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Cannabinoid-loaded receptor-targeted nanoparticles improve anticancer activity through delayed cell internalization

Durán Lobato Matilde[1], Álvarez Fuentes Josefa[1], Fernández Arévalo Mercedes *[1], Martín Banderas Lucía[1]

[1] Department of Pharmacy and Pharmaceutical Technology, Facultad de Farmacia, Universidad de Sevilla, c/ Prof. García González nº2 41012, Sevilla, Spain
*Correspondencia: mfarevalo@us.es

1. Introduction

Δ9-tetrahydrocannabinol (Δ9-THC) is known for its antitumor activity and palliative effects. However, its unfavorable physicochemical and biopharmaceutical properties, including low bioavailability, psychotropic side effects and resistance mechanisms associated to dosing make mandatory the development of successful drug delivery systems. In this work, several steps forward were taken by including a targeting ligand and a multifunctional fluorescent labeling strategy in the presented approach. Transferrin (Tf), a targeting moiety for cancer cells based on a higher expression of the Tf receptor in tumor cells [1-4], was coupled to Δ9-THC-loded PLGA NPs to modulate the interaction of the particles with the target cells. In addition, a double fluorescent labeling of the formulations, both through chemical linkage to the polymer and through dye encapsulation, was performed in order to selectively track the internalization pathway and intracellular fate of both the carrier and the cargo. The resulting formulations were evaluated in order to correlate the modulation of their anticancer effect with their cell internalization mechanics.

2. Materials and methods

Δ9-THC was provided by THC Pharma GmbH, (Frankfurt/Main, Germany). Poly(DL-lactide-co-glycolide) Resomer® RG 502H was obtained from Evonik-. Poly(vinyl alchohol) (87 – 90 % hydrolyzed, Mw: 30,000 – 70,000) PVA, Nile Red, Rhodamine B, human transferrin (Tf), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl), N-Hydroxysuccinimide (NHS), fluorescein isothiocyanate isomer I (FITC), sucrose and genistein were purchased from Sigma-Aldrich (St Louis, MO; USA). Glycerol was obtained from Acofarma Distribución S.A. (Barcelona, Spain). Trehalose was obtained from VWR International Eurolab S.L. (Barcelona, Spain). Solvents used ethyl acetate (EA), acetonitrile, methanol, pyridine, dimetylsulfoxide (DMSO) (HPLC-grade) and acetic acid were purchased from Panreac Química (Barcelona, Spain). Deionized and filtered water was used in all the experiments (Milli-Q Academic, Millipore, Molsheim, France).

The RP-HPLC analysis was carried out with a Hitachi LaChrom® (D-7000) Series HPLC (Waters column 3 µm, 4.6x100 mm, at 25.0 ± 0.1 ºC)

For cell line experiments, human colon adenocarcinoma cells (Caco-2) cells were obtained from the European Collection of Cell Cultures (ECACC); number 86010202 (Salisbury, UK).

PLGA NPs were produced by a modified emulsion solvent evaporation method (SEV) and Tf was conjugated on the surface of PLGA NPs and THC-PLGA NPs. PLGA was previously conjugated with FITC. FITC was covalently coupled to PLGA by the carbodiimide method reported elsewhere. Doubled-labeled fluorescent PLGA NPs weer obtained by encapsulating nile red into FITC-PLGA NPs.

3. Results and Discussion

We obtained monodispersed population of spherical nanostructures in agreement with the DLS characterization results and our previous works on THC-PLGA NPs, with no observable difference induced by the Tf coupling procedure or the presence of this moiety on the nanoparticle surface.

Caco-2 culture, a cancer cell model bearing cannabinoid receptors. Upon incubation with the cells, both plain THC PLGA NPs and Tf-THC PLGA NPs avoided moderate cell viability increases exerted by free THC at short incubation times, which have been associated in the literature to drug resistance mechanisms. Furthermore, Tf-THC PLGA NPs exerted a cell viability decrease down to 17 % vs. 88 % of plain nanoparticles in a cancer cell model, however their internalization was significantly slower than plain nanoparticles. Uptake studies in the presence of inhibitors indicated that the nanoparticles were internalized through cholesterol-associated and clathrin-mediated mechanisms. Overall, the observations suggested that the improved Δ9-THC antitumor effect was potentially due to increasing the presence of the nanocarriers, and hence maximizing the amount of drug locally released, at the surface of cells bearing cannabinoid receptors, instead of improving internalization.

4. Conclusions

The results obtained highlight the promising potential of Δ9-THC-loaded nanocarrier-based antitumor therapies, as well as exploring further strategies aimed at modulating the nanocarrier action at the cell surface.

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