of the PD biomarker. **Conclusions:** This work describes a novel PD biomarker to monitor TPL2 inhibition in whole blood. With this assay, GS-4875 demonstrated dose-dependent and reversible inhibition of the TPL2 pathway following oral administration in healthy rats.

Tu1208

NEUROPEPTIDE W ALLEVIATES HEPATORENAL OXIDATIVE DAMAGE IN SEPSIS-INDUCED RATS

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Background: Despite modern surgical, medical and intensive care treatments, sepsis is still one of the most frequent causes of morbidity and mortality due to multiple life-threatening organ dysfunctions. We aimed to investigate the possible protective effect of neuropeptide W (NPW), a novel peptide effective in regulating neuroendocrine functions, against sepsis-induced hepatorenal damage. **Methods:** In male Sprague-Dawley rats (200–250 g), sepsis was induced by cecal ligation and puncture under ketamine anesthesia (n=48). Immediately after surgery, saline or TNF-alpha inhibitor (etanercept; 1 mg/kg) plus antibiotic (ceftriaxon; 100 mg/kg) (ET+C) or NPW (0.1, 0.3, 1 or 3 mg/kg) was given subcutaneously, and repeated at 12th and 24th hours, while sham-operated control group (n=8) received three saline injections within twenty-four hours. Rats were decapitated at the 25th hour of surgery and C-reactive protein (CRP), corticosterone and IL-6 levels were measured in serum samples. Kidney and liver samples were obtained for the measurement of myeloperoxidase activity (MPO), malondialdehyde and glutathione levels and nuclear factor kappa-B (NF-kB) mRNA expression levels. Histopathological evaluations were performed in hematoxylin-eosin-stained samples. ANOVA and Student's t-tests were used for data analysis. **Results:** Elevated serum levels of IL-6, corticosterone and CRP ($p<0.05$-0.01) in saline-treated sepsis group, as compared to controls, were depressed in the ET+C- ($p<0.05$) or NPW- ($p<0.05$-0.001) treated groups. Hepatic malondialdehyde and MPO levels, which were increased in saline-treated sepsis group ($p<0.05$ and $p<0.001$), were decreased by ET+C- ($p<0.01$) or NPW ($p<0.05$-0.001) treatments. Similarly, increased renal malondialdehyde level was depressed by NPW ($p<0.05$), but not by ET+C; while none of the treatments had an inhibitory effect on renal MPO. In contrast to replenished renal glutathione levels by all treatments, hepatic glutathione content was not changed by any of the treatments. Hepatic and renal NF-kB mRNA expressions were similar in all groups. Severe hepatocyte degeneration, sinusoidal congestion and inflammatory cell infiltration were observed in saline-treated sepsis group, while parenchymal degeneration, congestion and Kupffer cell activation were mild in ET+C- and NPW-treated sepsis groups. Similarly, severe degeneration of renal corpuscles and tubules with glomerular and interstitial congestion in the saline-treated sepsis group was replaced by moderate glomerular and interstitial vascular congestion and mild tubular congestion in both NPW- and ET+C-treated groups. **Conclusion:** NPW, applied during the first 24 hours of sepsis, exerted a dose-dependent protective effect against hepatorenal damage, which appears to involve an inhibitory impact on neutrophil infiltration to the liver and an antioxidant action on the kidney.

Tu1209

CHIRALLY-MODIFIED LARAZOTIDE COMPOUND ANALOG #6 FACILITATES RECOVERY OF ISCHEMIC-INJURED PORCINE JEJUNUM VIA RE-ASSEMBLY OF INTRAEPITHELIAL TIGHT JUNCTIONS

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Intestinal epithelial tight junction integrity is crucial to maintain an intact intestinal barrier. Larazotide acetate (LA) facilitates assembly of interepithelial tight junctions (TJs) by blocking the endogenous zonulin pathway. LA is currently in P3 clinical trials for celiac disease and likely has applicability to other diseases characterized by increased epithelial permeability. We have previously explained LA's dose response profile and the inhibitory nature of the LA fragments. Our previous mass-spectral analysis of *in vitro* enzyme degradation of LA by brush-border aminopeptidase M revealed the formation of fragments (de Gly LA and de Gly-Gly LA) that are inhibitory to *ex vivo* recovery. Therefore, a chirally-modified LA, Analog #6 (A6, all D- amino acids) was generated to theoretically prevent its fragmentation while maintaining recovery efficacy. We hypothesized that A6 would exhibit similar recovery effects when compared to LA and would not be degraded into equivalent fragments, thereby allowing A6 to be effective at lower concentrations. To induce experimental intestinal injury, Yorkshire-cross pigs 6-8-weeks-of-age were anesthetized, followed by midline laparotomy and creation of a series of 10cm jejunal loops by ligating the intestinal lumen. The local mesenteric vasculature was ligated to select treatment loops for 45-min, whereas other loops were left as non-ischemic controls. Loops were subsequently resected, and the mucosal tissues were mounted in Ussing chambers. Tissues were treated with various doses of A6 (0.01μM, 0.1μM, 1.0μM and 10μM) and with LA (1μM). Apical Ringer's solution samples were collected at select timepoints for mass spectrometry analyses. Mucosal tissue samples were collected for tissue fractionation and western analyses were used to probe mucosal homogenates for tight junction protein localization. Transepithelial electrical resistance (TEER) data revealed similar recovery profiles between the three doses (0.1, 1 and 10μM) of A6, and these TEERs were not significantly different from LA treatment at 1μM. Mass spectrometry analyses of mucosal Ringer's samples revealed that degradation of A6 is slower than LA degradation. As opposed to LA fragment, A6 fragments did not show competitive inhibition. A6 facilitates TJ recovery in injured epithelium with greater stability than LA and with the potential for prolonged activity as a result of reduced inhibitory fragments.

Tu1210

GENOME-WIDE TRANSCRIPTIONAL RESPONSES TO ASPIRIN WITH AND WITHOUT INFLAMMATORY STIMULANTS IN HUMAN COLONIC ORGANOIDS

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Background: Aspirin (ASA) is an established colorectal cancer (CRC) chemoprotective agent endorsed by the USPSTF. However, mechanisms of ASA's chemoprotective effects in the colon and inter-individual differences of these effects are incompletely understood. One mechanism is likely through anti-inflammatory effects, though responses to ASA in an inflammatory milieu have not been systematically evaluated. To study differences in short-term transcriptional response in the colon, colonic organoid cultures were utilized, thereby eliminating several confounders. The aim of this study was to map genome-wide colonic transcriptional responses to treatment with ASA with and without pro-inflammatory stimulants in colonic organoids. **Methods**: Organoids were established from colonic biopsies obtained from 3 African American healthy males. After the 3rd passage, organoids were differentiated for 24 hours and then treated for 24 hours with 3nM ASA alone, stimulants (IL1β, IL6 and TNFα) alone or combined ASA + stimulants. RNA was then sequenced on 2 NovaSEQ lanes with 50bp single end reads. STAR and RSEM were used for alignment and gene quantification, respectively. DESeq2 was used to identify differentially expressed (DE) genes in the treatment groups. False discovery rate (FDR) of <5% was considered significant. Gene ontology analyses were performed using DAVID. **Results**: Principal components analyses showed that gene expression clustered by individual and by treatment (**Fig 1A&B**). Overall, the most DE genes were found with TNFα treatment alone, while IL6+ASA (compared with IL6 alone) showed the most DE genes among combined treatments (**Fig 2A**). Gene ontology analyses showed enrichment for a number of biological processes with ASA treatment alone such as fatty acid metabolism, PPAR signaling and acetylation among others (**Fig 2B**). Combined treatments with IL6+ASA and TNFα+ASA showed additional enrichment in ATP-binding and flavin adenine dinucleotide binding pathways, respectively. A potent anti-oxidant, Heme oxygenase 1 (*HMOX1),* was found to be upregulated by ASA and combination treatments (**Fig 2C). Conclusions**: This genome-wide transcriptional mapping study of ASA treatment with and without inflammatory stimulants (IL1 β, IL6 and TNFα) provides new insights into mechanisms of ASA's effects in the human colon. In short term organoid culture, ASA showed significant effects on genes involved in fatty acid metabolism and acetylation. Upregulation of *HMOX1* by ASA is an important observation as previous studies have characterized ASA's anti-oxidant effects mediated through *HMOX1* in other cancer models, but this effect has not been described previously in normal colon or CRC. Finally, treatment differences between individuals are notable and provide rationale for future study of inter-individual variation in ASA responses.

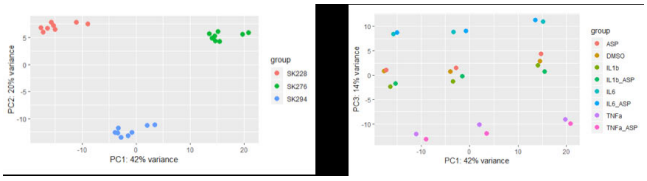

Fig 1: Principal components plots of gene expression by **A)** individual and **B)** treatments. ASP, aspirin; DMSO, vehicle control

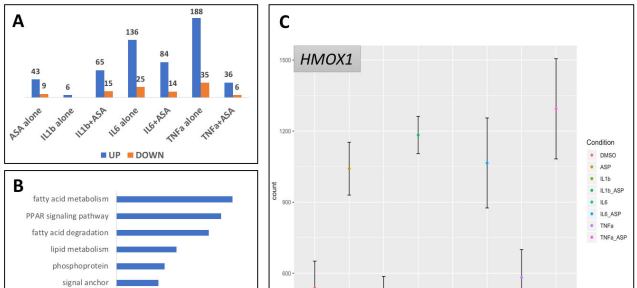

Fig 2: A) Number of differentially expressed genes (up- and down-regulated) in all treatments. **B)** Gene ontology results for ASA treatment by -$\log_{10}$ (Benjamini Hochberg p-value). **C)** *HMOX1* expression in all treatment conditions showing significant upregulation with aspirin as well as combination treatments.

Tu1211

AN ANTISENSE OLIGONUCLEOTIDE FOR MICRORNA-24-3P EXHIBITS SAFE AND EFFICIENT KNOCKDOWN ABILITY *IN VITRO* AND *IN VIVO* AND INHIBITS TRINITROBENZENE SULFONIC ACID (TNBS) COLITIS

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Background and Goals: Antisense oligonucleotides (ASOs) are short, single-stranded nucleic acids that target complementary endogenous RNA sequences to repress translation. ASOs have great potential as new therapeutics because of the specificity of the sequence as opposed to small molecule-based drugs that may affect other targets. MicroRNAs (miRs) are small