Oral L-arginine supplementation has been used in several studies to improve endothelium-dependent functions and may be also be involved in several pulmonary disorders and is confirmed by beneficial actions of NO synthase (NOS). L-arginine, a substrate for NOS, is synthesized by endothelial cells, airway epithelial cells, and immune cells and is converted to NO by NOS. NO may play a role in modulating airway inflammation and epithelial function.

Objectives:
- To assess the effect of L-arginine supplementation on NOx production from primary human airway epithelial cells exposed to Asymmetric Dimethylarginine (ADMA).
- To investigate the role of L-citrulline in NOx production from airway epithelial cells.
- To determine the potential therapeutic roles of L-arginine and L-citrulline in airway diseases, such as the obese-late-onset asthma phenotype, where lower L-arginine/ADMA ratios are associated with asthma.

Methods: Airway epithelial cells were obtained from bronchoscopic brushings of research volunteers with asthma and placed on air liquid interface (ALI). The media were made using DMEM L-arginine free medium. Primary human airway epithelial cells were cultured in ALI system with different concentrations of L-arginine or L-citrulline and ADMA for 10 days. The epithelial media were cultured at 37°C with 95% air and 5% CO2. The NOx production was measured by Griess reaction.

Results: Our data have shown ADMA decreases NOx levels in a dose-dependent manner in primary human airway epithelial cells: (NOx)basal = 65 uM±36 uM; (NOx)ADMA = 31 uM±11 uM; (NOx)ADMA/Arginine= 48 uM±17 uM; (NOx)ADMA/Citrulline= 50 uM±15 uM; (NOx)ADMA/Arginine= 20 uM±3 uM; (NOx)ADMA/Citrulline= 18.9±4.6 µM NOx (n=3).

Conclusions: An increase in the amount of substrate for NOS increases the formation of endogenous NO. L-arginine supplementation is able to rescue NOx production from the cells: (NOx)basal = 65 uM±36 uM; (NOx)ADMA = 31 uM±11 uM; (NOx)ADMA/Arginine= 48 uM±17 uM; (NOx)ADMA/Citrulline= 50 uM±15 uM; (NOx)ADMA/Arginine= 20 uM±3 uM; (NOx)ADMA/Citrulline= 18.9±4.6 µM NOx (n=3).

The Effect of L-arginine and L-citrulline on NOx production in primary human airway epithelial cells exposed to Asymmetric Dimethylarginine (ADMA)

Winnica DE, Baffi CW, Wenzel SE and Holguin F

Asthma Institute, Division of Pulmonary, Allergy & Critical Care Medicine. University of Pittsburgh, School of Medicine

Abstract

Background

Objectives

Materials & Methods

Figure 1. IL-13/INFγ stimulates the production of iNOS in airway epithelial cells in an air-liquid interface

Figure 2. Extracellular levels of L-arginine increase NOx production induced by IL-13/INFγ in airway epithelial cells.

Figure 3. ADMA decreased NOx levels in a dose-dependent manner.

Figure 4. Effect of L-citrulline 400µM on NOx production from human airway epithelial cells (n=4)

Figure 5. L-arginine supplementation is able to redirect iNOS toward NOx production (n=9)

Figure 6. Effect of L-arginine 100µM on NOx production from human airway epithelial cells (n=4)

Figure 7. ADMA decreased NOx levels in a dose-dependent manner.