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Molecular Predictors of Anakinra Treatment Success in Heart Failure Patients with Reduced Ejection Fraction

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Introduction

Heart failure with reduced ejection fraction (HFrEF) is a cardiovascular disease distinguished by low-grade chronic inflammation1. Literature suggests that disturbed metabolic pathways within cardiomyocyte mitochondria play a significant role in systemic wide inflammation seen in HFrEF. These mechanisms are linked to the activation of cytokine receptor Interleukin-1 (IL-1). This has led to the testing of repurposed drug therapies such as Kineret (Anakinra) that inhibits the activation of IL-1. To measure the outcomes of Anakinra, investigators have used cardiopulmonary exercise tests (CPET) and high-sensitivity CRP (hs-CRP)4. Clinical trials using these markers have demonstrated promise for reducing inflammation after 12 weeks of administration5. Little is known though as to how Anakinra impacts the heart’s metabolic pathways underlying inflammation. Therefore we have taken a hypothesis-driven approach to characterize the HFrEF metabolic pathways affected by Anakinra for different therapeutic durations for durations of 2 weeks (Ank-2) and for 12 weeks (Ank-12).

Methods

Post-hoc analysis was performed on 49 patients with reduced ejection fraction, mostly African American (79.6%) and male (75.5%) from the VCU REDHART study. Lipids from HFrEF patients’ plasma and serum were quantified via a SciX TripleTOF 6600 mass spectrometry paired to an Agilent 1290 binary chromatograph (LC). An acquly UPLC CSH C18 column (100 x 2.1 mm; 1.7µm) was used with the LC. Metabolites were acquired by a Leco Pegasus IV TOF mass spectrometer coupled to an Agilent 6890 gas chromatograph (GC) equipped with a Gerstel automatic liner exchange system (ALEX) that included a multipurpose sample (MPS2) dual rail, and a Gerstel CIS cold injection system. Metabolic data were filtered to exclude exogenous metabolites identified as medications or products of gut microbiota. Data were normalized, filtered using IOR, log transformed, and pareto scaled. Regularized Linear Discriminant Analysis (LDA) was used to discover any meaningful group separations based on acquired metabolites and lipids. Metabolic Pathway Analysis selected enriched metabolites that had been annotated on Kyoto Encyclopedia of Genes and Genomes (KEGG). A multivariate analysis generated a biologically valid testable hypothesis on the metabolic pathways participating in cardiac energy production. Effects are represented by color (green = low, red= high).

Results

Figure 1: Baseline and treatment groups’ separation obtained from analytes. Regularized Linear Discriminant Analysis (r-LDA) demonstrated group separation after stepwise selection of 30 lipids and metabolites. Selected predictors explain the group’s differences and spatial separation.

Pathway Impact

Figure 2: Metabolic pathways associated with Anakinra treatment. Ellipse diameter indicated the magnitude of the impact on the pathway expression. All pathways had a statistically significant impact in the enrichment analysis (p<0.05). Ellipse color represent p-value (yellow = low, red=high). Only metabolic pathways with p-value p<0.05 are labeled.

Figure 3: Comparison of significant CRP and acylcarnitine modulations from baseline and treatment groups. Treatment groups were Anakinra 2 weeks, Anakinra 12 weeks and placebo 12 weeks. All values (y-axis) are normalized. Analysis of variance (ANOVA) retrieved p≤0.05 per analyte.

Figure 4: Anakinra 12-weeks yields benefits compared to placebo. The derived hypothesis that Anakinra after 12 weeks would carry a different effect on the metabolome compared to placebo, tested with one-tailed t-test. All values (y-axis) are normalized. Metabolites statistically different (p<0.05) suggested metabolic changes following 12 weeks of Anakinra treatment.

Conclusions

Metabolites and lipids revealed inflammatory and energetic pathways responding to Ank-12. The TCA cycle and butanoyl metabolism were among those reported. Downstream IL-1, inflammatory hsCRP was expected to decrease. After Ank-12, hsCRP levels markedly decreased indicating reduced systemic inflammatory flux. Medium and long-chain acylcarnitines (Acyt C10:0 and C16:0) are elevated due to oxidative stress, an imbalance that leads to inflammation. It was hypothesized concentrations of Acyt would decrease after Ank-12; due to decreasing inflammation and restoring cardiac function re-enabling cardiac metabolism also improved with treatment indicated by decrease in hexose in response to treatment.

Table 1: Metabolomic functions affected by Anakinra following 12-weeks treatment. Hypothesis-driven test of Anakinra effect different from placebo after 12 weeks (one-tailed Student’s t-test). The hypothesis tested the downregulation of inflammatory markers, and upregulation of metabolites and pathways participating in cardiac energy production. Effects are represented by color (green = low, red= high).

<table>
<thead>
<tr>
<th>Metabolite Type</th>
<th>Treatment</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Ank-12-wks</td>
<td>p≤0.001</td>
</tr>
<tr>
<td>Acylcarnitine C16:0</td>
<td>Ank-12-wks</td>
<td>p≤0.05</td>
</tr>
</tbody>
</table>

References

6) High-deprivation of Western diet, low-grade chronic inflammation, and oxidative stress lead to the selective activation of the pro-inflammatory cytokine Interleukin-1 (IL-1β). The study of the metabolic changes induced by IL-1β provides insights into the mechanisms underlying inflammation and restoring cardiac function re-enabling cardiac energy production.

Limitations and Future Directions

Small sample size may have skewed the results of Anakinra effect towards representing an African American male subgroup. Baseline metabolic variability highly affected the power of the analysis in detecting metabolites post-treatment. Variability was overcome by a Random Forest outlier detection algorithm to select baseline values that did not significantly differ from the treatment groups. Nonetheless, meaningful changes resultant of IL-1 blockade were detected after 12 weeks of treatment. Future studies applying metabolomic techniques with more power could show Anakinra alterations earlier than 12 weeks.