A Brief Summary of the Research Work of Mr. Avik Chattopadhyay

Title: High Throughput Screening Identifies Novel Pharmacological Inhibitors of Interferon-gamma-induced Nitric Oxide Production, Alleviating Ulcerative Colitis and Bacterial Sepsis in Mice

Introduction: Interferon-gamma (IFN-γ) is a type II interferon primarily produced by T cells and natural killer cells. One of the key markers in IFN-γ signaling is the expression of NOS2 catalyzing the production of Nitric Oxide (NO). IFN-γ signaling and NO production combat infectious diseases like *Mycobacterium tuberculosis* and *Salmonella* Typhimurium infections. However, excessive IFN-γ-activated NO production is implicated in several inflammatory diseases, including ulcerative colitis, multiple sclerosis, systemic lupus erythematosus, and sepsis. Disease exacerbation in chronic inflammatory diseases is managed with steroidal medications; however, long-term use of corticosteroids often leads to unavoidable adverse effects. These problems necessitate identifying alternative non-steroidal anti-inflammatory drugs, potently targeting IFN-γ-induced NO hyperproduction.
Previous studies from our laboratory showed that H6 mouse hepatoma cells produce NO and ROS upon IFN-γ-activation and undergo cell cycle arrest and apoptosis. This study aimed to identify novel non-steroidal anti-inflammatory compounds against IFN-γ-induced NO production using the *in vitro* H6-IFN-γ-activation model system.

Methods: The chemical library: LOPAC®1280, was screened *in vitro* on the H6 mouse hepatoma cell line to identify novel inhibitors. The lead compounds were studied for mechanism and tested across tumor cell lines and primary cells: mouse peritoneal macrophages. The compounds were assessed *in vivo* in two distinct pre-clinical mice inflammatory disease models: DSS-induced ulcerative colitis and *Salmonella* Typhimurium-induced bacterial sepsis.

Results: The lead compounds were identified: pentamidine, azithromycin, rolipram, and auranofin. Auranofin was the most potent compound determined based on IC_{50} and goodness of fit analyses. Mechanistic investigations revealed that most lead compounds suppress the IFN-γ-induced transcription of *Nos2* without negatively affecting NO-independent processes, such as the IFN-γ-induced transcription of *Irf1*, *Socs1*, and MHC class 1 surface expression. However, all four compounds lower IFN-γ-induced ROS amounts. In addition, auranofin significantly reduced IFN-γ-mediated NO and IL6 production in residents and thioglycolate-elicited peritoneal macrophages. *In vivo*, pentamidine and auranofin protected mice from DSS-induced ulcerative colitis and *Salmonella* Typhimurium-induced sepsis.

Conclusion: The lead compounds: pentamidine, azithromycin, and auranofin are FDA-approved drugs. Roflumilast, a drug with a similar mechanism of action to rolipram, is FDA-approved.

Therefore, the lead compounds are suitable for drug repurposing. The *in vitro* high throughput screening and validation model against IFN-γ-activation is suitable for screening other large chemical libraries and identifying novel anti-inflammatory compounds. Auranofin and pentamidine alleviate pathogenesis in two distinct inflammatory disease models and may serve as therapeutic options in alternative or combinatorial regimens to treat the chronic inflammatory conditions of inflammatory diseases.

Additional information:

The study was published in **International Immunopharmacology** on 29 Jun 2023, titled "High throughput screening identifies auranofin and pentamidine as potent compounds that lower IFN-γ-induced Nitric Oxide and inflammatory responses in mice: DSS-induced colitis and Salmonella Typhimurium-induced sepsis." The DOI is: 10.1016/j.intimp.2023.110569.

An extension of the study was filed as an **Indian Patent** on 8 June 2023 by the Indian Institute of Science. The application number is 202341039378. The patent title is "MODEL(S) FOR IDENTIFICATION OF ANTIINFLAMMATORY COMPOUNDS AND COMPOUNDS DETERMINED THEREFROM."

The Institutional Animal Ethics Committee approved all procedures (**IAEC**) constituted per article number 13 of the CPCSEA rules laid down by the Government of India (**CAF/ethics/805/2021**).

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