**Detection and Modulation of Nitric Oxide in an In Vitro Asthma Model**

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**ABSTRACT**

Asthma is an inflammatory disease of the lungs and airways that presents challenges for management in diverse populations in the United States. These challenges reflect the conclusion that asthma is not caused by a single agent and is not infectious, but rather represents a disregulated host response to a number of inflammatory stimuli. Moreover, unlike many acute diseases, asthma is marked by a pattern of recurrent exacerbations, which may have relatively benign early signs and symptoms but may then progress to life-threatening manifestations. An optimal strategy for management of asthmatic patients would benefit from a simple means of monitoring the early signs of lung inflammation that mark an asthma attack. Our team has been developing a tool for monitoring a well-recognized biomarker of lung inflammation that employs a portable sensor for nitric oxide, a gas in exhaled breath that is produced at levels that are correlated with severity of asthma. The device is intended to provide a readout of nitric oxide that can be recorded in a home setting by non-medically trained people and then transmitted remotely to professionals for rapid adjustment of therapeutic interventions for the asthmatic patient. This capacity to evaluate the extent of lung inflammation becomes so severe as to require medical intervention. Our detector should also be a useful tool in evaluating the efficacy of curcumin with improved bioavailability for future use in management of asthma.

**CURIUM AND ITS DERIVATIVES**

Curcumin (diferuloylmethane) is a low molecular weight molecule that has multiple anti-inflammatory activities, including inhibition of a large list of destructive proteolytic enzymes from mammalian and bacterial sources and down-regulation of intracellular inflammatory cascades. As a component of the spice turmeric, it has been consumed for centuries in large quantities without toxic side effects. The extremely hydrophobic nature of curcumin may be related to its biologic activities, but it presents a challenge for oral administration. The laboratory of our colleague, Dr. Francis Johnson, in the Department of Chemistry at Stony Brook, has been developing derivatives of curcumin that are more water-soluble than the parent compound bioavailable and therefore have a reduced loss of the inhibitory activity characteristic of curcumin itself. One of these chemically modified curcuminic, CMC 2.24, is being examined as a possible candidate for control of a variety of inflammatory conditions.

**CURIUM AND CMC 2.24**

Curcumin and CMC 2.24 are both inhibitors of a representative metalloproteinase, thermolysin, that is a very close homolog of the human enzyme Angiotensin-Converting Enzyme, a key regulator of blood pressure. The pattern of inhibition by both curcumin and CMC 2.24 indicates binding at multiple sites on the target enzyme to achieve IC50 values in the micromolar range without toxicity. The pattern of inhibition is similar to that observed for other bacterial and mammalian enzymes that contribute to inflammatory tissue damage.

**INHIBITION OF TARGETED ENZYMES BY CURCUMIN AND CMC 2.24**

**RECENT RESULTS**

We have shown that production of nitric oxide and nitrate ions derived from NO released by A549 cells can be increased by culturing the cells in the presence of a classic pro-inflammatory stimulus, bacteria lipopolysaccharide (LPS, endotoxin). When the cells are cultured in the presence of LPS and 20 μM curcumin, the levels of nitrate and nitrite ions are significantly lowered without any evidence of cytotoxicity. These results support the previous observations of Moon et al. that curcumin inhibits formation of nitrate and nitrite ions from A549 cells stimulated with gamma-interferon, another known pro-inflammatory stimulus.

**CONVERSION OF NO TO NITRITE AND NITRATE IN WATER**

It is especially straightforward to collect aliquots of supernatant medium from stimulated or unstimulated A549 cultures for measurement of nitrate and nitrite ions by first eliminating high molecular weight components from the culture medium by ultrafiltration and then assaying the filtrates with a two-step sequence of reactions with the Griess Reagent. The analysis of nitrate and nitrite ions in the filtrates is first converted to all nitric oxide reductase and the nitric oxide is then reacted with sulfanilamide and naphthyl ethylenediamine to form the intensely colored azo compound. The color from the azo compound is linearly proportional to the amounts of nitrate and nitrite in the filtrates.

**REFERENCES**

11. Curcumin (Turmeric) (2014) Natural Medicines Comprehensive Database, Therapeutic Research Faculty, 3120 W March Lane, Stockton, CA 95219.