Milk exosomes as natural curcumin nanocarriers in liver fibrosis

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***Introduction*:** Exosomes are natural extracellular nanovesicles involved in cell-cell communication. These natural nanovesicles are currently emerging as alternative platform against synthetic nanoparticles. Their nanometric size and lipidic structure, similar to liposomes, allow the incorporation of hydrophilic and hydrophobic drugs in their structure for their controlled delivery in desirable tissue [1, 2]. Hydrophobic drugs such as curcumin present limitations as low bioavailability and solubility. These limitations can be overcome using milk exosomes as nanocarriers of targeted therapy improving their bioavailability and keeping their anti-inflammatory activity [3, 4].
***Materials*:** An active cargo method employing saponin was optimized for the incorporation of curcumin in the exosome structure. Curcumin (1000 µg), saponin (2 mg) and milk derivate exosomes (200 µg) were mixed at 37ºC for 20' and purified by size exclusion columns (ExoCur). Physicochemical characterization of ExoCur was carried out by nanodrop, nanophotometry and flow cytometry (FC) for cargo and delivery analysis and by NTA, DLS and TEM for morphology. Cellular assessment of ExoCur cytotoxicity and uptake were performed by MTT assay in RAW264.7 and HepG2 cells. FC and confocal microscopy were used to assess the *in vitro* uptake of the nanoconjugate. *In vivo* evaluation of the therapeutic effect of ExoCur was performed in hepatic model of acute chronic liver disease, produced by CCl_4 agent. Mice treated with CCl_4 received 3 injections of ExoCur (30 µg, 100 µL PBS) or corn oil (control).
***Results*:** Our active approach employing detergent (saponin) allowed curcumin incorporation in goat´s milk exosomes structure. Fluorescence properties of curcumin confirmed the presence of the drug in the exosome structure with a cargo of 3.6 µg of curcumin/µg exosome. No membrane and size modification were confirmed by NTA, DLS and TEM (ExoCur: 120±6.1 nm). The controlled release of the curcumin from ExoCur was confirmed by absorbance, measuring a maximal curcumin delivery of 44.7% after 48 h of incubation. Cytotoxic activity of ExoCur was confirmed in both cells in a dose and time- dependent effect, with higher cytotoxicity in macrophages (% viability, RAW264.7: 18.6% and HEPG2: 36.1%). Preliminary *in vivo* results confirmed therapeutic effect of ExoCur, showing a reduction of the fibrotic tissue in chronic liver model.
***Conclusions*:** We have developed the novel nanometric drug delivery system ExoCur based on the encapsulation of the therapeutic molecule curcumin in the structure of nanometric milk exosomes. The novel nanocarrier ExoCur confirmed a therapeutic effect on targeted hepatic tissue, showing a reduction of fibrotic tissue in CCl_4 treated-mice.

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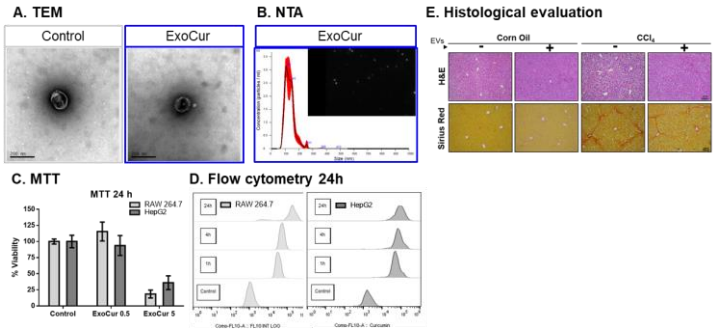

Figure 1: (A)-(B). Physicochemical characterization. (C)–(D) *In vitro* evaluation in cell lines. (E) *In vivo* assessment in CCl_4-treated mice.