Audriana Li
High School
Haynes Academy School for Advanced Studies, New Orleans, LA

Dr. Guoshun Wang
LSUHSC, Department of Microbiology, Immunology, and Parasitology

"Investigating the Role of Inducible Nitric Oxide Synthase in Gut Dysmotility and Inflammation in Cystic Fibrosis"

Background: Cystic Fibrosis (CF) is an inherited disease caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene. Intestinal inflammation and obstruction constitute the major manifestations of the disease. The underlying mechanisms remain poorly understood. We hypothesize that nitric oxide (NO), produced by inducible nitric oxide synthase (iNOS, encoded by the NOS2 gene), causes smooth muscle relaxation, which contributes to CF intestinal obstruction.

Methods: To test the hypothesis, CF mice were treated with the selective iNOS inhibitor GW274150 or control, and gastrointestinal transit was measured using a gut transit assay. Based on the pharmacological findings, *Nos2* and *Cftr* double gene-knockout mice were generated by crossbreeding *Nos2*$^{+/-}$ mice with *Cftr*$^{+/-}$ *mice*. Multiple experiments were conducted from these animal models, including polymerase chain reaction (PCR), Griess Reagent Assay (GRA), and intracellular iNOS immunostaining. First, PCR genotyping was performed to identify *Nos2*$^{+/+}$*Cftr*$^{-/-}$ and *Nos2*$^{-/-}$*Cftr*$^{-/-}$ mice. Second, a GRA was performed to determine NO production under lipopolysaccharide (LPS)-stimulation and conditions of no stimuli or treatment of GW274150. Finally, intracellular iNOS immunostaining and flow cytometry were used to determine iNOS expression in macrophages and neutrophils.

Results: Pharmacological inhibition of iNOS with GW274150 significantly shortened gastrointestinal transit time in CF mice compared with vehicle-treated controls, suggesting improved intestinal motility. After crossbreeding, mouse tails were cut for PCR-genotyping using specific primers to *Nos2* and *Cftr. G*el electrophoresis of the PCR amplicons revealed the desired *Nos2* and *Cftr* double knockout (*Nos2*$^{-/-}$*Cftr*$^{-/-}$*)* or the corresponding control (*Nos2*$^{+/+}$*Cftr*$^{-/-}$*)* mice. Leukocytes from both genotypes were isolated, cultured, and stimulated with LPS, and the supernatants were subject to nitrite measurement by GRA. Results show that the *Nos2*$^{+/+}$ *Cftr*$^{-/-}$ cells produced high levels of NO. Selective iNOS inhibitor GW274150 abolished such NO production. Moreover, the *Nos2*$^{-/-}$*Cftr*$^{-/-}$ cells produced little NO. Intracellular immunostaining of iNOS and flow cytometry demonstrate that LPS stimulation augmented iNOS expression in the control cells, but not *Nos2*$^{-/-}$*Cftr*$^{-/-}$ cells. Treatment of the control cells with GW274150 decreased frequencies and lowered iNOS expression in the macrophages/neutrophils.

Conclusion: These findings support a role for iNOS-derived NO in CF-associated gut dysmotility. We established a iNOS gene-knockout in CF mouse model. With this model we can further determine the role of iNOS in intestinal inflammation/obstruction. Specific iNOS inhibition could be beneficial in restoring gut motility and mitigating inflammatory effects in CF patients.