#A-E-030 Phenotypic Profiling in Asthma and Allergy: Effect of prednisone on cell surface and soluble factors


Abstract

Concurrents are making oral anti-inflammatory drugs (prednisone, etc.) widely used in the treatment of asthma, but no evidence exists or has been suggested of the role they play in the treatment of asthma. This study was intended to examine the role of oral prednisone in the treatment of asthma by characterizing the leukocyte and lymphocyte subsets, the expression of immune mediators, and the levels of cytokines and soluble factors. A subset of normal controls was also characterized.

Methods

20 mg of prednisone was administered orally daily to 80 subjects (10 females and 60 males) with mild asthma (N = 37) or allergic asthma (N = 43). Blood samples were taken at baseline and 3 days post treatment. A new microvolume laser scanning cytometry (MLSC) was used to characterize white blood cells (WBC) and cell subsets, including T cells, B cells, NK cells, and monocytes. The relative cell surface expression of cell surface antigens, cell surface intensity, and HLA-DR expression levels were determined by flow cytometry. Since multiple tests were performed to analyze the data, a Bonferroni correction was used to determine statistical significance. Significant differences were determined by ANOVA or Student’s t-test. The differences were determined to be significant if the p-value was less than 0.005.

Results

Significant changes were observed in the number of leukocytes, lymphocytes, T cells, and monocytes. Eosinophils and basophils decreased significantly post treatment, while granulocytes increased significantly. The number of B cells and monocytes also increased significantly, while little change occurred in the number of T cells. Cytokines and chemokines also increased post treatment. CRP, decreased, however SAA, and IL-1sR2 increased post prednisone. The most significant changes were seen in MMP-3 plasma level (5-fold increase post prednisone). For soluble cytokine receptors, IL-6R decreased, while another acute phase protein, IL-6, increased post prednisone. The most significant change was seen in IL-6R levels.

Conclusions

The authors acknowledge Michael G. Derks for help in protocol development, Chellew for help in protocol development, Chellew for help in protocol development, and San Diego Institute of HealthCare Assessment, Inc. for providing assistance for this study. The authors thank Dr. Alan Helal and Dorothy Schneider from San Jose Clinical Research, Dr. Theodore Current/Research, and Dr. Harold Guy from Bay Area Research Center and Dr. Harold Guy from San Jose Institute of HealthCare Assessment, Inc. for providing assistance for this study. The authors thank Dr. Alan Helal and Dorothy Schneider from San Jose Clinical Research, Dr. Theodore Current/Research, and Dr. Harold Guy from Bay Area Research Center and Dr. Harold Guy from San Jose Institute of HealthCare Assessment, Inc. for providing assistance for this study. The authors thank Dr. Alan Helal and Dorothy Schneider from San Jose Clinical Research, Dr. Theodore Current/Research, and Dr. Harold Guy from Bay Area Research Center and Dr. Harold Guy from San Jose Institute of HealthCare Assessment, Inc. for providing assistance for this study.

Phenotypic Profiling in Asthma/Allergy

- Surrogate proof of principle study
- 80 Subjects
  - asthma/allergy (mild asthma)
  - Prednisone vs. placebo
    - oral, 20 mg, 2x/day
  - two blood samples per subject
  - 80 Subjects

Phenotypic Profiling in Asthma/Allergy

- Soluble factors: ELISA
  - Cytokines, chemokines, Ig, Acute phase proteins, MMPs, TIMPs, soluble receptors, soluble cell adhesion molecules.
- Cell populations and intensities: MLSC
  - Subsets T cells, B cells, NK cells, Granulocytes, Eosinophils, Monocytes
  - Markers of activation, adhesion, costimulation, naïve/memory cells, HLA class II, etc.

Microvolume Laser Scanning Cytometry (MLSC)

- Proprietary instrumentation, reagents, consumables and software for quantitation of cell populations in small volumes of whole or processed blood = integrated solution
- Uses combinations of fluorophore-tagged antibodies to cell surface markers
- 64 assays / 10 µL each = 200 populations
- Instrument control, data processing, uploading completely automated
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Prednisone affects levels of cells in blood (cell count)

<table>
<thead>
<tr>
<th>Population</th>
<th>Trend</th>
<th>Mean Before Drug (± SD)</th>
<th>Mean After Drug (± SD)</th>
<th>Adjusted P-value</th>
<th>Expected</th>
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<tbody>
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<td>WBC</td>
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Prednisone

- Significant differences post prednisone
  - Expected results based on literature
  - Adjusted p-value < 0.05
  - No differences observed for placebo group

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Conclusions

- Robust data collection: 160 samples, 725 variables
  - Big drug vs. placebo effect
  - Broad spectrum anti-inflammatory and immuno-suppressive agent
  - Significant differences observed in all types of bioanalytical measurements
    - absolute cell counts
    - relative cell types
    - cell surface antigen expression
    - soluble factors
  - Some disease group differences
    - Total IgE higher in allergic subjects
    - Eosinophils higher in asthmatic subjects

Acknowledgements

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- The authors thank Dr. Alan Helal and Dorothy Schneider from San Jose Clinical Research, Dr. Theodore Current/Franken, Chiefes from Bay Area Research Center and Dr. Harold Guy from San Diego Institute of HealthCare Assessment, Inc. for providing assistance for this study.
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