A lipidomics analysis of influenza infection and PPARγ activation

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Abstract

While vaccines are a reliable preventative of influenza infection, useful therapeutics for treatment of the disease would be key to minimizing the impact of a major pandemic. In this study we have focused on the use of the anti-inflammatory compounds Abscisic Acid (ABA) and NSC61610 (NSC) in the presence of PPARγ to reduce lung inflammation and damage. The mechanisms by which these therapeutics activate PPARγ have not yet been identified, but a promising avenue is through the modification of known lipid mediators produced during Arachidonic Acid metabolism. In this study, we present a lipidomics analysis of influenza infected C57BL6 Wild Type mice treated with ABA and NSC. Our data demonstrate a rise in lipid mediator levels due to infection as well as in the presence of ABA, and to a lesser extent, NSC. Quantitative Real-Time PCR was performed in order to link the expression of lipid metabolic regulators to observed changes in lipid levels. In addition to this, molecular docking studies of PPARγ were performed to compare predicted binding affinities of previously identified ligands to other eicosanoids. The results of the simulations rank many previously identified PPARγ ligands below other metabolites, indicating that studies analyzing for the presence of previously unexplored eicosanoids may be fruitful in identifying new lipid mediators involved in anti-inflammatory pathways.

Introduction

Recent threats of Influenza pandemics have demonstrated the inability of the current vaccine-based system to quickly respond to the ever-changing and highly adaptable strains of the virus. The current system for vaccine development takes up to six months in order to produce an effective vaccine. The usefulness of identifying novel therapeutics for reducing the severity of an influenza infection becomes apparent. Since the innate immune response and resultant lung inflammation are the most damaging results of Influenza infection, it is appropriate to focus on immunomodulatory compounds as possible therapeutics. ABA and NSC have been identified as useful anti-inflammatory agents, however, future work in this area is contingent upon a more comprehensive understanding of the pathways by which these drugs modulate the immune response. This type of research would also hopefully lead to the discovery and development of other possible treatments, as well as a more complete understanding of the immune response to Influenza.

It has been found that both ABA and NSC only produce therapeutic effects in the presence of PPARγ, a gene known to regulate inflammatory pathways during infection. However, it has also been demonstrated by Lu, et al that these compounds may not directly interact with PPARγ binding proteins. At this point in time, it is unknown whether the effect is through an upstream or downstream pathway of PPARγ activation. Promising is the known ability of various lipid mediators belonging to the eicosanoid family to bind and modulate PPARγ. Eicosanoids are inflammatory mediators that contribute to the initial phases of the inflammatory response but more importantly are required for the resolution phase. They are generated from the metabolism of arachidonic aiod through the lipoxygenase and lipoxygenase pathways (figure 1). In this study, we examine the effects of the anti-inflammatory compounds ABA and NSC on eicosanoid pathways and hope to shine some light on the way in which these drugs activate PPARγ. Lung tissue of Influenza A/California/09 infected mice was collected and analyzed every other day after infection for the presence of lipid mediators. In addition, a molecular docking model was generated in order to compare the binding potential of the eicosanoid class as a whole to known PPARγ endogenous ligands, thus aiding in the identification of future candidates for immune modulation. From this data we hope to develop a basis for which to build a larger model for the regulatory role of PPARγ on the eicosanoid pathway and the effects of ABA and NSC on these pathways.

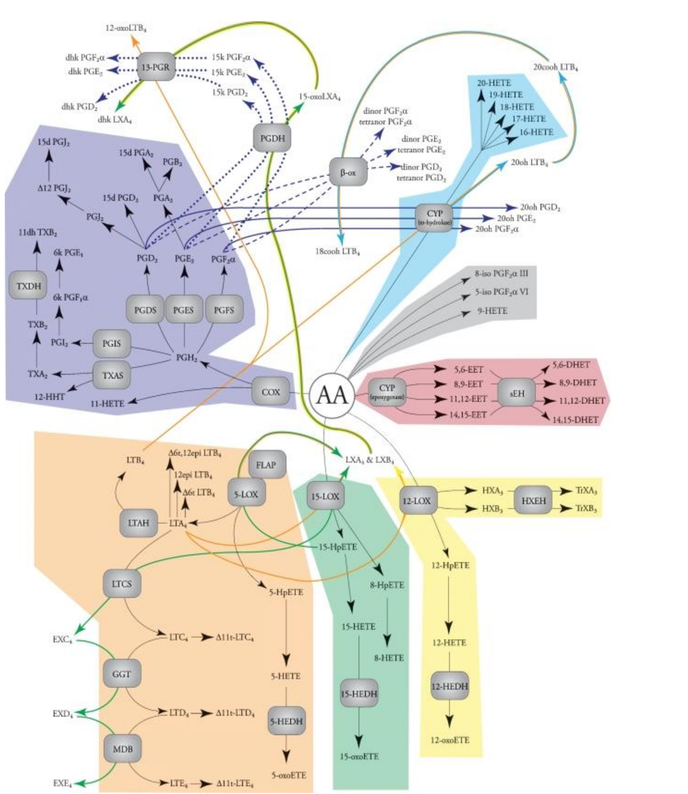

Fig.1: Schematic representation of the generation of eicosanoids from the metabolism of arachidonic acid through the cyclooxygenase and lipoxygenase pathways (from Buczynski et al., PMID:19244215).

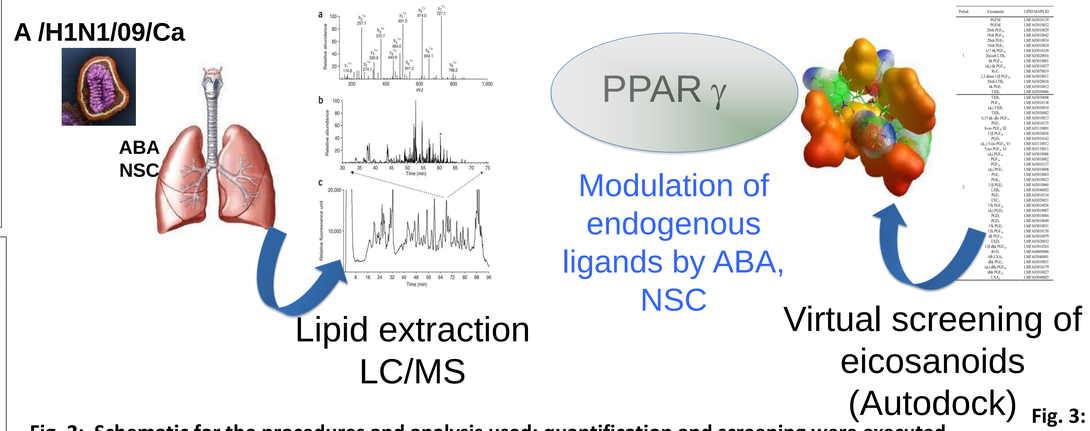

Fig. 2: Schematic for the procedures and analysis used; quantification and screening were executed together in order to gain a more complete picture of overall system interactions.

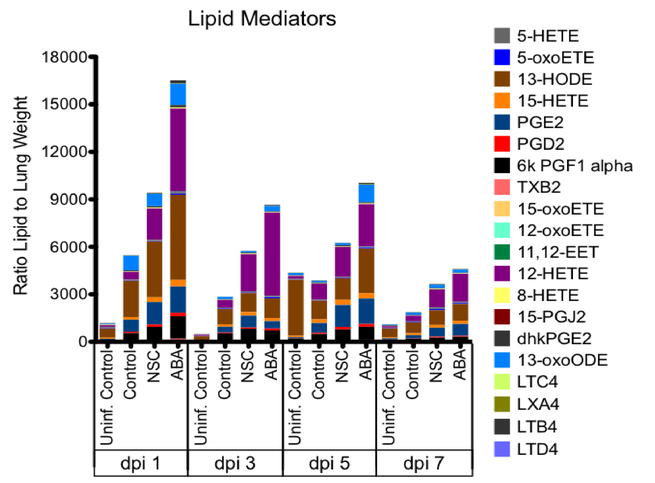

Fig. 3: Total lipid mediator levels for each experimental group on all days post infection. Total lipid levels are broken down by color for a direct comparison of levels between lipids.

Cyclooxygenase Pathway

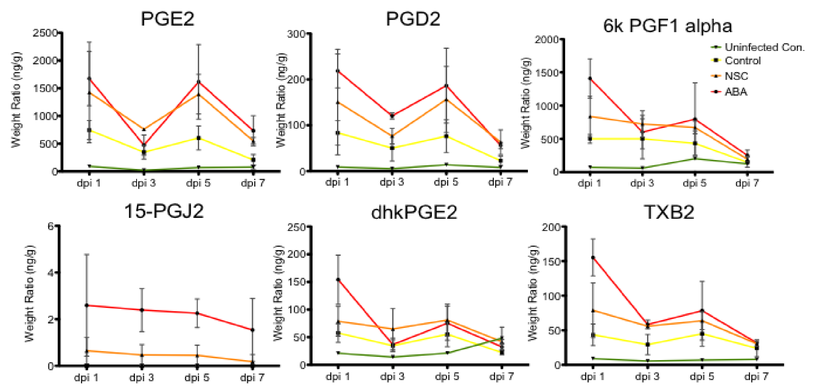

Fig. 4: Levels of quantified metabolites along the cyclooxygenase pathway are shown. Note the high levels of 15d-PGJ2 in ABA treated mice, compared to NSC and infected control mice at all time points. Lipid extraction and subsequent mass spectrometry were based on a modified procedures from Blaho, et al, (PMID: 19487688).

Lipoxygenase Pathway

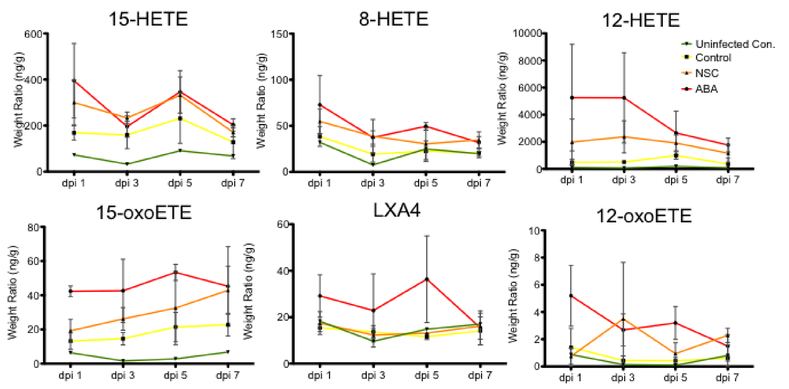

Fig. 5: Levels of quantified metabolites along the lipoxygenase pathway are shown. Note the high levels of LXA4 in ABA treated mice, compared to NSC and infected control mice at all but dpi7.

Quantified Eicosanoids

Name	Mode	Affinity	RMSD_lb	RMSD_ub
LTC4	1	-8	0	0
LTD4	1	-7.4	0	0
PGE2	1	-7.2	0	0
15d-PGJ2	1	-7.1	0	0
6k PGF1 alpha	1	-7	0	0
15-oxoETE	1	-7	0	0
LTB4	1	-7	0	0
dhkPGE2	1	-7	0	0
TXB2	1	-6.9	0	0
LXA4	1	-6.9	0	0
PGD2	1	-6.9	0	0
11,12-EET	1	-6.8	0	0
15-HETE	1	-6.8	0	0
12-HETE	1	-6.7	0	0
5-HETE	1	-6.6	0	0
8-HETE	1	-6.6	0	0
5-oxoETE	1	-6.6	0	0
12-oxoETE	1	-6.5	0	0
13-oxoODE	1	-6.4	0	0
13-HODE	1	-6.1	0	0

Table 1: Binding affinities for quantified eicosanoids. More negative affinities indicate lower potential energy binding, and thus higher affinity ligands. Molecular docking was performed with Autodock, focusing on the ligand binding domain of PPAR-γ.

Top 20 of All Screened Eicosanoids

Name	Mode	Affinity	RMSD_lb	RMSD_ub
Δ11t LTC4	1	-8	0	0
bicyclo PGE2	1	-8	0	0
LTC4	1	-8	0	0
LTC4	2	-7.9	3.026	7.983
LTC4	3	-7.8	2.828	7.284
LTC4	4	-7.8	2.934	8.124
10s, 17s-DiHDoHE	1	-7.7	0	0
8-iso PGF2a III	1	-7.7	0	0
bicyclo PGE2	2	-7.7	3.904	6.066
Δ17 6k PGF1α	1	-7.7	0	0
PD1	1	-7.6	0	0
10s, 17s-DiHDoHE	2	-7.6	1.309	1.849
8-iso PGF2a III	2	-7.6	3.365	7.836
Δ11t LTC4	2	-7.6	1.349	2.438
bicyclo PGE2	3	-7.6	3.263	5.138
Δ17 6k PGF1α	2	-7.6	3.613	5.871
Δ17 6k PGF1α	3	-7.6	1.37	2.216
LTC4	5	-7.6	1.768	2.781
LTC4	6	-7.6	2.726	8.751
8-iso PGF2a III	3	-7.5	2.341	3.771

Table 2: Binding affinities for the top twenty conformations of the 97 eicosanoids analyzed. Mode indicates the pose or relative position of the lipid being docked, with 10 poses for each eicosanoid tested. Molecular docking in Autodock was focused on the ligand binding domain of PPAR-γ. Lipid structures in .sdf format were obtained from PubChem compound[13], and the Human Metabolome Databas.

Conclusion/Key Points

- In all infected groups, lipid levels were increased throughout infection, as expected. With most metabolites, ABA and NSC increased levels throughout the study above those for infected control mice, with ABA treatment increasing eicosanoid levels even higher than treatment with NSC.
- Exceptions to this were LTD4 and LTC4, which showed nearly undetectable levels in all groups throughout the study.
- Lipoxin A4 and 15-deoxy-prostaglandin-J2 showed a marked difference between ABA the other two infected control groups, with levels in ABA treated mice much higher and those in other infected mice only slightly above levels for uninfected mice.
- This could explain the increased recovery time in spite of decreased weight loss characteristic of NSC treated mice, but not seen in ABA treated mice. ABA seems to increase not only pro-inflammatory, but also pro-resolution metabolites above levels induced through treatment with NSC.
- With increased lipid metabolism and a close link between the therapeutic effects of ABA, NSC and PPAR-γ, it would be expected that this and other genes involved in lipid metabolism would increase for infected and treated mice. PPAR-γ and Angiopoietin-Like4 gene expression seemed to increase in all infected mice, but there were no clear differences between treated and non-treated mice or between ABA and NSC treated mice.
- Only LTC4 was within the top twenty binding affinities for all eicosanoids screened, however, this molecule was nearly undetectable in all experimental groups.
- 15d-PGJ2 PGE2 were two of the highest affinity quantified eicosanoids, and are on the same pathway of the cascade as Δ17-6-keto-PGF1α and bicyclo-PGE2, which both bound with very high affinity during the overall eicosanoid screening.
- Screenings accurately rank many metabolites, but cannot “fine-tune” rankings due to an inability to model key covalent interactions with Cys285 (Waku, et al, 2010).

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