In Vitro Evaluation of the Potential Anti-inflammatory Effect of Raspberry Ketone on RAW 264.7 cells for Guided Bone Regeneration

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Statement of Purpose: Guided Bone Regeneration (GBR) membranes are clinically placed above bone-grafting materials to act as a barrier between soft tissue infiltration and bone defect sites [1]. Electrospun chitosan membranes (ESCM) have shown potential for local therapeutic delivery due to the high surface area of nanofibers [2]. One approach to promote healing is to modulate the inflammatory response to aid in transition from pro-inflammatory macrophages (M1) to anti-inflammatory macrophages (M2). Raspberry ketone (RK), a naturally occurring phenolic compound, has exhibited potential to aid transition of M1 to M2 macrophages [3]. This work aimed to evaluate potential anti-inflammatory effect of RK released in cell culture medium on growth and macrophage polarization of RAW 264.7 cells, macrophage-like murine cell line. Experiments were conducted using culture medium containing with and without Lipopolysaccharide (LPS) to induce inflammatory response to imitate bacterial presence that would be present naturally in healing defects.

Methods: RAW 264.7 cells (ATCC-TIB-71), a murine macrophage-like cell line were used. Cells were seeded into 48-well plates at 30,000 cells/ml and cultured in DMEM with 10% FBS, 1% penicillin, streptomycin, and neomycin (Thermo Fischer, MA) for 24 hours. After 24 hours, polarization to M1 was induced by incubating with medium containing 2 µg/ml Lipopolysaccharide (LPS) and varying concentrations of RK (12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml). Identical groups were included which did not receive LPS stimulation. Negative control contained no RK or LPS, and positive control contained 2 µg/ml LPS in medium. Cells were assayed at 24 hr, 48 hr, and 72 hr timepoints for NO concentration (Invitrogen Griess Reagent Kit, CA). ANOVA and pairwise comparison was used for statistical analysis (α=0.05; n=4/grp/time point).

Results: No difference in NO content between media negative control and other groups were observed for 24-hour timepoint. NO content for LPS+200 µg/ml RK, 12.5 µg/ml RK and 200 µg/ml RK were significantly less than LPS positive control for 24-hour timepoint (Fig 1a). By 48-hour timepoint, LPS positive control was significantly higher than all experimental groups except LPS+12.5 µg/ml RK and LPS+25 µg/ml RK. Only LPS containing groups had significantly higher NO concentration compared to media negative control (Fig 1b). By 72-hour timepoint, LPS positive control, LPS+12.5 µg/ml RK, LPS+25 µg/ml RK, LPS+50 µg/ml RK, LPS+100 µg/ml RK, and LPS+200 µg/ml RK were significantly higher NO content compared to media negative control. All groups except LPS+12.5 µg/ml RK were significantly less compared to LPS positive control during 72-hour timepoint (Fig 1c).

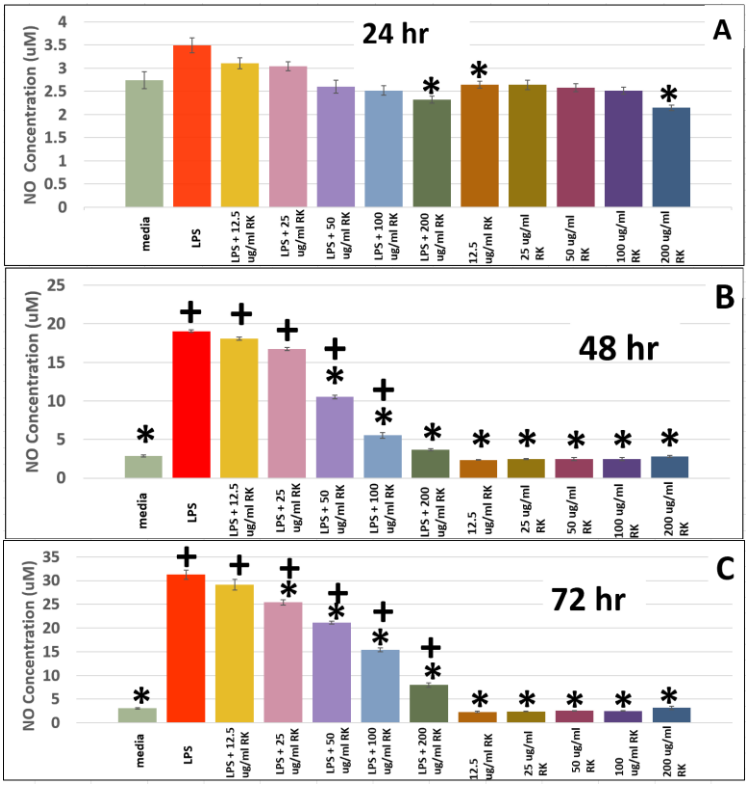

Figure 1. (a) Avg (± std dev) NO Concentration (uM) for 24 hr timepoint. **(b)** Avg (± std dev) NO Concentration (uM) for 48 hr timepoint. **(c)** Avg (± std dev) NO Concentration (uM) for 72 hr timepoint (*Indicates significant difference from LPS control, + Indicates significant difference from Media control).

Conclusions: RK resulted in a dose dependent decrease in pro-inflammatory NO release from LPS-stimulated monocyte cells. RK by itself did not cause an increase in NO production indicating it did not induce the cells to a pro-inflammatory state. These data suggest that RK may be an useful for modulating inflammatory responses.

References:

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