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Background: Ulcerative Colitis (UC) is a chronic, idiopathic intestinal inflammatory disease whose diagnosis requires colonoscopy with biopsy. There is an unmet need for sensitive, and easy-to-detect biomarkers helping a rapid and non-invasive diagnosis, and suitable for following disease progression. Extracellular vesicles (EVs) have been proposed as promising carriers of biomarkers. Nevertheless, their plasma levels remain very low and thus difficult to detect. We explored Low-Intensity Pulsed Ultrasound (LIPUS) as a novel and safe approach to enhance the mucosal release of EVs in experimental models of UC.

Methods: LIPUS was applied at a frequency of 38 kHz and at an intensity of 150 mW/cm² for 3 min, using devices dedicated to *in vitro* or *in vivo* studies. Primary intestinal fibroblasts, Human Intestinal microvasculature endothelial cells (HIMEC) and peripheral blood mononuclear cells (PBMC) were isolated from UC patients and healthy volunteers (n=6). Cell viability was tested in Caco-2 and all primary cells using MTT assay. Computer acoustic simulations, allowing a reliable control of the energy dose in the colon, were carried out using k-Wave software to translate *in vivo* the LIPUS. Acute and chronic colitis models were induced in C57BL/6N mice by administration of dextran sodium sulfate (DSS) respectively 2 and 2.5% *ad libitum* in their drinking water for seven days for the acute and three cycles of DDS for the chronic. Mice were monitored daily for body weight loss, bleeding and stool consistency. EVs were characterized in supernatants, plasma and mucosa at NanoSight and inflammatory mediators detected 1, 2 and 24 h after LIPUS by ELISA assay. Apoptosis and cell proliferation were evaluated by TUNEL and Ki67 stainings.

Results: After LIPUS, all cells appeared 100% viable and no pro-inflammatory changes were observed. In Caco-2 cells, fibroblasts and HIMEC, LIPUS significantly decreased the levels of IL-8. The maximum release of EVs, range in size from 100 to 120 nm, was recorded after 60 min in resident cells from both UC and healthy-derived cells. Conversely, PBMC displayed a progressive decrease. In both acute and chronic models, the physiological cellular turnover of the mucosa resulted unchanged after LIPUS. A significant increase of EVs was observed after 2 h of stimulation, and dropped down after 24 h. The levels of EVs were higher (p<0.01) in the chronic rather than acute phase of colitis.

Conclusion: LIPUS proved to be a safe and non pro-inflammatory approach to boost the release of EVs accumulated into the mucosa increasing their levels in the bloodstream. This feature is transient and more evident in the short term. Overall, the LIPUS could pave the way to a new era of a gut disruptive liquid biopsy.

Abstract citation ID: jjac190.0247

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Low-Intensity Pulsed Ultrasound as a new approach of gut disruptive liquid biopsy to boost the release of mucosal extracellular vesicles in Ulcerative Colitis

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