Inclusion cyclodextrin complex of cannabinoids: A promising option for improving physicochemical properties and biological performance

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ABSTRACT

Purpose: The acidic cannabinoids are naturally occurring and have recently been highlighted as potential drug candidates for treating different types of cancer. However, their anticancer activity is limited by instability and low water solubility. The objective of this study was to develop an acidic cannabinoid/cyclodextrin inclusion complex for improving the solubility and potentially biological performance of acidic cannabinoids via cyclodextrin (CD) complexation. ***Method:*** Phase solubility studies of acidic cannabinoids were performed with methylated-β-CDs (M-β-CD). The preparation methods of inclusion complexes were optimized by loading capacity and complexation efficiency. The inclusion complexes were characterized by solubility determination, differential scanning calorimetry (DSC), field emission scanning electron microscopy (FE-SEM). Cytotoxic effects of the acidic cannabinoid/M-β-CD complex were performed to investigate the inhibitory effect on MCF-7 breast cancer cells after M-β-CD complexation with each acidic cannabinoid (THCA or CBDA). ***Results:*** The aqueous solubility of THCA significantly increased to 520.3 μg/mL as the acidic cannabinoids/M-β-CD complexes were prepared by spray freeze drying method at the same molar ratio (1:2); this was 3-folds higher compared to those made by spray drying or freeze-drying methods. The spray freeze-drying process obtained the optimum cannabinoids/M-β-CD inclusion complexes. Thermograms of the M-β-CD complex showed two endothermic peaks while the characteristic peak of acidic cannabinoids was absent, indicating the successful inclusion of THCA or CBDA into the cavity of M-β-CD. In vitro cytotoxicity studies exhibited that cannabinoids/M-β-CD complexes had better anticancer activity than acidic cannabinoids alone. ***Conclusion:*** An improved physicochemical and physiological performance of acidic cannabinoid/M-β-CD complexes in breast cancer cells was demonstrated. These cyclodextrin-based formulations are a promising option for optimizing the efficacy of cannabinoid delivery.

INTRODUCTION

Two major active cannabinoids derived from the cannabis plant, cannabidiolic acid (CBDA) and tetrahydrocannabinolic acid (THCA), are thermally unstable and highly lipophilic. During the growth of the cannabis plant, cannabinoids primarily exist in an acidic form with THCA and CBDA. It has promising and effective cannabinoids with various pharmacological activities, including anti-inflammatory, anti-epileptic, and anti-cancer. Several studies have shown that THCA and CBDA are associated with the biological functions of inhibiting cyclooxygenase (COX) and cytokine (TNF-alpha, Interleukin). However, these acidic cannabinoids can be readily decarboxylated when exposed to the thermodynamic system. Therefore, these physicochemical properties of acidic cannabinoids have major challenges for designing stable drug products.

Cyclodextrins (CDs) have been widely used as an inactive ingredient for preparing drug/inclusion complexes[1-3]. CDs can load the various kinds of drug molecules via non-covalent host-guest interaction[4]. The CD complexation has many advantages in enhancing the solubility and stability of poorly water-soluble drugs[5]. It also contributes to improving bioavailability. Especially, CD complexation is a versatile option for solubilizing and stabilizing the hydrophobic molecules with high loading capacity. Recently, there have been various types of CD formulations encapsulating cannabinoids[6].

OBJECTIVES

The objectives of this study were to optimize the complexation process for acidic cannabinoids among the different types of CD and to evaluate their solubility, permeability, and cytotoxicity in MCF-7 breast cancer cell lines.

EXPERIMENTAL METHODS

Phase solubility studies of cannabinoids were performed according to the method established by Higuchi and Connors. Different concentrations of α-CD, β-CD, γ-CD, Hydroxypropyl-β-CD (HP-β-CD), and Methyl-β-CD (M-β-CD) (0.625 ~ 10mM) in distilled water were prepared. In this study, the cannabinoids/M-β-CD complex was prepared by spray drying, freeze-drying, and spray freeze-drying methods. Each cannabinoid extract was dissolved in an aliquot of 15% ethanol and added to an aqueous solution of M-β-CD under shaking for 48 hours at 120 rpm. Different molar ratios (1:1, 1:2, and 1:5) of cannabinoids and M-β-CD were weighed. The acidic cannabinoids THCA or CBDA (equivalent to 1 mg) and cannabinoids/CD inclusion complexes (equivalent to 1 mg cannabinoids) were dissolved in 5 mL distilled water. The surface morphologies of physical mixtures, spray-dried complex, freeze-dried complex, and spray freeze-dried complex were observed by field emission scanning electron microscope (FE-SEM) (JSM-6700F, JEOL, Japan). The thermograms of the cyclodextrin inclusion complex with cannabinoids were determined using a differential scanning calorimeter (DSC Q2000, TA instruments, United states). The cytotoxicity of cannabinoids/M-β-CD complexes was determined by the Alamar blue® assay. As the Alamar blue® fluorescence intensity changes indicate the reduction action, the active cell could mediate the metabolic activity of mitochondrial enzymes. When cells were exposed to trypsin until complete the detachment at 37℃ for 20 min, cells were resuspended and counted (Neubauer chamber), diluted in culture media at a density of 2.5 x 104 cells/mL, seeded onto 96-well microplates (100 μL per well; 2.5 × 103 cells/well) and cultured for 48 h.

RESULTS

A phase solubility plot of cannabinoid acids in five different CDs was shown in **Fig. 1**. The plot depicted a Higuchi AL type phase solubility behaviors for all complexes, a linear increase in cannabinoids solubility with increasing concentration of CDs.

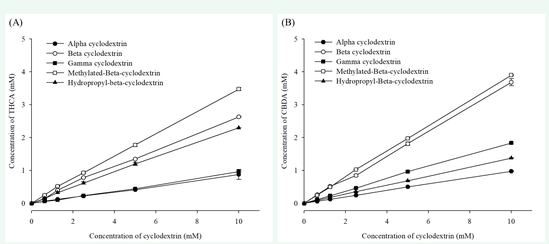

Figure 1. Phase solubility plots of (A) THCA and (B) CBDA in different types of cyclodextrins, Mean ± SD (n=3).

Table 1. Complexation parameters of cannabinoids in different types of cyclodextrin. Mean ± SD, (n=3)

Types of cannabinoids	Types of cyclodextrin (CD)	Slope	Y-intercept (S_o)	Stability constant(Ks)	R^2	Complexation efficiency (%)	Molar Ratio (Cannabinoids: CD)
THCA	α-CD	0.086	0.052	641.503	0.998	9.433	1.00 : 11.39
	β-CD	0.262	0.359	2135.672	0.997	35.483	1.00 : 3.80
	γ-CD	0.096	0.109	1278.237	0.998	10.632	1.00 : 10.38
	M-β-CD	0.344	0.448	2361.731	0.999	52.486	1.00 : 2.88
	HP-β-CD	0.227	0.241	1395.723	0.999	29.366	1.00 : 4.35
CBDA	α-CD	0.098	0.018	190.995	0.998	10.816	1.00 : 10.27
	β-CD	0.115	0.018	159.391	0.997	12.994	1.00 : 8.64
	γ-CD	0.185	0.035	198.804	0.998	22.639	1.00 : 5.45
	M-β-CD	0.390	0.141	420.164	0.999	63.854	1.00 : 2.55
	HP-β-CD	0.138	0.023	171.973	1.000	15.942	1.00 : 7.25

Table 1 summarized the complexation parameters for all cannabinoid acids-cyclodextrin inclusion complexes. The molecular ratios of cannabinoids/M-β-CD varied the solubility of THCA and CBDA in the cannabinoids/M-β-CD complex (**Table 2**). When an initial molar ratio of 1:2 (Cannabinoids: M-β-CD) was selected, the water solubility of THCA and CBDA was respectively 931.68μg/mL and 1092.42μg/mL. Interestingly, the solubility of THCA increased to 931.68 μg/mL as the cannabinoids/M-β-CD complex was prepared by spray freeze drying method at the same molar ratio (1:2), respectively 2 times, 5 times higher than that of the spray drying and freeze-drying method.

Table 2. The water solubility of cannabinoids in M-β-CD inclusion complex according to the preparation method (Data were given as mean ± SD, n=3)

Preparation method	Types of cannabinoids	The water solubility of cannabinoids (μg/mL)		
		The molar ratio (Cannabinoids: M-β-CD)		
		1:1	1:2	1:5
Spray drying	THCA	217.97 ± 9.44	476.37 ± 20.31	338.19 ± 3.25
	CBDA	298.37 ± 67.88	554.53 ± 3.75	438.22 ± 9.25
Freeze-drying	THCA	77.63 ± 4.73	186.22 ± 10.55	107.34 ± 25.45
	CBDA	93.39 ± 7.87	204.35 ± 20.42	90.24 ± 12.32
Spray freeze-drying	THCA	313.37 ± 17.12	931.68 ± 18.54	473.56 ± 57.22
	CBDA	454.31 ± 40.86	1092.42 ± 39.42	544.38 ± 30.14

As shown in **Fig. 2**, the preparation methods affect the morphologies with remarkable CD molecules on the surface of the powder. The shapes of the physical mixture showed that the composition mainly consisted of CD molecules, and cannabinoids were partially mixed and adsorbed to the CDs.

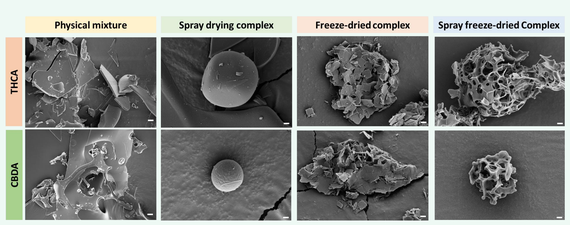

Figure 2. FE-SEM images of cannabinoids/M-β-CD inclusion complex using different preparation methods (Scale bar: 2μm).

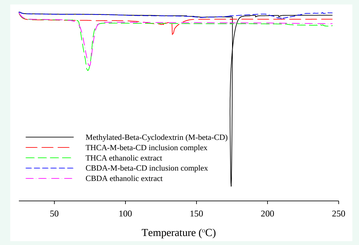

Figure 3. DSC spectrums of methylated-beta-cyclodextrin(M-beta-CD), cannabinoids (THCA or CBDA), and cannabinoids/M-beta-CD inclusion complex

As shown in **Fig. 3**, THCA or CBDA has a typically sharp endothermic peak between 65 and 75℃. M-β-CD has a significant endothermic peak at 180 ℃. Intermolecular action of cannabinoids and CD would be potentially caused by forming inclusion complex.

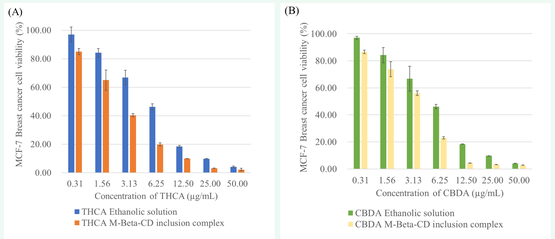

Figure 4. Cell viability of cannabinoids formulations against MCF-7 breast cancer cell lines for (A) THCA, and (B) CBDA. The mean ± standard deviations are presented on the graph.

As shown in **Fig. 4**, the treatment of MCF-7 cells with cannabinoids-M-Beta-CD inclusion complexes for 48h resulted in a significant increase in cytotoxicity, compared to the ethanolic extracts of THCA or CBDA.

CONCLUSION

- The acidic cannabinoids/cyclodextrin inclusion complexes are successfully prepared by different methods.
- Solid-state characterization of acidic cannabinoids/methylated-beta-cyclodextrin (M-β-CD) inclusion complex is characterized by FE-SEM, DSC analysis.
- In vitro cytotoxicity studies exhibited that cannabinoids/M-β-CD complexes had better anticancer activity than cannabinoids alone.

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6. Cyclodextrin-enabled Cannabinoid Formulations – Cyclolab

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