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Phagocytosis of apoptotic neutrophils by macrophages is enhanced by nano-sized vesicles derived from Brassica oleracea L. (broccoli)

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INTRODUCTION & AIM

Efferocytosis, the process by which macrophages clear apoptotic cells, is crucial for resolving inflammation and is often impaired in various inflammatory conditions [1]. Previous studies suggest that natural products and their bioactive components can modulate macrophage functions, including activation, recruitment, polarization, and metabolism. Broccoli-Derived Nanovesicles (BDNVs) are emerging as promising candidates for cancer modulation and immune regulation due to their intrinsic antioxidant components and nano-scale structure [2,3]. In this context, our research explores the potential of BDNVs to enhance macrophage efferocytic capacity. We propose that pretreating macrophages with varying doses of BDNVs may improve their ability to remove apoptotic neutrophils.

METHODS

For isolation of BDNVs, fresh sprouts of Brassica oleracea L. (Broccoli) were subjected to ultracentrifugation and their amount were expressed as weight/volume.

To assess efferocytic activity, THP-1 monocytes were differentiated into adherent macrophages using phorbol myristate acetate (PMA) for 72 h. Human neutrophils were isolated by Ficoll density gradient, induced to undergo apoptosis by heat shock at 43°C for 60 min followed by a 24 h incubation at 37°C, then labeled with PKH-26. Labeled apoptotic neutrophils were co-incubated with BDV-treated macrophages and phagocytic activity was assessed by epifluorescence microscopy and flow cytometry.

RESULTS & DISCUSSION

In the initial phase of the study, we observed that treatment with PMA was capable of inducing differentiation of the THP-1 cell line into macrophages. Moreover, we found that heat stress induced apoptosis in the isolated neutrophils (**Fig. 1**). In the epifluorescence microscopy experiments, it was observed that treatment at different time points with various doses of BDNVs had effects on the percentage of cells showing a strong red fluorescent signal within the cytoplasm (**Fig. 2**). This observation was confirmed by flow cytometric analysis, which revealed a statistically significant increase in the percentage of efferocytic macrophages, both after 24 and 48 hours of treatment with BDNVs, compared to basal efferocytosis (i.e., that of macrophages not treated with BDNVs) (**Fig. 3**)

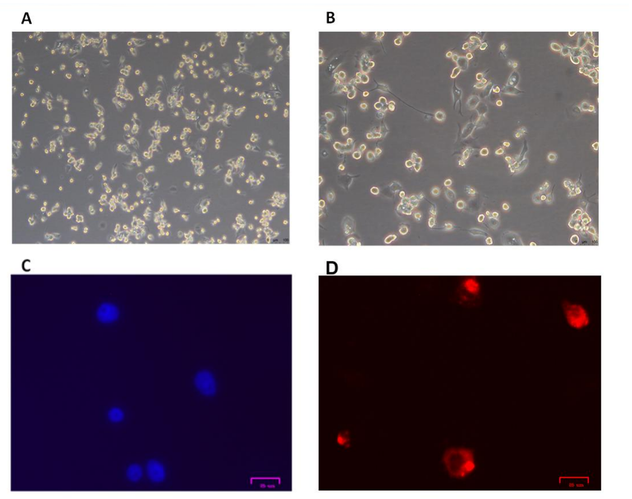

Figure 1. THP-1 cells exposed to PMA for 72 h became adherent. These cells acquired a rounded and flat morphology and some of them became highly elongated (A and B). They were amenable to be discriminated as fluorescent nuclei upon DAPI staining (C). Importantly, no background fluorescence in the red channel, identifying apoptotic neutrophils, was visible. Original magnification: 10X (A); 20X (B). (C) DAPI staining of macrophages nuclei. (D) PKH-26 staining of apoptotic neutrophils. Bar = 25 µm.

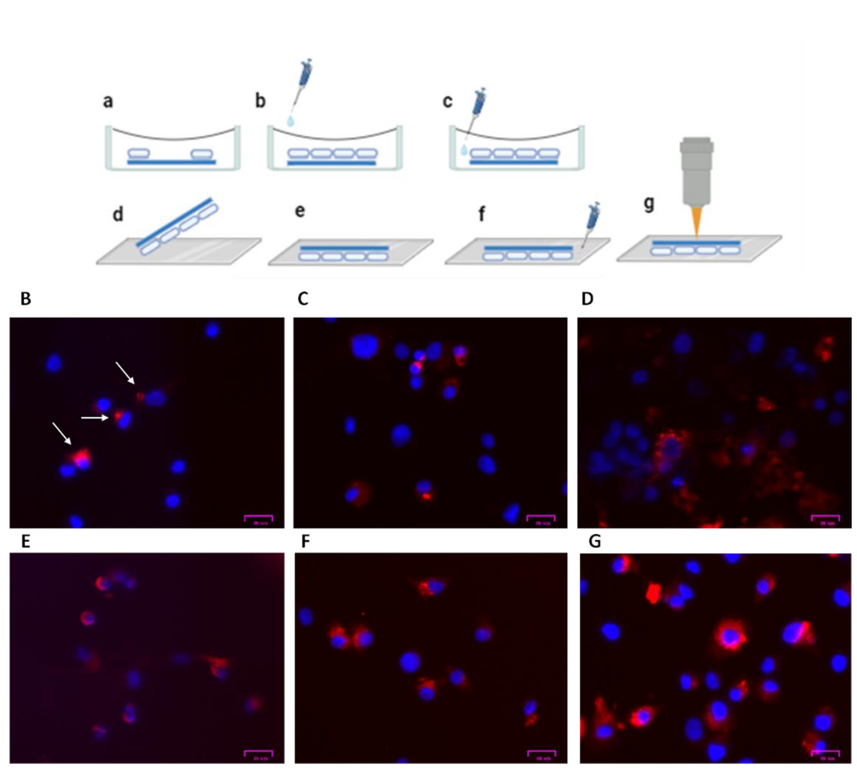

Figure 2. *BDNVs enhance the efferocytosis capacity of macrophages. (A) Protocol for seeding macrophages on coverslips placed in a 24-well plate. (a) Cells were seeded into the wells of a 24-well plate. (b, c) Cell detachment was avoided by slowly adding the nanovesicle suspension without damaging the cells. (d, e, f). The coverslip was then removed from the well and placed on a glass slide containing the dye/mounting medium. (g) The resulting preparation was analyzed under a fluorescence microscope. (B-G) Efferocytosis by macrophages pre-treated with different weight/volume ratios of BDNVs: (B) no pre-treatment (white arrows denote red apoptotic bodies engulfed by macrophages); (C) 5 µg/mL; (D) 11 µg/mL; (E) 22 µg/mL; (F) 45 µg/mL; (G) 100 µg/mL. Bar = 25 µm.*

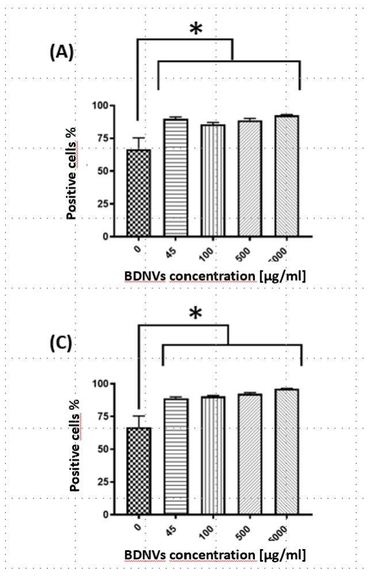

Figure 3. Flow cytometric analysis of efferocytosis mediated by macrophages treated for 24 hours (A) or 48 hours (C) with BDNVs at different concentrations. *p < 0.05; n = 2, experiments performed in duplicate."**

CONCLUSION

Collectively, these results suggest that BDNVs promote the clearance of apoptotic neutrophils through enhanced efferocytosis. BDNVs might be considered as pro-resolvent agents in the treatment of chronic inflammatory conditions.

FUTURE WORK / REFERENCES

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