines, regardless of the *APOE* genotype.

CCF cells were treated with 20 μ M Lansoprazole for 48 h and controls with veh or T0 (1 μ M). (A) WB for ABCA1; B and C, cellular (B) and secreted Apo-E (C) protein level was measured by WB using anti-human Apo-E antibody. Data are results of two experiments in quadruplicate. ABCA1 and Apo-E levels were normalized on b-actin and presented as fold of veh treatment. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ versus vehicle.

3.4. Transcriptional upregulation by Lansoprazole is LXR dependent

To test if the effects of Lansoprazole are LXR dependent we used primary astrocytes derived from WT, *LXR α ^{-/-}*, *LXR β ^{-/-}* and *LXR α/β -dco* (double knockout) newborn pups. Cells were treated with 20 μ M Of Lansoprazole For 48 Hrs, control cells with veh or T0. We analyzed mRNA expression levels of two LXR-target genes with substantial impact on AD, namely *Abca1* and *ApoE*. As shown on Figure 4A, Lansoprazole increased *Abca1* mRNA expression in WT, *LXR α ^{-/-}* and *LXR β ^{-/-}* astrocytes but not in *LXR α/β -dco* cells proving that the effect is LXR-dependent. Figure 4B demonstrates that the same is true for *ApoE* mRNA expression level. *ApoE* mRNA expression in *LXR α/β -dco* cells was too low (the amplification reached the detection level later than 37th cycle).

3.5. Lansoprazole upregulates Abca1 and Apo-E protein levels in astrocytes and CCF cells

We also examined if Lansoprazole induces upregulation of intracellular Abca1 and Apo-E protein levels, as well as the secretion of Apo-E by primary glial cells and human astrogloma CCF cell line. First, primary astrocytes derived from WT mice were treated for 48 hrs with 20 μ M of Lansoprazole. The western blots on Figure 5 demonstrate that Abca1 and Apo-E protein levels were increased more than 2-fold, which corresponded to 1.5 fold increase in the amount of secreted Apo-E in the medium (Figure 5C). Second, we examined if Lansoprazole affects ABCA1 and Apo-E protein levels in CCF cells. ABCA1 and intracellular Apo-E protein levels were measured by WB using anti-human ABCA1 and Apo-E antibodies. As shown on Figure 6A and B, the intracellular levels of ABCA1 and Apo-E were increased more than 3-fold ($p < 0.05$). Accordingly, there was more than 3-fold increase in the level of secreted APOE in the media (Figure 6C).

4. Discussion

The gastric H,K-ATPase (gastric proton pump) is the primary target for the treatment of the acid-reflux disease. The benzimidazole derivative Lansoprazole is a PPI that consists of two heterocyclic moieties - a pyridine and benzimidazole moiety, linked via a methylsulfonyl group (Figure 2). All PPIs once delivered to the parietal cell act as prodrugs. Intracellularly they accumulate in the secretory canaliculus of the parietal cell and after sequential two step protonation of the pyridine and benzimidazole undergo chemical rearrangement, convert into sulfenic acid and become less membrane permeable. The dehydrated form of the sulfenic acid is the active form of the drug that reacts with cysteine sulfhydrylates of the proton pump thus inhibiting its activity [15]. The activation of PPIs in a particular chemical environment means that their pharmacological activity is highly specific, with a very large margin of safety given the pH of activation (≤ 2.0).

In a cell based reporter transactivation assay, we identified Lansoprazole as an LXR activator, presumably due to its interaction with the LXR ligand binding domain. The results of secondary screenings and additional *in vitro* assays using established cell lines, as well as primary cells from WT and LXR knockout mice demonstrated that Lansoprazole acts as an LXR agonist. These assays confirmed that Lansoprazole can activate endogenous LXR in a concentration dependent manner followed by transcriptional up-regulation of LXR responsive genes and increased levels of their proteins. These proteins are considered critical in various steps of atherosclerosis and AD pathogenesis. LXR mediated up-regulation of ABCA1 expression and function is a promising target for treatment of atherosclerosis, but is also of significant interest towards understanding the tightly connected ABCA1 and APOE function and their role for development and progression of Late Onset Alzheimer's disease. It is now well known that A β binds and forms complexes with Apo-E, ApoA-I, other apolipoproteins and HDL *in vitro* and *in vivo* [16,17,18,19,20]. A β binding to lipid-poor APOE, however, facilitates its aggregation, while it's binding to lipidated Apo-E, as well as ApoA-I, has an inhibitory effect on aggregation [18,21]. The critical point is that the lipidation of Apo-E in the brain depends entirely on the presence of functional ABCA1. In the absence of functional ABCA1 the cholesterol/phospholipid efflux is impaired resulting in poorly lipidated Apo-E particles which are unstable and rapidly degraded [22,23]. In contrast, treatment with LXR ligands increases the expression of Abca1, cholesterol efflux, generation of lipid-rich Apo-E lipoproteins and thus, the stability and the half-life of Apo-E [24]. Thus, the formation of Apo-E lipid complexes, which is mediated through functional Abca1, has a modulatory/inhibitory effect on A β aggregation and pharmacologically active compounds that facilitate this process will have a therapeutic application in AD [25,8]. While the performance of a set of experiments that would prove Lansoprazole treatment could lead to these effects *in vitro* and/or *in vivo* was beyond the scope of this study, it is worth mentioning that until now in all published studies treatment with LXR ligands has consistently, and in a highly reproducible manner, shown an inhibitory effect on A β deposition and possibly facilitated clearance [9,24,10].

The LXR mediated upregulation of mRNA and protein levels of ABCA1 and APOE in the context of all assays as presented, including those with primary cells from LXR knockout mice strongly suggests that the application of Lansoprazole may also lead to other effects of activated LXR and in particular inhibition of inflammatory reactions elicited in response to bacterial lipopolysaccharide (LPS) or A β . In fact the antiinflammatory effect of Lansoprazole has been known for a long time now and is considered when a decision has to be made for clinical use of Lansoprazole. Moreover, in a very recent study McGeer's group demonstrated that PPIs Lansoprazole and Omeprazole exert antiinflammatory effects and inhibit neurotoxicity of supernatants from human microglia and THP-1 cells stimulated with LPS combined with IFN- γ [26]. Both PPIs (and more efficiently in combination with S-ibuprofen) significantly reduced the TNF- α secretion from stimulated THP-1 cells in a concentration dependent manner. Like

in other similar studies with stimulated cells from the monocytic lineage, the authors did not suggest a plausible mechanistic explanation of their findings. However, considering the importance of microglia activation and inflammatory reactions in general, they suggested that the administration of PPIs combined with NSAIDs may be effective in the treatment of AD and other neuroinflammatory disorders. The results of our study suggest a possibility for LXR mediated inhibition of NF- κ B controlled inflammatory response. The antiinflammatory effect of PPIs mediated by NF- κ B pathway inhibition in cells where the primary biological target of PPIs is missing suggests that other than in parietal cells, the pharmacological activities of PPIs when found, are linked to biological processes and molecules unrelated to proton pumps. [27,28].

A critical issue related to the pharmacological effects of PPIs, as presented in this report, are the possibilities that they are mediated by the primary biological target of the drugs - the gastric proton pump, or through the activation of other nuclear receptors. In addition to the specificity of the transactivation screening assay used to identify Lansoprazole as an LXR activator, we consider the first possibility very unlikely primarily due to the cell and tissue specific expression of the proton pumps and their subunits. The gastric H-K-ATPase is a member of X-K-ATPase family that also includes Na-K-ATPase and non-gastric H-K-ATPase. These ion pumps are composed of two subunits and are located in plasma membranes where they function as cation pumps that transport K⁺ into the cell in exchange for Na⁺ and/or H⁺. The catalytic α -subunits of X-K-ATPases (6 isoforms) are large polytopic proteins (100 kDa) that perform ATP hydrolysis and ion translocation. The β -subunit (five genes have been identified coding for 5 isoforms) plays a crucial role and is indispensable for the structural and functional maturation of the functionally active X-K-ATPase molecule and modulation of the enzymes' affinities for cations. In this respect, **1)** while the expression of the α subunit in the choroid plexus has been documented in rats and rabbit [29,30], it is difficult to reconcile Lansoprazole binding to functionally inactive α -subunit in a cell culture system derived of brain structures with dissected and separated away choroid plexus (see Materials & Methods) and up-regulation of mouse *Abca1* and *ApoE* mRNA; **2)** on the other side, while it has been suggested that the β -subunit may have a role in gene expression in association with transcriptional coregulators, such a function has been restricted to perinatal myocytes [31]; and finally **3)** even though a functional member of X-K-ATPase family has been identified in microglia [32], it is very unlikely to expect a separation of the β -subunit and a role in transcriptional up-regulation following an inhibition by PPIs.

In some studies it has been demonstrated that the synthetic LXR agonist T0 can activate the nuclear receptor Farnesoid X Receptor (FXR) [33]. However, our data rule out the possibility that Lansoprazole functions as an FXR agonist in the biological context examined by this study. Specifically, Lansoprazole was unable to upregulate any of the target genes, e.g. *Abca1* and *ApoE*, in LXR double knockout cells, suggesting that its effects on the transcription of these genes are mediated through LXRs. Nevertheless, and even though our data point to an LXR-dependent effect of Lansoprazole on genes like *Abca1* and *ApoE* in brain cells, we cannot rule out that it has additional biological activities in the brain, given its known actions as a proton pump inhibitor.

There are other important aspects of LXR agonist-like activity of Lansoprazole stemmed from the results of this study, supporting the idea that Lansoprazole is a specific LXR agonist and it's activity is separate from the pharmacological activities related to the inhibition of the gastric proton pump:

First, while Lansoprazole is the strongest LXR activator in a group of PPIs that are structurally very similar, at least one of them – the magnesium salt of Esomeprazole does not activate LXR. A reasonable explanation for the lack of LXR agonist like activity of this compound could be

the size of the molecule and lack of binding to the ligand binding domain of LXR. These results open the possibility for chemical optimization studies in the future and synthesis of novel LXR agonists. **Second**, the cell type specific activity of Lansoprazole found in established astrocytoma cell lines of human origin and H4 cells may have important reflection on the therapeutic potential and use of Lansoprazole, or its derivatives, in neurodegenerative disorders. Similarly to the cell type specific effects of synthetic LXR ligands, this can be either a result of cell type specific and different representation of the two receptors or cell type specific differences in the release of co-repressors following receptor-ligand binding and thus different level of transcriptional regulation of certain genes. Support for this conclusion comes also from the differences in transcriptional regulation of Abca1 in response to Lansoprazole found in $LXR\alpha^{-/-}$ and $LXR\beta^{-/-}$ primary cells (Figure 4).

In summary, in this preliminary report we show that Lansoprazole, a FDA approved widely used and available over the counter inhibitor of gastric proton pump, acts as an LXR agonist and activates LXR in mouse and human cell types. The final effect is a transcriptional up-regulation of responsive genes with critical role in atherosclerosis and AD. The results establish a unique perspective of new class of remarkably safe LXR activators and demand a new set of investigations (including chemical optimization) regarding their effect on AD, atherosclerosis and neuroinflammatory diseases.

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Figure 1. Lansoprazole induces LXR α/β mediated transcriptional activity in a transactivation assay

The assay was performed using Cos7 cells transfected with a Firefly luciferase reporter gene containing 3 copies of LXR response element, an expression plasmid for LXR α (A) or LXR β (B), and a Renilla luciferase reporter for normalization. LXR α/β dual agonist T0901317 (Cpd T0) with EC₅₀=25nM was used for comparison. **A.** Lansoprazole (Cpd L) at 5 μ M activates LXR α to 80% of the T0 EC₅₀. **B.** At 10 μ M Lansoprazole activates LXR β to 120% of T0 EC₅₀. Means are fold of veh. *, p<0.05, **, p< 0.01 versus veh

Figure 2. PPI structural analogs increase *ABCA1* mRNA expression in H4 human neuroglioma cell line

Cells were treated with 20 μ M of Lansoprazole (Cpd L), Pantoprazole (Cpd P), Esomeprazole (Cpd E) and Omeprazole (Cpd O) for 24 h and *ABCA1* mRNA expression was analyzed by QPCR. Controls were treated with veh and T0 (25 nM) for the same time. Means are presented as fold of veh. *, $p < 0.05$, ***, $p < 0.001$ versus veh.

Figure 3. Lansoprazole upregulates *ABCA1* expression in human glial-derived cell lines in a concentration-dependent manner

Cells were treated as indicated for 24 h and *ABCA1* mRNA expression was analyzed by RT-QPCR. **A.** H4 neuroglioma cell line. **B.** U-87 (astrocytoma, APOE3/3). **C.** CCF (astrocytoma, APOE3/4) **D.** U-118 (astrocytoma, APOE2/4). Means are presented as fold of vehicle (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$)

Figure 4. Lansoprazole upregulates LXR target genes expression in WT but not in LXR α / β -dko primary glial cells

Primary astrocytes derived from WT, LXR α ^{-/-}, LXR β ^{-/-} and LXR α / β -dko newborn pups were treated with 20 μ M Cpd L for 48 h. Control cells were treated with veh or T0 and *Abca1* and *Apoe* mRNA expression were determined by RT-QPCR. **A.** Increased expression of *Abca1* in all cell types but LXR α / β -dko; **B.** *Apoe* mRNA is upregulated in WT and LXR β ^{-/-} astrocytes (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 5. Protein levels of Abca1 and Apo-E are increased in response to Lansoprazole treatment
 Primary astrocytes derived from WT newborn pups were treated with 20 μ M Cpd L for 48 hrs. Control cells were treated with veh or T0 (1 μ M) and Abca1 (**A**), cell associated Apo-E (**B**) and secreted Apo-E (**C**) were determined by western blot as described in the text (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Figure 6. Lansoprazole increases ABCA1 and Apo-E intracellular protein level, and Apo-E secretion from CCF cells expressing *APOE3/4* alleles

CCF cells were treated with 20 μ M Lansoprazole for 48 h and controls with veh or T0 (1 μ M). ABCA1 (A) and Apo- E (B and C) protein level was measured by WB using anti-human Apo-E antibody. Data are results of two experiments in quadruplicate. ABCA1 and Apo-E levels were normalized on β -actin and presented as fold of veh treatment. *, $p < 0.05$, **, $p < 0.01$ versus vehicle.