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Photodynamic Activity of Hypericin-Loaded Liposomes in Breast Cancer Cells

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INTRODUCTION & AIM

Photodynamic therapy (PDT) is based on the light activation of a photosensitizer, leading to the generation of reactive oxygen species (ROS) and subsequent cell damage. Hypericin is an effective natural photosensitizer activated by visible light, particularly in the orange range (around 590 nm), enabling efficient singlet oxygen generation [1]. However, its application is limited by poor solubility and restricted intracellular accumulation. Liposomal encapsulation may improve its stability, delivery, and photodynamic performance. To evaluate the photodynamic activity of liposomal hypericin in breast cancer models, focusing on cellular uptake, photocytotoxicity, and inhibition of cell migration.

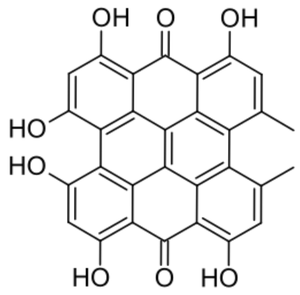

Figure 1. Chemical structure of hypericin, a naturally occurring photosensitizer used in photodynamic therapy.

METHODS

Human breast cancer cell lines MCF-7 and MDA-MB-231 were cultured under standard conditions (37°C, 5% CO_2). Free hypericin was dissolved in ethanol to obtain a stock solution and subsequently diluted in culture medium to achieve the desired concentrations, while liposomal hypericin was prepared using the thin-film hydration method using HSPC/DSPE-PEG 2000 followed by extrusion to obtain nanosized vesicles.

Dark cytotoxicity and phototoxicity were evaluated using the MTT assay. Cells were seeded in 96-well plate and treated with free or liposomal hypericin (0.5 and 1 μM). Dark toxicity was assessed after 24 h incubation without irradiation. For photodynamic treatment, cells were incubated with hypericin for 24h, washed, irradiated with orange light at 7.2 J/cm^2, and further incubated for 24 h. MTT solution was added, and after incubation, formazan crystals were dissolved in DMSO. Absorbance was measured at 490 nm, and cell viability was expressed relative to control.

Cellular uptake was analyzed by flow cytometry. Cells were seeded in 12-well plates and incubated with free and liposomal hypericin (1 μM) for 4 h and 24 h. After washing, cells were trypsinized, resuspended in PBS, and analyzed using a Cytoflex flow cytometer. Uptake was quantified based on hypericin fluorescence and analyzed with FlowJo software.

Wound healing assay was performed using Culture-Insert 2 Well in μ-Dish. Cells were seeded to form a confluent monolayer and treated with liposomal hypericin (1 μM) followed by photodynamic irradiation. After insert removal, fresh medium was added, and cell migration into the wound area was monitored at 0, 24 and 48 h using light microscopy. Control cells were not treated. Wound closure was quantified using Fiji software [2].

All experiments were performed in replicates, and results were expressed as mean ± SD. Statistical analysis was conducted using one-way ANOVA, with $p < 0.05$ considered statistically significant.

RESULTS & DISCUSSION

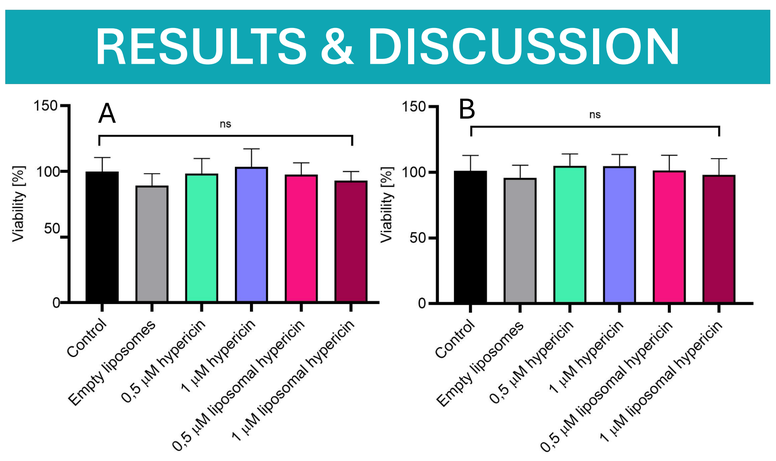

Figure 2. Cell viability of (A) MCF-7 and (B) MDA-MB-231 cells after 24 h incubation with free and liposomal hypericin (0.5 and 1 μM) under dark conditions, assessed by the MTT assay.

No significant dark cytotoxicity was observed (Fig. 2) for either free or liposomal hypericin in MCF-7 and MDA-MB-231 cells, as cell viability remained close to control levels across all tested concentrations. Empty liposomes also showed no cytotoxic effect.

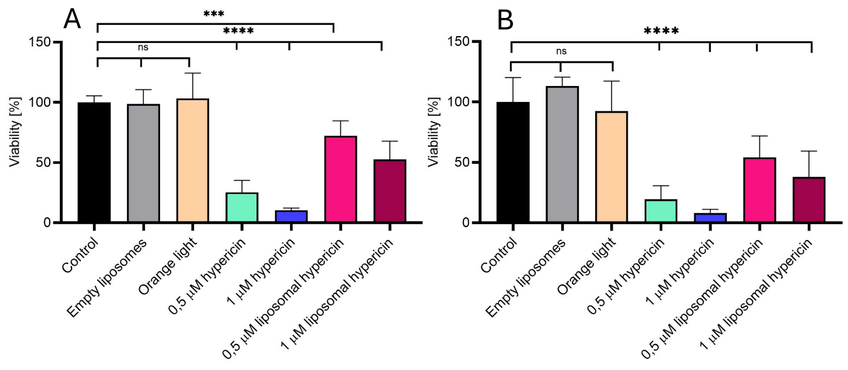

Figure 3. Cell viability after photodynamic treatment (7.2 J/cm^2) with free and liposomal hypericin (0.5 and 1 μM) in MCF-7 and MDA-MB-231 cells.

We observed that upon photodynamic activation (Fig. 3), liposomal hypericin significantly reduced cell viability in a concentration-dependent manner, confirming its effective light-triggered cytotoxicity. Although free hypericin showed stronger phototoxic effects, the liposomal formulation maintained high efficacy while exhibiting minimal dark toxicity. No significant effect of light alone was observed. Overall, liposomal hypericin demonstrated a favorable balance between safety and photodynamic activity, supporting its potential as a controlled photosensitizer delivery system.

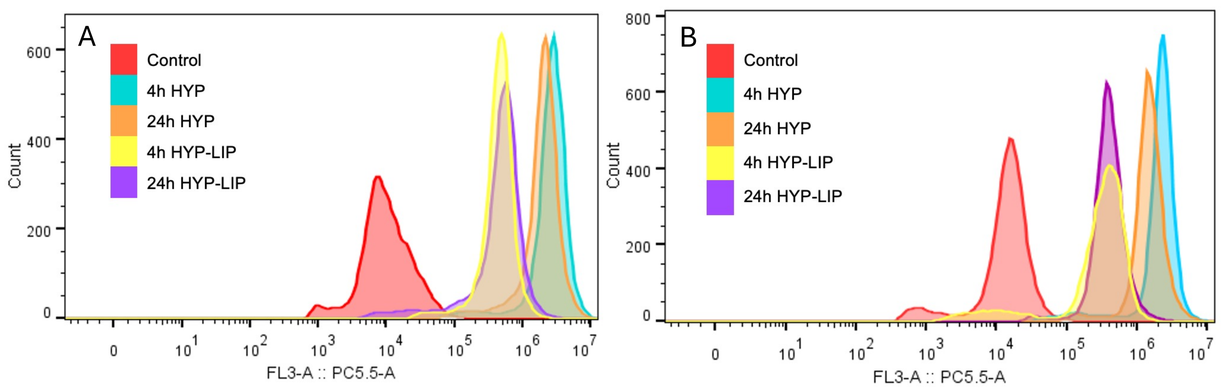

Figure 4. Cellular uptake of free and liposomal hypericin in MCF-7 (A) and MDA-MB-231 (B) cells after 4 h and 24 h incubation at 1 μM.

Although free hypericin showed higher cellular uptake (Fig. 4) at both 4 h and 24 h in both (A) MCF-7 and (B) MDA-MB-231 cells, with high fluorescence intensity, liposomal hypericin showed cell line–dependent differences. In MCF-7 cells, higher fluorescence was observed at 4 h, whereas in MDA-MB-231 cells, higher signal was observed at 24 h. Despite lower overall intensity compared to the free form, liposomal hypericin showed measurable and time-dependent high intracellular accumulation, which may support its use as a controlled delivery system for photodynamic therapy.

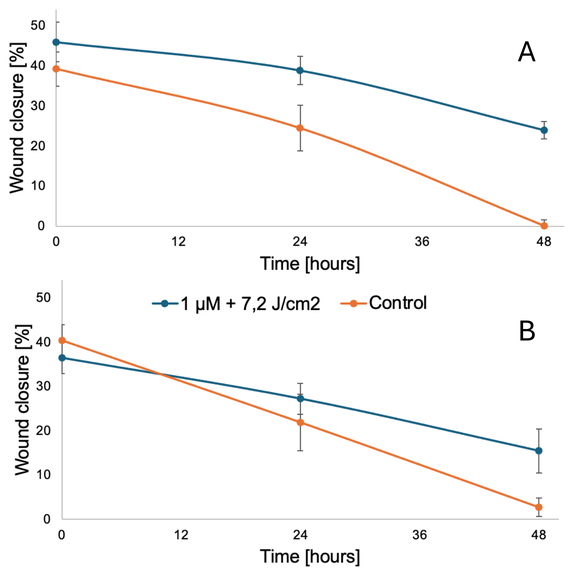

Figure 5. Wound healing assay in (A) MCF-7 and (B) MDA-MB-231 cells after PDT.

Photodynamic treatment with liposomal hypericin inhibited cell migration in both MCF-7 and MDA-MB-231 cells (Fig. 5). Compared to control, reduced wound closure was observed at 24 h and 48 h, indicating impaired migratory capacity. The effect was more pronounced at later time points, with minimal wound closure observed after 48 h following photodynamic treatment.

Together, these findings indicate that liposomal hypericin enables effective photodynamic activity despite lower cellular uptake, supporting its role as a controlled and biologically active photosensitizer delivery system.

CONCLUSIONS

Liposomal encapsulation enhances the photochemical safety and therapeutic applicability of hypericin by enabling controlled delivery and effective PDT responses. These findings support liposomal hypericin as a promising photosensitizer with improved translational potential for photodynamic cancer therapy.

REFERENCES

The authors declare no conflicts of interest.

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