Comparison of liposomal encapsulated and non-formulated fulvic acid on proliferation, oxidative stress and cell death in cancer cell lines

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Background

Cancer is an international- and national health concern resulting in deaths worldwide. Research has indicated that the phytochemical, fulvic acid, potentially exerts antiproliferative activity in tumorigenic cells. Furthermore, research into nanocarriers has identified liposomes as a potential method of improving the bioavailability of a variety of molecules. Liposomes can be modified to carry a vast number of different molecules. The aim of this study was to investigate the influence of liposomes in neuroblastoma (SH-SY5Y) and prostate (DU-145) cells exposed to fulvic acid.

Materials and Methods

- Neuroblastoma tumorigenic cell line (SH-SY5Y)
- Prostate cancer carcinoma cell line (DU 145)
- Fulvic acid (FA)
- Fulvic acid embedded in liposomes (LiFA)

- **Cell growth:** Spectrophotometry (Crystal violet staining)
- **Cell morphology:** Light microscopy
- **H_2O_2 production:** Fluorescent staining with 2′,7′-Dichlorofluorescin diacetate (DCFDA)
- **Lysosomal acidity:** Fluorescent staining with Acridine orange
- **Cell cycle progression:** Flow cytometry (propidium iodide and triton-x)

Results:

The influence of liposomes (1.5%) on the effects fulvic acid has on the SH-SY5Y- and DU 145 cell lines was studied and the following results were obtained

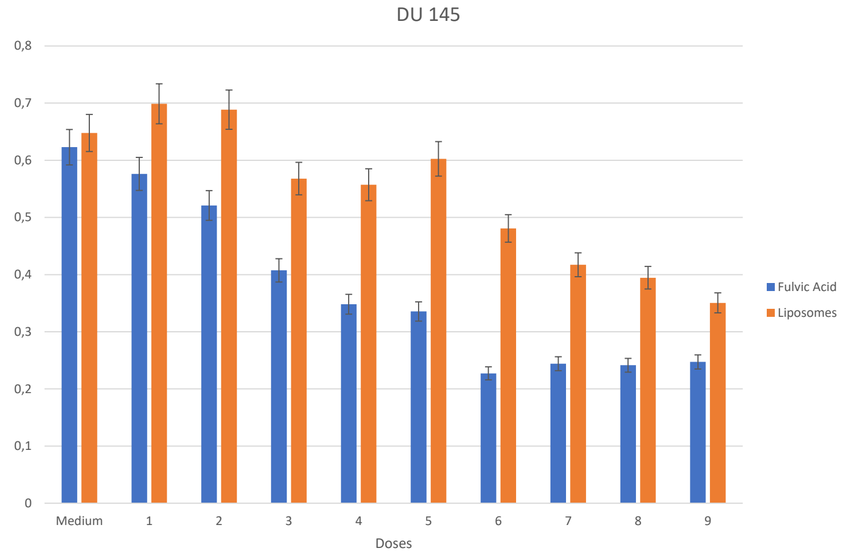

Figure 1: DU 145 cell growth relative to a control well containing medium only, 24 hours after exposure. FA indicates fulvic acid only; LiFA indicates Fulvic acid + liposomes (1,5%)

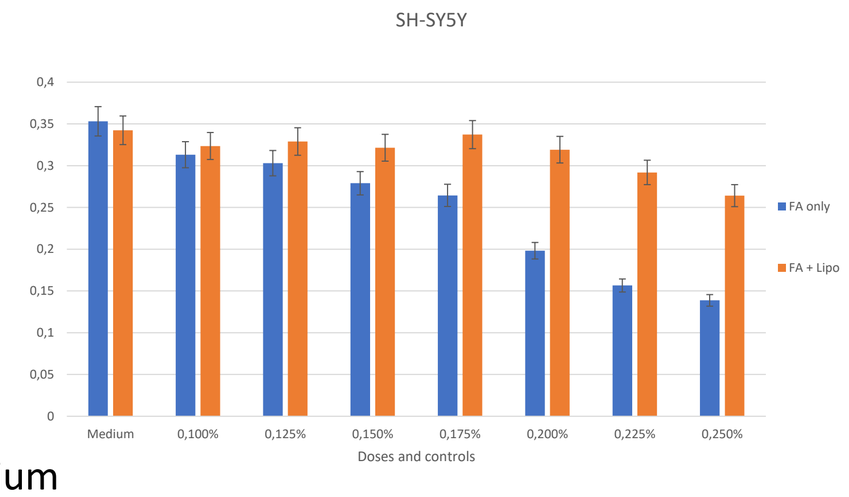

Figure 2: SH-SY5Y cell growth relative to a control well containing medium only, 24 hours after exposure. FA indicates fulvic acid only; LiFA indicates Fulvic acid + liposomes (1,5%)

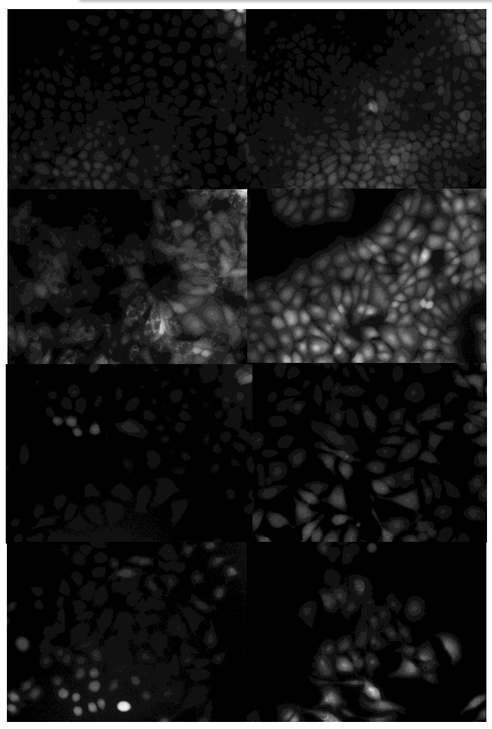

Figure 4: Results from DCFDA staining, showing ROS production in (clockwise starting from top left): SH-SY5Y cells exposed to only medium; H_2O_2; 0.25% FA; and 0.25% LiFA

Figure 5: Results from DCFDA staining, showing ROS production in (clockwise starting from top left): DU 145 cells exposed to only medium; H_2O_2; 0.5% FA; and 0.5% LiFa

DU 145

SH-SY5Y

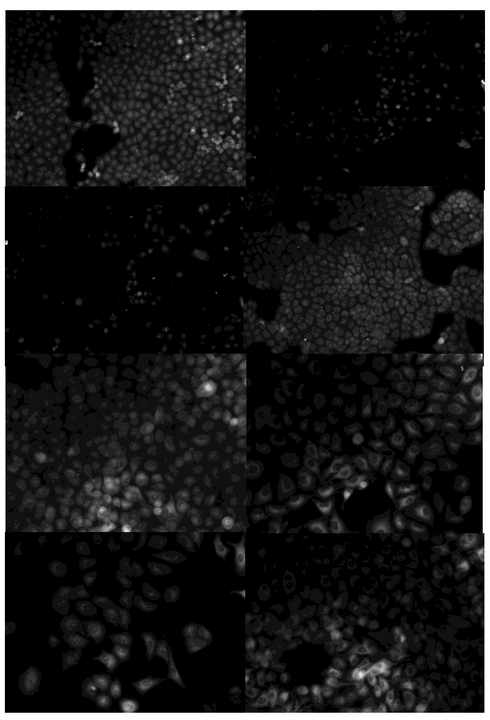

Figure 6: Results from acridine orange staining, showing cell viability in (clockwise starting from top left): SH-SY5Y cells exposed to only medium; C16; 0.25% FA; and 0.25% LiFA

Figure 7: Results from acridine orange staining, showing cell viability in (clockwise starting from top left): DU 145 cells exposed to only medium; C16; 0.5% FA; and 0.5% LiFA

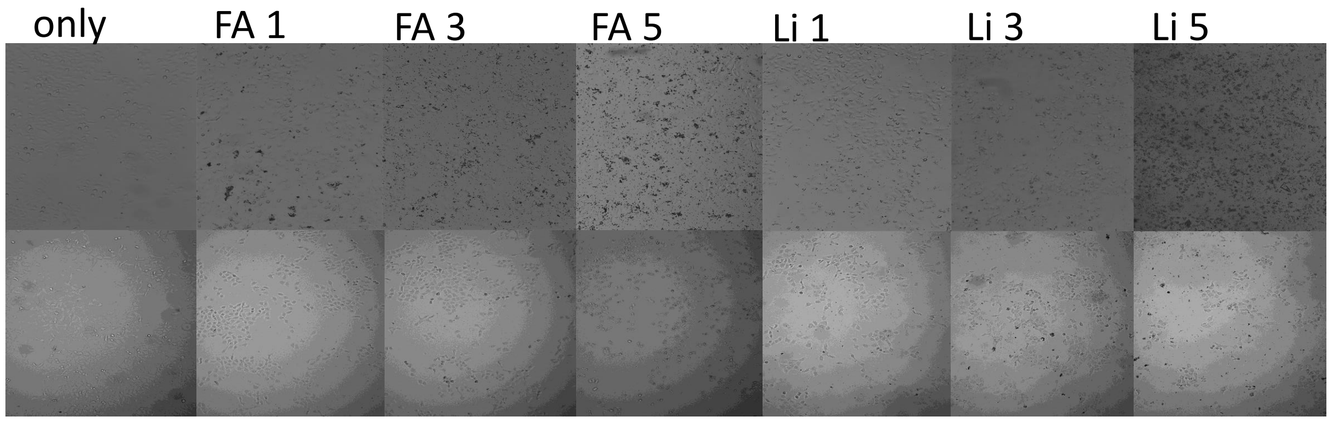

Figure 3: Results from light microscopy comparing the cells exposed to fulvic acid (FA) and fulvic acid embedded in liposomes (Li) from both DU 145 and SH-SY5Y cell lines

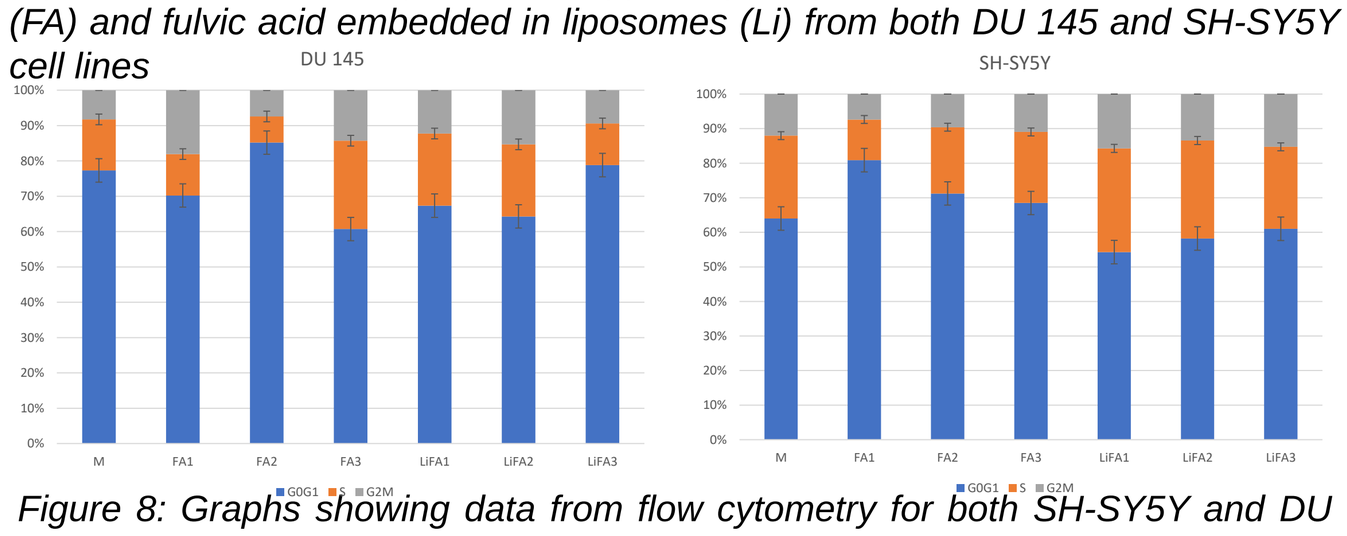

Figure 8: Graphs showing data from flow cytometry for both SH-SY5Y and DU 145 cell lines, showing proportions of living cells in each phase of the cell cycle after exposure to different concentrations of FA indicates fulvic acid only; LiFA indicates Fulvic acid + liposomes (1,5%)

Discussion and conclusion

This study suggests that fulvic acid exhibits antiproliferative activity and induces cell rounding in a dose-dependent manner in both the SH-SY5Y AND DU-145 cell lines. However, when cells are co-exposed to fulvic acid and liposomes, the antiproliferative effects are reduced, yet cell rounding is increased. Neither compounds seem to affect the cell cycle significantly after 24 hours of exposure. This study contributes to the understanding of the potential benefits of using liposomes as nanocarriers for phytochemicals.

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