The role of intestinal CYP2E1 in pathophysiology of the gastrointestinal tract disorders: new therapies for leaky gut syndrome and inflammatory bowel diseases

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Introduction:

CYP2E1 is one of the main members of cytochromes P450 superfamily, which is primarily found in the liver and to a lesser extent, in extrahepatic tissues, including intestines. CYP2E1 is responsible for the metabolism of many compounds with toxicological importance, such as ethanol. The enzyme generates toxic intermediates and may produce excessive amounts of reactive oxygen species (ROS). Therefore, the activity of CYP2E1 has been implicated in various pathophysiological conditions.

Current literature shows that overactivity of intestinal CYP2E1 leads to the loss of intestinal wall integrity which is maintained by tight junction proteins (TJP) and contributes to the development of the leaky-gut syndrome (LGS). Namely overactivity of CYP2E1, alterations in TJP, and resulting LGS were observed after treatment with fructose. Fructose in high doses is one of the main dietary factors increasing intestinal permeability.

LGS is observed in numerous gastrointestinal (GI) disorders, including inflammatory bowel diseases (IBD) characterized by chronic and relapsing inflammation affecting the GI tract. It is well-established that the activity of CYP2E1 may contribute to the severity of inflammation due to the production of ROS.

In preliminary studies, we observed that diallyl sulfide (DAS), a selective inhibitor of CYP2E1, maintained intestinal epithelial barrier integrity and alleviated inflammatory response of Caco-2 cells. Thus, we hypothesized that other selective CYP2E1 inhibitors may share similar beneficial properties.

The main aim of the project is to evaluate the therapeutic effects of CYP2E1 inhibitors in *in vitro* and *in vivo* models of intestinal hyperpermeability and inflammation. For this purpose, I used DAS analogs which are less cytotoxic and display similar or better CYP2E1 inhibitory properties: 1) Allyl methyl sulfide (AMS), 2) Diallyl ether (DE), 3) Thiophene (T).

Methods (*in vivo* Phase):

DSS (dextran sulfate sodium)-induced colitis: Acute colitis was induced by the addition of 3% DSS to drinking water from day 0 to day 4. On days 5 to 7, animals received water without DSS. Chronic colitis was induced by 3 cycles of 5 days treatment with 2% DSS in drinking water followed by 4 days of water without DSS. DE was administered orally (*p.o.*) or rectally (*i.c.*) on days 0-6 (acute) or 10-27 (chronic).

Fructose-induced intestinal hyperpermeability: Obesity was induced through free access to water containing 30% fructose solution for 8 weeks. DE was administered *p.o.* or *i.c.* every 3 days.

Macroscopic assessment of mouse colon: Total macroscopic damage score was calculated for each animal and include stool consistency, colon epithelial damage and colon length and weight loss scores.

Determination of tissue myeloperoxidase activity: The MPO activity was measured using the commercially available kit (Merck) **Measurement of intestinal permeability with FITC-dextran:** Mice were fasted and gavaged with FITC-dextran. Serum was obtained from blood and analyzed for its fluorescein intensity.

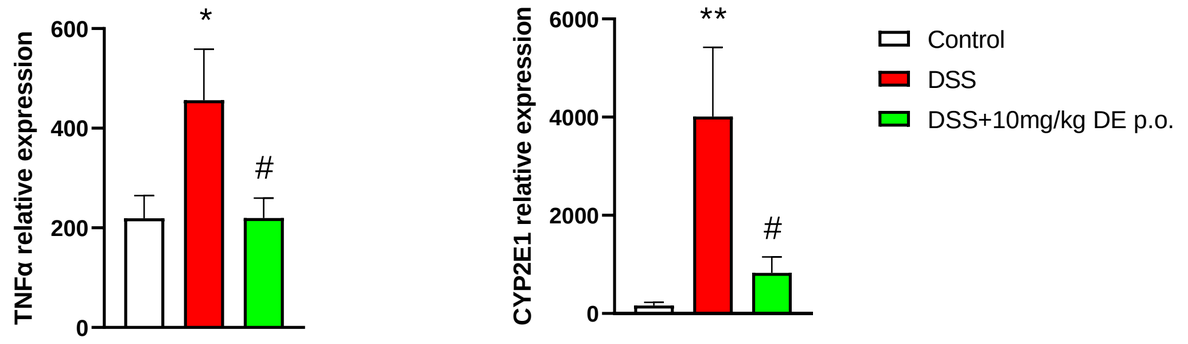
qPCR: RNA was isolated from mouse tissues. Then, RNA was transcribed onto cDNA. Quantitative analysis was performed using fluorescent probes. The threshold cycle (Ct) values for studied genes were normalized to Ct values obtained for HPRT. Following genes were assessed: occludin, ZO-1, CLDN-1, TNFα, IL-1β, IL-6, CYP2E1.

Results:

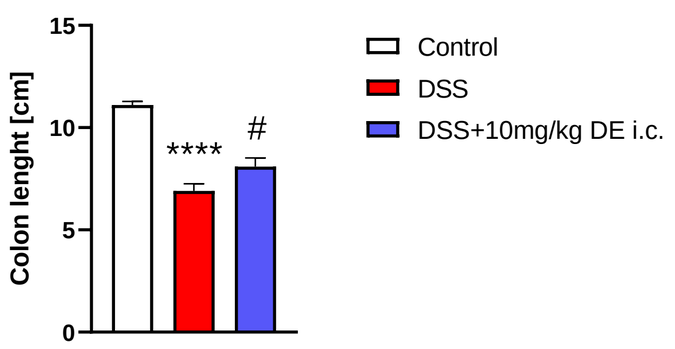
During the previous year of my doctoral studies, I performed *in vitro* research on Caco-2 cells. Initially, I did not observe any toxic effect of CYP2E1 inhibitors. Further research showed that monolayers of cells pretreated with DE and T were significantly resistant to the increase in its permeability induced by fructose. Moreover, DE completely prevented the inflammatory response of stimulated cells.

The results of the *in vitro* part of the study allowed to choose DE as the best candidate to evaluate the anti-inflammatory effect of CYP2E1 inhibitors in the mouse GI tract. For this purpose, we used three mouse models of intestinal inflammation and increased permeability. In all experiments, DE was administered at a dose of 10 mg/kg body weight. Most of the *in vivo* experiments were done during the current academic year.

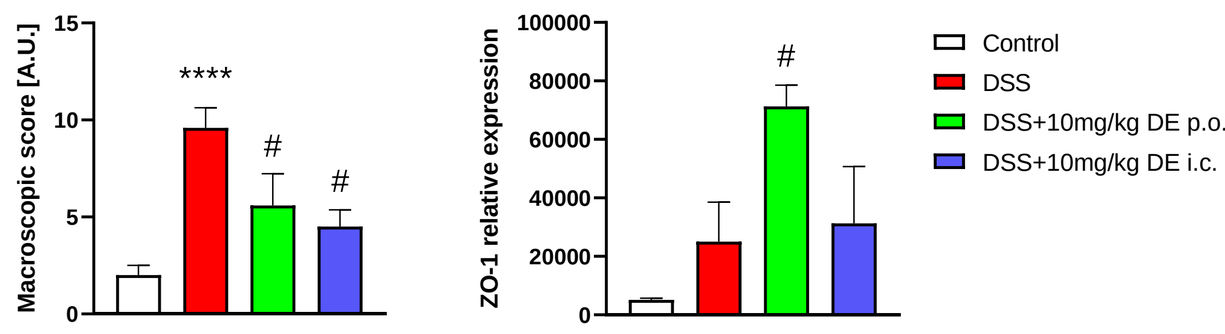
In the first experiment, we assessed the effect of DE administered *p.o.* on acute intestinal inflammation induced by DSS. DSS resulted in the development of macroscopic inflammation. Additionally, there was an increase in intestinal permeability and MPO activity. The mRNA expression of occludin, ZO-1, CLDN-1 decreased. The expression of TNFα, IL-6, CYP2E1 increased. DE treatment significantly prevented DSS-induced increases in TNFα (p=0.034) and CYP2E1 (p=0.018) expression. (Fig. 1)↓



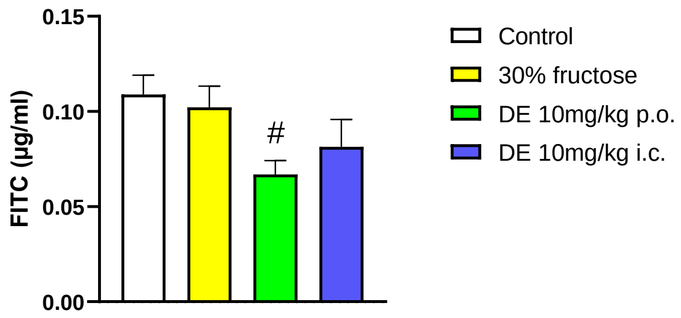
In the next step of *in vivo* research, we used the same model of acute colitis induced by DSS. In this experiment, we administered DE *i.c.* once daily. The expression of occludin and CYP2E1 decreased. Contrarily, the expression of ZO-1, TNFα, IL-6 increased. DE significantly prevented from the decrease in colon length caused by DSS (p=0.035). (Fig. 2)↓



Then, we used a model of chronic intestinal inflammation induced by DSS. DE was administered *p.o.* and *i.c.*. DSS resulted in the development of macroscopic inflammation. Additionally, there was an increase in intestinal permeability and MPO activity. The expression of ZO-1 and CYP2E1 increased. DE *p.o.* (p=0.034) and *i.c.* (p=0.01) significantly prevented DSS-induced increase in macroscopic intestinal damage. Additionally, DE *p.o.* significantly increased ZO-1 expression compared to the DSS group (p = 0.039). (Fig. 3)↓



In the fourth experiment, we assessed the effect of DE administered *p.o.* and *i.c.* on the gut permeability in the model of LGS induced by high doses of fructose. Fructose at a concentration of 30% resulted in increased intestinal weight. Under its influence, the expression of occludin and ZO-1 mRNA decreased. IL-1β expression increased. DE *p.o.* significantly reduced intestinal permeability compared to the fructose group (p=0.044). (Fig. 4)↓



Conclusion:

Current *in vivo* studies showed that DE administered *p.o.* or *i.c.* significantly prevents some of the alterations caused by DSS and fructose. My results so far suggest that realization of this project will increase the knowledge about the role of CYP2E1 in the pathological states of the gut. Evaluation of the effects of CYP2E1 selective inhibitors may bring additional opportunities to develop novel therapeutics for IBD and LGS. Moreover, effective drugs for LGS are also desirable since current treatment is based on dietary recommendations alone.

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