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CBD-loaded NPs increases glucose uptake and attenuates palmitate-induced lipid accumulation in human HepG2 hepatocytes

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1. Introduction

Cannabidiol (CBD) is a non-psychoactive cannabinoid that exhibits pain relief, anti-inflammatory, anti-oxidant, anti-tumor and neuroprotective properties. Furthermore, many studies have concluded that CBD exhibits several effects on both lipid and glucose metabolism through its action on various receptors and several metabolites. Accordingly, it has been found to decrease total cholesterol levels and to increase HDL levels, reduce intracellular lipid content and attenuate liver steatosis. Additionally, CBD has shown the ability to alleviate the symptoms of insulin resistance, minimize incidence of diabetes in mice and reduce the possibility of the metabolic syndrome among cannabis users.

So, the objective of present work is to prepare CBD-loaded PLGA nanoparticles (NPs) and test their ability to increase glucose uptake and inhibit lipid accumulation in human HepG2 hepatocytes.

2. Materials and methods

PEG-PLGA-based nanoparticles were prepared using a nanoprecipitation method. The cytotoxicity of CBD NPs was evaluated by the MTT proliferation assay.

Additionally, glucose uptake was assessed by pre-treatment of HepG2 with the NPs followed by incubation with 2-NBDG (2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose) and finally measuring the cellular fluorescence resulted from the internalization of the fluorescent glucose analog. HepG2 cells were seeded at 5 x 104 cell/well in medium supplied with serum, seeded in 96-well black plates with transparent bottom. Cells were incubated for 24 h. After this, cells were divided into groups of 5 wells. Each group receives different treatment (control; Metformin 5 μg/ml (positive control); Free CBD 1 μg/ml; Free CBD 5 μg/ml; CBD NPs 1 μg/ml; CBD NPs 5 μg/ml; Cells were incubated for 24 h. On day 3, glucose uptake measurement – fluorescence were carried out. Cells were washed with PBS (200 μl), and incubated with 2-NBDG

50 µM (glucose analog) in glucose-free medium for 3 h. After incubation, cells were washed with ice-cold PBS twice (200 µL). 200 µL ice-cold PBS and fluorescence was measured at Excitation / Emission: 485 nm / 535 nm. Experiment was performed in triplicate.

Furthermore, we explored effects of NPs on lipid accumulation induced by sodium palmitate in HepG2 cells. Cells were seeded on six-well plates at 5 × 105 cells/well, and incubated overnight. Day 2, the cells are incubated with free or encapsulated CBD in the presence of 0.35 mM sodium palmitate for 24 h in serum-free MEM containing 1 % w/v fatty acid free BSA. Day 3 Lipids and protein accumulation are measured via cholesterol and TG reagent kits (Sigma) according to the manufacturer's instructions. Proteins were measured using BCA kit.

3. Results and Discussion

CBD-loaded NPs had a mean diameter of 166.8±5.9 nm, a PdI of 0.245±0.019 and a zeta potential of around -32.8±1.9.

IC50 of CBD NPs and free CBD was 11.3±1.0 µg/mL and 9.9±1.9 µg/mL, respectively, with no significant difference between the two groups.

Cells incubated with CBD NPs showed significant improvement in cellular uptake of glucose, in comparison with control cells, and the effect was similar to that introduced by metformin.

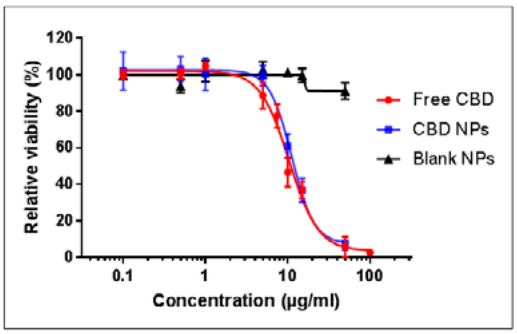

Figure 1. IC50 values for free and CBD loaded-PLGA nanoparticles

Furthermore, treatment with CBD NPs (5 µg/ml) significantly inhibited palmitate-induced accumulation of triglyceride and cholesterol in HepG2 cells, in comparison with free CBD. Likewise, HepG2 cells incubated with palmitate and CBD NPs and subsequently stained with oil red showed remarkably lower levels of the fat-soluble dye when compared to cells treated with free CBD.

4. Conclusions

The CBD-loaded PLGA NPs may be potentially useful in preventing and treating obesity-associated insulin resistance and elevated levels of cholesterol and triglyceride.

Acknowledgments

We specially thank funding from Instituto de nvestigación Sanitaria IDIVAL (Ref. INNVAL18/30. Authors also thank Biology and Microscopy 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 Mazen M, Vázquez Bourgon J, Berrocoso E, Crespo Facorro B, Suárez Pereira I, Martin Banderas L. CBD-loaded NPs increases glucose uptake and attenuates palmitate-induced lipid accumulation in human HepG2 hepatocytes. Rev Esp Cien Farm. 2021;2(2):200-1.