Two curcumin extracts modify the composition of gut microbiota, tight junction protein, and neuroinflammation in rats with neuropathic pain: microbiota-gut-brain axis

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ABSTRACT

Objectives: Emerging evidence suggests that gut microbiota may serve at the intersection between microbiome-gut-brain and neuroinflammation in the development of neuropathic pain (NP). This study evaluated the effects of curcumin C3 Complex® (CUR) and bisdemethoxy curcumin (CMO), on the composition of gut microbiota and intestinal permeability- and neuroinflammation-associated gene expression in animals with NP.

Methods: 23 male rats were randomly divided into: sham, spinal nerve ligation (SNL group, pain model), SNL+100mg CUR/kg BW (CUR group), and SNL+50mg CMO/kg BW (CMO group) for 4 weeks. Fecal samples were collected for microbiota composition analysis using 16S rRNA gene sequencing. The mRNA expression level of tight junction proteins (Claudin-1, Occludin) and neuroinflammation (NF-κB) in the colon, amygdala, and spinal cord using qRT-PCR. Data were analyzed statistically.

Results: Using a beta-diversity weighted UniFrac distance metric, the microbiome profile of the CMO-treated group was significantly different than other groups ($P<0.05$). Regarding alpha-diversity, while most groups did not differ with respect to richness or evenness the CMO group improved microbiome evenness compared to the SNL group ($P=0.016$). The relative abundance of several microbiome amplicon sequence variants (ASV) changed with different treatments. The SNL group showed a depletion in *Rothia nasimurium* compared to the sham group ($P<0.01$). In contrast, *Streptococcus* and Clostridia ASVs (f_Oscillospiraceae;g_UCG−005) were enriched in the SNL group ($P<0.01$). CUR or CMO treatments induced changes in multiple species compared to SNL. CUR and CMO reversed the enrichment effect of SNL on Clostridia ASV ($P<0.01$). Compared to the sham group, the SNL group exhibited increased Claudin-1 mRNA expression levels in the amygdala. Relative to the SNL group, both CUR and CMO groups suppressed the mRNA gene expression of Claudin-1 (spinal cord, amygdala), Occludin (spinal cord, colon), and NF-κB (amygdala) in SNL-operated animals.

Conclusions: This study suggests CUR and CMO administration modifies multiple species of gut microbiome in an NP model. These effects may be associated with a reduction in SNL-induced intestinal permeability and neuroinflammation.

Funding Sources. Texas Tech University Health Sciences Center, Lubbock, TX.

BACKGROUND/OBJECTIVES

The relationship between gut microbiota and neuropathic pain (NP), including SNL-induced NP, has received increasing attention. Gut microbiome is considered as a pivotal regulator in immune, neural, endocrine, and metabolic signaling pathways, thus affecting the development of NP directly or indirectly, via microbiota-gut-brain axis. However, the exact mechanism is still unknown. Thus, this study examine the effect of dietary curcumin C3 Complex® (CUR) and bisdemethoxy curcumin (CMO), on the composition of gut microbiota, and intestinal permeability- and neuroinflammation-associated gene expression in animals with NP.

HYPOTHESIS

CUR and CMO administration would modify gut microbiome composition, tight junction protein-associated mRNA expression, and neuroinflammation-associated mRNA expression in SNL-induced NP rats.

METHODS

Animals treatments: 23 male SD rats (5-week-old) into 4 groups: Sham+vehicle (Sham group n=5), SNL+vehicle (SNL group n=6), SNL+100 mg/kg BW CUR (CUR group n=6), and SNL+50m/kg BW CMO (CMO group n=6) for 4 weeks.

Gut microbiota composition in cecal feces by 16S rRNA gene sequencing

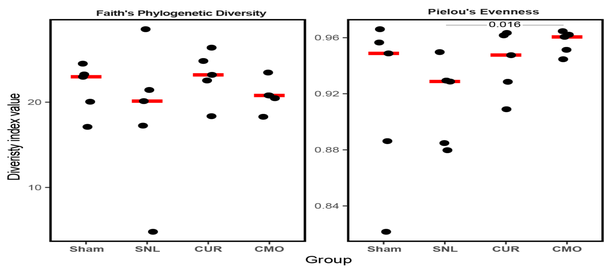
mRNA expression of claudin-1 and NF-kB in colon and amygdala by qRT-PCR.

Statistical analysis: mRNA expression levels were analyzed by one-way ANOVA followed by Tukey's post hoc analysis. Gut microbiome composition and diversity were analyzed by QIIME2.

RESULTS

Figure 1. Effect of CUR and CMO on beta-diversity. Weighted UniFrac distance metrics for beta-diversity indicated that microbiome profiles changed significantly as a result of treatment ($P = 0.032$, PERMANOVA). Specifically, CMO group was structurally different than other groups, i.e., Sham ($P = 0.013$, pairwise PERMANOVA), SNL ($P = 0.031$, pairwise PERMANOVA), and CUR ($P = 0.014$, pairwise PERMANOVA). Distance between other groups were not statically significant ($P > 0.1$, pairwise PERMANOVA).

Figure 2. Effect of CUR and CMO on alpha-diversity. Alpha-diversity was assessed with respect to species richness and evenness. First, in terms of species diversity or richness all groups were not different ($P > 0.05$, Faith's Phylogenetic Diversity). Second, while most groups didn't differ with respect to evenness, CMO improved microbiome evenness in comparison to SNL ($P = 0.016$, Pielou's Evenness), which suffered from a slight decrease that was not statically significant.



ACKNOWLEDGEMENTS

This study was supported by Department of Pathology, Texas Tech University Health Sciences Center, Lubbock, TX.

Figure 3. Effect of CUR and CMO on species abundance. SNL did alter the abundance of several species when compared to Sham. For example, ASVs belonging to *Rothia nasimurium*, *Streptococcus*, and *Ruminococcus* were depleted in SNL. CUR or CMO treatments induced changes in multiple species as well in comparison with SNL. Importantly, CUR and CMO reversed the enrichment effect of SNL on Clostridia ASV (f__Oscillospiraceae;g__UCG−005;s__uncultured_Clostridiales), as this ASV was only detected in SNL group.

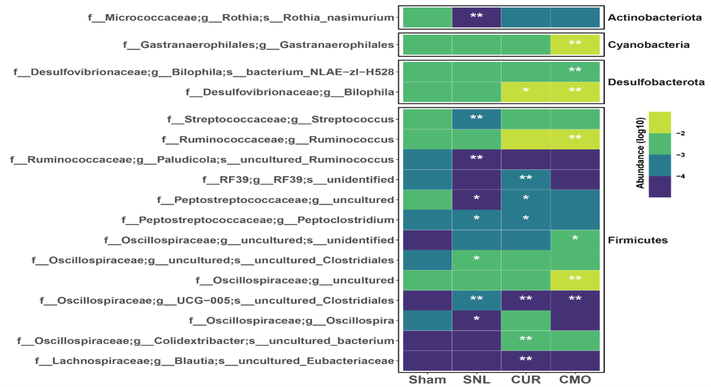
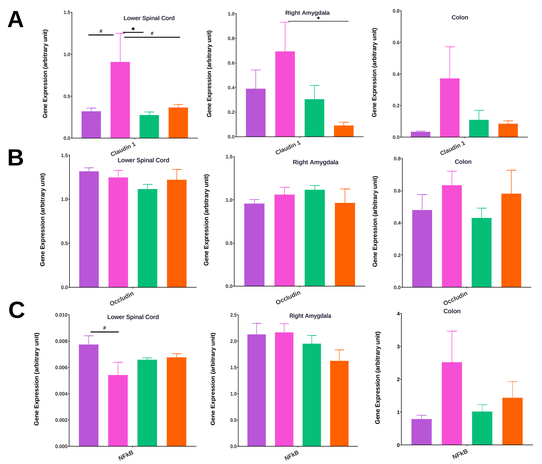


Figure 4. Effect of CUR and CMO on mRNA expression. (A) Claudin-1 in the lower spinal cord, the right amygdala, and colon; (B) Occludin in the lower spinal cord, the right amygdala, and colon; (C) NF-kB in the lower spinal cord, the right amygdala, and colon. *p<0.05.



CONCLUSIONS

Our results suggest that the administration of the curcumin extracts CUR and CMO to rats with neuropathic pain: 1) modifies the composition of the gut microbiota; 2) alters the mRNA expression of the tight junction protein Claudin-1; and 3) suppresses the expression of the NFkB one marker for neuroinflammation.