Encapsulated Cannabidiol in Nano-Structured Lipid Carriers for Treating Radiation Dermatitis

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INTRODUCTION & OBJECTIVES

Over fifty percent of cancer patients receive radiation therapy, but many suffer from radiation dermatitis, which significantly impacts their quality of life. Current treatment options often fall short in reducing pain and inflammation. Improved solutions are therefore necessary for providing relief. Cannabidiol (CBD), a non-psychoactive compound from the cannabis plant, shows promise in reducing inflammation and pain.[1] Most research has focused on CBD in consumption or inhalation, with limited studies on its topical use. Since the endocannabinoid system (ECS) is crucial for skin function, topical cannabinoids may effectively treat radiation-induced dermatitis.[2] CBD's physicochemical properties present significant challenges for formulating lotions. These challenges include a high log P value that indicates its lipid/water partitioning, poor water solubility, and slow permeability and absorption through the skin. To improve the solubility of CBD in aqueous lotion formulations and to enhance its skin permeability, we encapsulated CBD in nano-structured lipid carriers. We employed modeling and simulation techniques to assess formulation partitioning and diffusion into the skin, allowing us to identify suitable formulations that enhance CBD's permeability.

MATERIALS AND METHODS

CBD (PureForm Global), Olive oil (Millipore Sigma), Span 80 (Croda), Transcutol (Gattefossee), Tween 80 (Croda), Sapenio P600 (Seppic), Cetostearyl alcohol (Croda), and Purified water (Pharmco) were used as received. The CBD topical cream formulation is prepared using our patented procedure (US 12,059,393), which includes several lipid compositions, methods for creating nanostructured lipid carriers for CBD encapsulation, and their application in treating radiation dermatitis.

Skin Permeation was estimated using the GB Kasting skin permeation model[3] and a proprietary model developed by Amjad Basha.[4] Cytotoxicity was assessed with a lactate dehydrogenase (LDH) assay using media collected from the tissue wells. Gene expression in the skin was assessed using a 3D in vitro skin model containing epidermal keratinocytes and dermal fibroblasts (Mattek EFT-400). The efficacy of our topical cream for the treatment of radiation dermatitis was evaluated using BALB/c Mouse Model.

RESULTS AND DISCUSSION

Achieving balanced skin hydration in the stratum corneum and ensuring effective permeability of the active ingredient, CBD, into the epidermis and dermis are key factors in reducing pain and inflammation caused by radiation. CBD is insoluble in water, and based on our physicochemical calculations, merely solubilizing CBD in oil, such as olive oil, would not allow CBD to permeate through the epidermis and dermis. For instance, we calculated for a dosage scenario of 10 µg/cm^2 of CBD when applied to the skin with 1 mg /cm^2 of olive oil and presented the CBD distribution (Table 1).

CBD did not diffuse through the skin to provide any therapeutic benefit. Minimizing the evaporation of CBD from the skin surface and maximizing its permeability through the stratum corneum are key to successful cream development.

Table 1: Distribution of CBD on skin using olive oil alone as a vehicle

Calculated Permeant Distribution			
Removed	0.0 %	0.0	µg/cm²
Evaporated	64.28 %	0.643	µg/cm²
Surface	0.0 %	0.0	µg/cm²
Stratum Corneum	2.61E-02 %	2.610E-04	µg/cm²
Viable Epidermis	2.22E-02 %	2.216E-04	µg/cm²
Dermis	0.27 %	2.742E-03	µg/cm²
Systemically Absorbed	35.41 %	0.354	µg/cm²
Total	100.01 %	1.000	µg/cm²

Several experiments were designed with support from molecular modeling to arrive at an optimal topical crème formulation (Table 2). The CBD cream is prepared by weighing out the specified quantities of Olive oil, Span 80, Cetostearyl alcohol, and Transcutol. Add CBD to this mixture and stir at room temperature until the CBD is completely dissolved, resulting in a clear oil phase. In a separate vessel, combine Tween 80 with purified water. Mix thoroughly using an overhead mixer until a homogeneous and transparent solution is obtained. While continuously mixing the aqueous phase, slowly introduce the oil phase. Maintain homogenization throughout the addition to ensure the formation of a stable emulsion. Once the oil phase is fully incorporated, gradually add Sepineo™ P 600 (polymeric thickener) to the emulsion. Continue mixing until a uniform cream with a consistent texture is obtained.

There are several methods reported for the nano-structured lipid preparation.[5] We employed the microemulsion method as it is effective in stabilizing the crème formulations.

Table 2: Topical CBD crème formulation

Ingredient	Amount (%)
CBD	3
Olive oil	30
Span 80	4
Cetostearyl alcohol	4
Transcutol	6
Tween 80	4
Sepineo™ P 600 (Polymeric thickener)	4
Water	45

For the LDH Cytotoxicity Assay, the culture medium from each tissue sample was diluted 1:10 with sterile Phosphate Buffered Saline (PBS). A background control (diluted culture medium that was not used for cell culture), a "Low Control" (diluted treatment medium collected from the Untreated culture wells), and a "High Control" (diluted culture medium collected from the 1% Triton X-100 treated culture wells) were included in the assay. Each diluted sample was added to an optically clear, flat-bottom 96-well plate in duplicate. The LDH reaction mixture (Takara) was prepared and added to each aliquot diluted medium (1:1). The reaction plate was incubated for ~20 minutes at room temperature, protected from light. Stopping solution (1.0N HCl) was then added to each well and absorbance was measured at 492nm with a reference filter at 620nm. Each sample absorbance value was calculated as the mean OD492-0D620 value for the duplicate reaction wells, with the blank absorbance value subtracted. The % Cytotoxicity was then calculated relative to the Untreated (negative control, set to 0% cytotoxicity) and the Triton X-100 treated (positive control, set to 100% cytotoxicity) absorbance values, according to kit instructions: % Cytotoxicity = [(Test Media Value —Low Control)/(High Control —Low Control)] *100

The cytotoxicity results are presented in Figure 1. Increased LDH activity is an indicator of damaged or dead cells. Neither CBD nor the vehicle is cytotoxic. Statistical data analysis (unpaired t-test, $p<0.05$) was performed to compare the TM groups and the Vehicle group to an Untreated Control Group. Tissues treated with Triton X-100 served as a positive control.

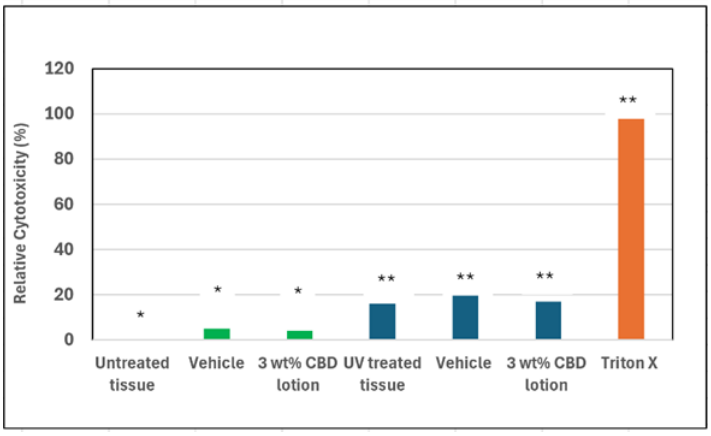

Figure 1: Cytotoxicity of CBD lotion compared at various scenarios. The * ($P \leq 0.05$) and ** ($P \leq 0.001$) symbol designates statistical significance after performing unpaired t-test

Gene expression was assessed using Genemarkers CBD Panel, a qPCR-based gene expression panel which contains 163 target genes, 5 endogenous control genes, and 10 additional target genes (CDKN1A, BAX, GADD45A, CHEK1, H2AX, GDF15, AKT1, CDKNA2, BCL2, TGFB1). Tissues were irradiated with 200 mJ UVB/cm^2 prior to the application of the TMs.

Table 3: Statistically Significant Percent Changes in Gene Expression of CBD cream Relative to vehicle (Olive Oil)

Gene ID	**Function**	**Vehicle**		**30 mg CBD cream**	
		FC	**Δ**	**FC**	**Δ**
BCL2	Antiapoptotic	-2.53	-60%	1.53	53%
KRT14	Cell Renewal/ Regeneration	1.59	59%	-2.25	-56%
KRT5	Cell Renewal/ Regeneration, pigmentation and fibrosis	n.s	n.s	-2.67	-63%
LAMA3	Tissue Integrity / Remodeling	-1.66	-40%	-3.26	-69%
SERPINH1	Extracellular matrix integrity	-2.94	-66%	2.07	107%

Gene expression was assessed 24 hours following the application of the TMs. Statistically significant (unpaired t-test, $p<0.05$, N=4) changes in gene expression are shown in Table 3. Statistically significant changes that correspond to linear fold change values > 2.0 are shown in bold; linear fold change values (FC) < 1.5 are shown in grey text, "n.s." = not Statistically significant. Linear fold-change values of 2 or greater are typically considered biologically relevant, but in the personal care industry, fold-change values of 1.5 are often seen in marketing materials.

The addition of CBD to the UV-exposed tissues produced effects at the lowest and highest doses, with different genes being impacted at the different doses. The lower dose (30 mg) regulated genes associated with cell renewal, tissue remodeling, anti-apoptotic pathway, and the extracellular matrix integrity, such as BCL2, KRT14, KRT5, LAMA3 and SERPINH1. Whereas the 120 and 250 mg doses produced significant increases in antioxidant/stress response genes such as, SLC30A1, MT1F, HAL MT1G and HMOX1. (not shown in the Table)

For the mice study, 144 female Balb/c mice were randomized into six (6) groups of 24 animals each. Mice had the skin on the back shaved and depilated prior to Day 0. For dermatitis induction on Day 0, animals in Groups 2-6 were anesthetized via a single intraperitoneal injection of xylazine and ketamine prior to being administered a single dose of 30 Gy of radiation directed to the dorsal skin. The animals in Groups 1 and 2 did not receive treatment. The skin redness was evaluated starting

on Day 8 and continuing every other day until Day 28. The results are reported in Figure 2

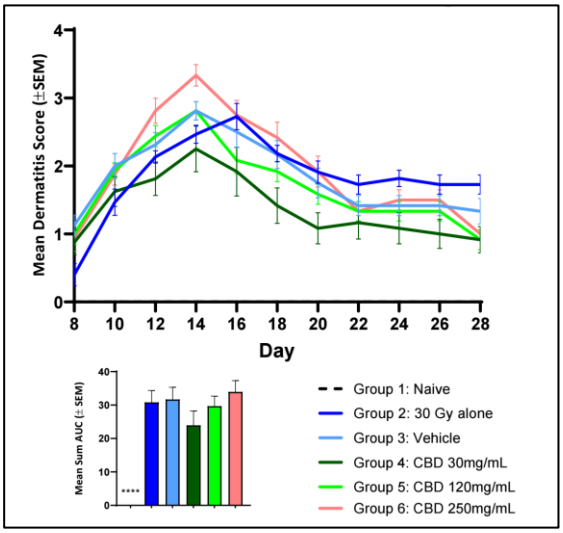

Figure 2: Blinded Clinical Dermatitis Scoring

Naïve animals experienced significantly less weight loss and had lower dermatitis and redness scores compared to those treated with a vehicle or exposed to radiation. Animals treated with 30 mg/mL CBD showed modest improvements in weight loss and consistently reduced dermatitis and redness scores compared to both vehicle-treated and radiation-only groups. Combined, all CBD-treated groups had notably lower dermatitis and redness scores than radiation or vehicle groups at most timepoints. Additionally, CBD treatment, especially at 30 mg/mL, decreased the percentage of days with moderate or severe dermatitis. Higher CBD doses yielded less consistent benefits. No naïve animals developed dermatitis scores of 2 or above at any time point.

CONCLUSIONS & PERSPECTIVES

The CBD topical cream formulation reported in this paper was developed by following our patented procedure (US 12,059,393), which details innovative lipid compositions and methods for creating nano-structured lipid carriers. These carriers are specifically designed to encapsulate cannabidiol (CBD), enhancing its stability and permeability for topical applications. The encapsulated CBD is then utilized in formulations intended for the treatment of radiation dermatitis. This approach ensures that the active CBD ingredient is efficiently delivered to the affected skin areas, potentially providing therapeutic benefits by minimizing evaporation from the skin surface and maximizing its absorption through the stratum corneum. The patented method supports the preparation of a stable and uniform cream that addresses key challenges associated with CBD's physicochemical properties, thereby improving its effectiveness in managing pain and inflammation related to radiation dermatitis.

Quantum mechanical (QM) calculations are instrumental in the development and optimization of such lipid-based delivery systems. These computational methods allow researchers to model and predict the molecular interactions within the lipid matrix, facilitating the design of structures that can modulate gene expression. Specifically, QM calculations can help tailor the lipid composition to influence genes associated with pain and inflammation, which is especially relevant for managing the discomfort and tissue damage resulting from radiation exposure. By adjusting the lipid structure at the molecular level, it is possible to enhance the efficacy of CBD in mitigating radiation-induced pain and skin damage.

Despite these encouraging findings, further research is essential to confirm the safety and effectiveness of this therapeutic approach. Rigorous preclinical studies, followed by comprehensive clinical trials, are necessary to validate the benefits of CBD/nano-structured lipid formulation in patients suffering from radiation-induced dermatitis. These studies will provide critical data on dosage, long-term effects, and potential side effects, paving the way for the eventual adoption of this therapy in clinical practice. Ultimately, the goal is to offer an innovative and effective treatment option for individuals experiencing skin complications and pain after radiation therapy.

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