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

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Abstract

Introduction: Nitric oxide (NO) plays an important part in the regulation of many physiological functions and may also be involved in several pulmonary diseases and is synthesized by different isoforms of NO synthase (NOS) from L-arginine. Asthma is characterized by bronchial hyper responsiveness, epithelial damage, inflammation and increased cytokine production. Recent evidence also suggests that the NOS inhibitor asymmetric dimethylarginine (ADMA), which is found in asthmatic airways and is inversely associated with exhaled NO, may play a role in obesity-related asthma.
Objectives: We determine: a) whether the addition of ADMA reduces NO-related metabolites in primary airway epithelial cells under IL-13/ IFNγ stimulation, b) supplementation of L-arginine/L-citrulline to cultures with high ADMA/iNOS expression is able to redirect iNOS towards NOx production.
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 media and L-arginine (100 µM) was re-added with each media change. At day 6, the epithelial cells were stimulated IL13 + IFNγ (both at 10 ng/ml). Subsequently, ADMA (100µM, 250µM, 500µM) was added to these conditions, 48 hours prior to cell harvest. To determine the effect of L-arginine/L-citruline on NOx production, additional amount of them were added at day 9 to airway epithelial cells culture in presence of 100µM ADMA.
Results: Our data have shown ADMA decrease NOx levels in a dose-dependent manner in primary human airway epithelial cells; No-ADMA:48,3±4,1µMNOx; ADMA-100µM:28,2±3,5µMNOx; ADMA-250µM:21,7±1,1 µMNOx and ADMA-500 µM:18,9±4,6 µMNOx (n=3).
Interestingly, supplementations of different concentrations of L-arginine or L-citrulline to the medium were able to rescue NOx production from the cells: (NOx)basal=65uM±36uM; (NOx)ADMA=31uM±11uM; (NOx)ADMA/suppArg= 48uM±17uM (n=6) and (NOx)basal=50uM±15uM; (NOx)ADMA=20uM±3uM; (NOx)ADMA/suppCitrulline= 32uM±4uM (n=2)
Conclusion: An increase in the amount of substrate for NOS increases the formation of endogenous NO. L-arginine and L-citrulline may have a therapeutic potential in diseases in which there is a defective production of NO.

Background

Oral L-arginine supplementation has been used in several studies to improve endothelium-dependent, nitric oxide (NO)-mediated vasodilation. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of all three isoforms of NOS and it is circulating at low µM concentrations in humans. The ratio of L-arginine over ADMA (arginine/ADMA ratio) is one determinant of NO production by NOS.
There is evidence showing that oral L-citrulline supplementation raises plasma L-arginine concentration and augments NO-dependent signaling in a dose-dependent manner. Discovery of L-citrulline as arginine and NO precursor, and the close linked between arginine and citrulline, has urged considerable interest and is being explored further.

Objectives

- Describe the effect of L-arginine/L-citrulline supplementation on human airways epithelial cells expose to Asymmetric Dimethylarginine (ADMA).

Materials & Methods

- Primary human airway epithelial cells obtained from bronchoscopy of research volunteers.
- Human airway epithelial cells cultured in air-liquid interface (ALI) system
- Cells cultured stimulated with IL-13/INFγ (10 ng/ml) at day 6 **(+).**
- Western blot to evaluate iNOS
- Assessments of Nitrite and Nitrate (NOx) by Griess reaction.
- DMEM L-arginine free medium.
- Regular BEMG medium (1200µM L-arginine)

Results

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

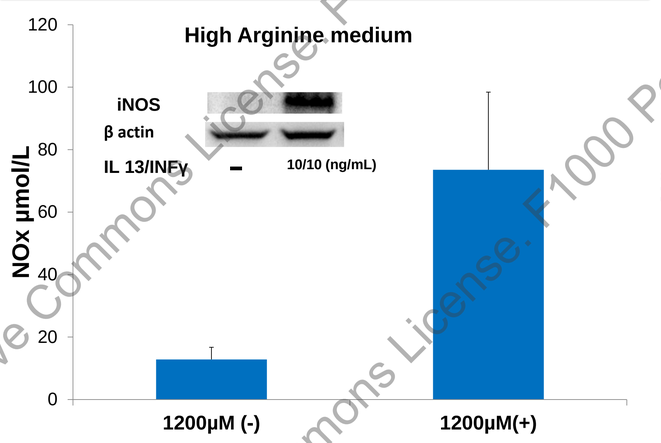


Figure 2. Extracellular leves of L-arginine increase NOx production induced by IL-13/INFγ in airway epithelial cells.

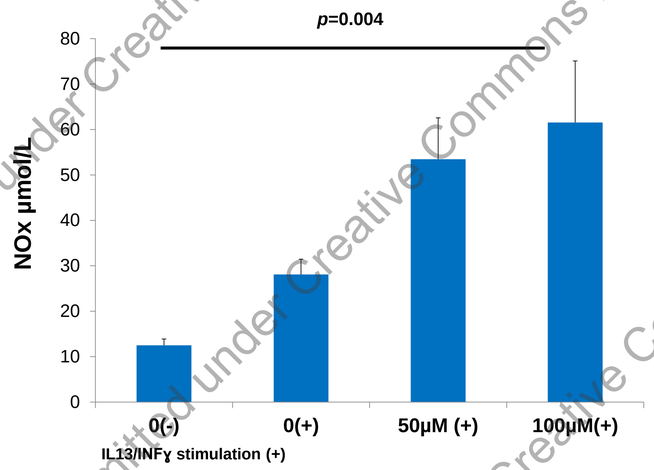


Figure 3. ADMA decreased NOx levels in a dose-dependent manner

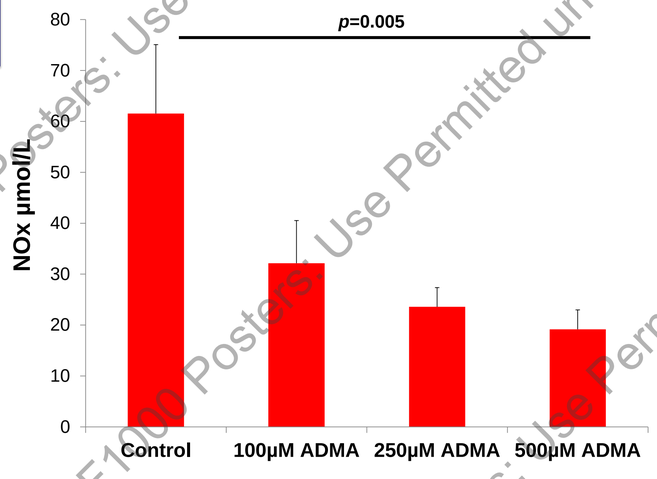


Figure 4. effect of L-arginine supplementation on NOx production from airway epithelial cells

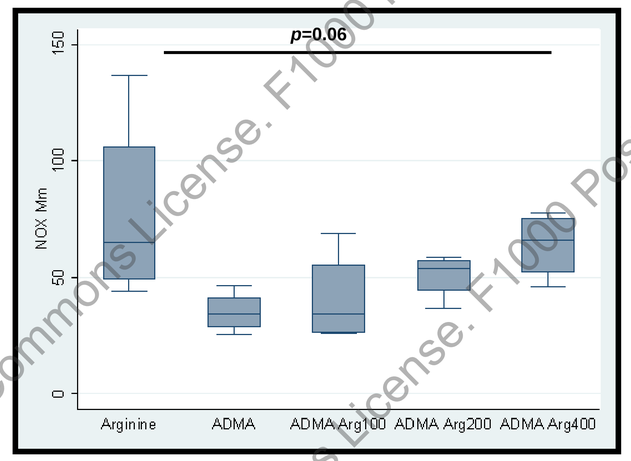


Figure 5. L-arginine supplementation is able to redirect iNOS toward NOx production (n=9)

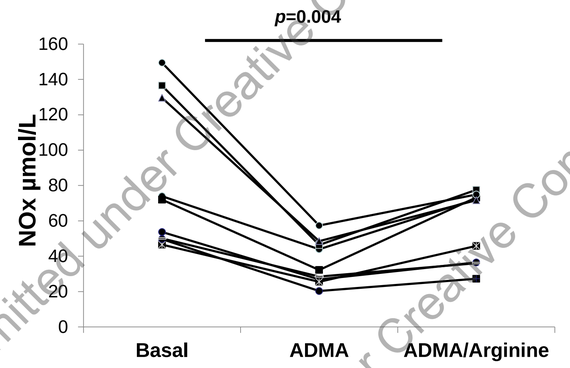
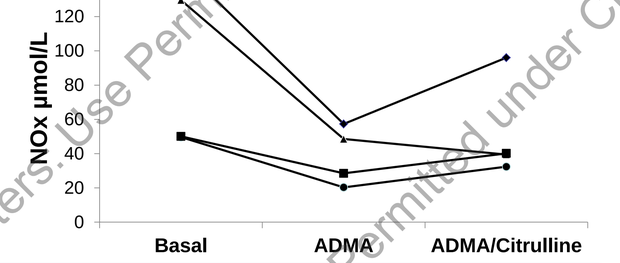


Figure 6. effect of L-citrulline 400µM on NOx production from human airway epithelial cells (n=4)



Conclusions

- L-arginine and L-citrulline supplementation are able to increase the formation of NOx (nitrites + nitrates) in human airways epithelial cells, despite treatment with the endogenous iNOS inhibitor Asymmetric Dimmethylargine (ADMA)

Speculations

- Further studies are nedeed to explore the potential therapeutic roles of L-arginine and L-citruline in airway diseases, such as the obese – late onset asthma phenotype, where lower L-arginine/ADMA ratios can lead to reduced airway NO bioavailability.