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Intracellular Fate of Bovine Milk Exosomes in Murine Bone Marrow-Derived Macrophages

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Objectives: Milk exosomes (MEs) and their microRNA cargos constitute novel bioactive food compounds, and bovine MEs (BMEs) are being considered for use in drug delivery. The internalization of MEs by macrophages and degradation in lysosomes (in all cells) limit biological activities. Here, we determined whether BMEs internalized by murine bone marrow-derived macrophages (BMDMs) retrofuse with the intralumenal membrane in the multivesicular body (MVB) for subsequent release into the extracellular space or whether BMEs are destined for degradation in lysosomes.

Methods: BMDMs were treated with HiLyte^{TM} Fluor 750 hydrazide-labeled BMEs, washed, and the release of BMEs into the extracellular space was assessed by quantifying HiLyte^{TM} fluorescence in culture media and exosomes purified from BMDM media (BMDM-Exo). The lysosomal localization of BMEs in BMDMs was assessed by confocal microscopy analysis of pH-sensitive pHrodo red-labeled BME in lysosomes counterstained with LysoTracker^{TM} Green DND-26 (LysT) and confocal microscopy analysis of pHrodo red signals in the presence of the autophagy inhibitor, bafilomycin A1 (Baf A1). Parametric and non-parametric tests were used for statistical analyses; $P < 0.05$ was considered statistically significant.

Results: HiLyte^{TM} signal was not detected in BMDM culture media and BMDM-Exo ($P > 0.05$ compared to untreated control). Treatment with Baf A1, resulted in an 84% decrease in pHrodo-BME signal in BMDMs ($P < 0.05$), and pHrodo-BMEs were found to co-localize with LysT in BMDMs.

Conclusions: The retrofusion of BME-derived intralumenal MVBs and secretion of BME into the extracellular space is quantitatively minor in BMDMs. The majority of BMEs localizes to lysosomes for degradation in BMDMs.

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