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Cannabis-based terpenes loaded in PEG-PLGA nanoparticles for pain management

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1. Introduction

In the recent decade, more attention has been paid to the cannabis-based extracts and products to fill the gap left by analgesics currently available in the clinic. The resin of Cannabis sativa is highly rich in terpenes, many of which have been found to exert various biological activities. Among terpenes found in cannabis, beta-myrcene, beta-caryophyllene and nerolidol may be promising in pain management1 However, the volatile and hydrophobic nature, and associated poor solubility and low bioavailability, may limit their suitability as pharmaceutical agents.

We hypothesized that the encapsulation of these terpenes in polymeric nanoparticles may enhance their pharmacological effects, in particular in pain management.

2. Materials and methods

2.1. NPs production and loading capacity

Beta-myrcene (MC), beta-caryophyllene (CPh) and nerolidol (NL) were loaded into poly(ethylene glycol)-poly(lactide-co-glycolide) (PEG-PLGA) nanoparticles (NPs) using the emulsion-solvent evaporation method 2-3.

The encapsulation capacity was measured using a GC-MS method, as previously described.14 Peaks of MC, CPh, cis-NL, and trans-NL were observed at a retention time of 6.92, 5.73, 6.77, and 7.16 min, respectively. For quantification purposes, the characteristic ions for terpenes at m/z 69, 93 and 133 were monitored by selected ion monitoring (SIM) mode.

2.2. In vitro cytotoxicity

The cytotoxicity of the terpene-loaded NPs was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide (MTT) proliferation assay. Briefly, cells seeded into 96-well plates and incubated overnight were treated with various concentrations of free terpenes, terpene-loaded NPs, and blank NPs. After 24 h of incubation, MTT solution (0.5 mg/mL in cell culture medium) was added for 4 h and the resulting formazan crystals were dissolved with DMSO. Untreated cells and cells treated with Triton X 1 % served as controls. Finally, the optical density (OD) at 570 nm was determined using a microplate reader (Synergy HT, BioTek Instruments, Inc., Vermont, USA). The relative cell viability (%) was calculated as [(OD treated cells/OD control (untreated) cells)×100].

2.3. Calcium signaling assay

The terpene-loaded PEG-PLGA NPs were tested in HEK cells that express the nociceptive transient receptor potential vanilloid-1 ion channel (TRPV1), a non-selective ligand-gated cation channel that is involved in pain sensation. The activation of TRPV1 leads to an influx of Ca2+,

which was measured by the fluorescent indicator Fluo-4 acetoxymethyl (AM). Fluorescence was measured using a plate reader (Synergy HTX, BioTek, USA), at an excitation/emission wavelengths of 485 nm and of 528 nm, respectively and the intensity of intracellular green fluorescence was observed using Nikon inverted microscope Eclipse Ti (Japan).

3. Results and Discussion

The terpenes were successfully encapsulated in the PEG-PLGA NPs which had a mean size between 250 – 350 nm, a narrow size distribution, and a negative zeta potential of around -20 mV.

The half maximal inhibitory concentration (IC50) values of all terpene-loaded NPs were approximately 4-5-fold less than that of the corresponding free terpene ($p < 0.001$ for all terpenes). Moreover, blank PEG-PLGA NPs exhibited negligible cytotoxicity in this cell line at the dilutions corresponding to that of MC-loaded NPs (particles with the lowest EE%). This finding signifies that the cytotoxicity of the terpene-loaded NPs is mainly caused by the encapsulated terpenes, with minimal or no effect from other formulation components.

The calcium signaling assay showed that the nanoparticle formulations significantly increased the intensity of fluorescence in comparison with free terpenes.

When combinations of different types of formulations were assayed, we found that in general, all combinations of free terpenes improved the calcium influx in comparison with individual terpenes, and this effect was significant in the combination of MC/NL. Impressively, it was found that all combinations of NP formulations produced remarkably higher calcium responses when compared to combinations of their respective free terpenes

4. Conclusions

Our findings suggest that the newly developed PLGA-based nanosystems can remarkably increase the ability of the tested cannabis-based terpenes to enhance the fluorescence intensity driven by the intercellular calcium ion influx. This effect is correlated with the activation of TPRV1 channels which indicates that the terpene NPs may have great potential for application in pain management.

Acknowledgments

We specially thank Biology and Spectrophotometry Services from Centro de Investigación, Tecnología e Innovación (CITIUS, Universidad de Sevilla) for technical assistance.

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Este trabajo debe ser citado como:

El Hammadi MM, Small Howard AL, Fernández Arévalo M, Martin Banderas L. Cannabis-based terpenes loaded in PEG-PLGA nanoparticles for pain management. Rev Esp Cien Farm. 2021;2(2):198-9.