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Effects of RM-β-CD on sublingual bioavailability of Δ^9-tetrahydrocannabinol in rabbits

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Abstract

The purpose of the present study was to develop novel cyclodextrin-containing sublingual formulations of cannabinoids.

Complexation of model cannabinoids, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), with randomly methylated β-cyclodextrin (RM-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD), were studied by the phase-solubility method. Due to better complexation efficiency, RM-β-CD was selected for further studies. Solid THC/RM-β-CD and CBD/RM-β-CD complexes were prepared by freeze-drying. The dissolutions of both THC and CBD in the presence and absence of RM-β-CD were determined. THC was selected for in vivo studies: the pharmacokinetics of THC after both sublingual and oral administrations of ethanolic THC and THC/RM-β-CD complex solutions were studied in rabbits.

The aqueous solubility of CBD and THC increased as a function of CD concentration, showing A_L- and A_P-type diagrams for HP-β-CD and RM-β-CD, respectively. Dissolution rates of THC/RM-β-CD and CBD/RM-β-CD complexes were significantly ($p < 0.05$) higher than those of plain THC and plain CBD, respectively. The absolute bioavailability (F) of THC decreased in the following order: sublingual THC/RM-β-CD solution ($F = 12.1 \pm 1.4\%$; mean ± S.D.; $n = 4$) > oral THC/RM-β-CD solution ($F = 4.0 \pm 6.0\%$) ≥ sublingual ethanolic THC solution ($F = 3.8 \pm 2.8\%$) > oral ethanolic THC solution ($F = 1.3 \pm 1.4\%$).

These results demonstrate that RM-β-CD increases both the aqueous solubility and dissolution rate of these cannabinoids, making the development of novel sublingual formulation possible. These results also suggest that the sublingual administration of a THC/RM-β-CD complex substantially increases the bioavailability of THC in rabbits.

Keywords: Cannabinoids; Cyclodextrins; Delta(9)-tetrahydrocannabinol

1. Introduction

Cannabinoids are a group of compounds that originate exclusively from *Cannabis sativa* L., the plant source of marijuana and hashish. Cannabinoids have been used for thousands of years for both recreational and medicinal purposes. Over the last few years, cannabinoids have been recognized as being useful in the treatment of various medical conditions, such as nausea, AIDS associated wasting, anorexia, MS, pain and glaucoma (Plasse et al., 1991; Jarvinen et al., 2002). The two main cannabinoids in both marijuana and hashish are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Currently, synthetic THC is available in USA as an orally administered capsule formulation (Marinol®, United Pharmaceuticals). The oral use of THC is, however, limited by substantial first-pass metabolism and its hydrophobic nature. Although THC is almost completely (>90%) absorbed from this oral encapsulation, only 10–20% of the administered dose escapes the first-pass metabolism (Brenneisen et al., 1996). Over the last few years, methods, such as pulmonary, rectal and sublingual administration of cannabinoids have

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been explored to overcome first-pass metabolism (Mattes et al., 1993; Whittle et al., 2003).

Systemic drug delivery through sublingual mucosa is one of the best-known methods to bypass hepatic first-pass metabolism (Rathbone, 1996). However, sublingual drug delivery also has limitations, for in order to enter systemic circulation through sublingual mucosa, the drug molecule must first dissolve into the saliva before it is lost down the esophagus through swallowing. Due to the small volume of saliva (<1 ml) in the oral cavity, the therapeutic dose of a sublingual drug must be relatively small and in most cases dissolution enhancers are applied (Jain et al., 2002).

Cyclodextrins (CDs) are a group of cyclic oligosaccharides, which have been shown to improve aqueous solubility, dissolution rate and bioavailability of various lipophilic drugs (Martin Del Valle, 2004). CDs have also been successfully studied in a few sublingual and buccal formulations, e.g. hydroxypropyl-β-cyclodextrin (HP-β-CD) led to the effective absorption of sublingual testosterone (Pitha et al., 1986; Wang et al., 1996), androsteronediol (Brown et al., 2002) and clomipramine (Yoo et al., 1999). In addition, HP-β-CD and sulfobutylether-β-cyclodextrin (SBE-β-CD) has been shown to increase the absorption for the buccal administration of danazol (Badawy et al., 1996; Jain et al., 2002). It is generally assumed that only free drug, and not the complex of CD and drug, can penetrate across biological membranes (Irie and Uekama, 1997; Rajewski and Stella, 1996). Thus, the drug must be released from the complex to have the desired therapeutic action. Dilution of the formulation into biological fluids usually releases a drug molecule from the inclusion complex, according to its dissociation equilibrium. This may cause problems in sublingual drug delivery due to low volume of saliva. Moreover, the complex will be removed from the sublingual area after only a few minutes, and thus may not have enough time to release the drug before it is swallowed.

In the present study, the complexation of both THC and CBD with randomly methylated β-cyclodextrin (RM-β-CD) and HP-β-CD were studied by the phase-solubility method. Solid THC/RM-β-CD and CBD/RM-β-CD complexes were prepared and the dissolution properties of these complexes were determined. THC was selected for in vivo studies, the bioavailability of THC after sublingual administration of THC/RM-β-CD complex solution was studied in rabbits.

2. Materials and methods

2.1. Materials

The Δ^9-tetrahydrocannabinol was purchased from THC PHARM GmbH (Frankfurt, Germany) and deuterated THC (THC-d_3) from Cerilliant™ (Austin, TX, USA). *Bis*-(trimethylsilyl)trifluoroacetamide (BSTFA; 1% TMCl), pyridine, hexane and trifluoroacetic anhydride (TFAA) were all of GC-grade and were purchased from Sigma–Aldrich (Steinheim, Germany). HP-β-CD (Cavasol® W7 HP) and RM-β-CD (Cavasol® W7 M) were purchased from Wacker Chemie (Burghausen, Germany). Lactose monohydrate, Pharmatose® 110 M, was purchased from DMV International (The Netherlands) and gelatine capsules (size 1) were purchased from Tamro (Vantaa, Finland). All other reagents used were of analytic grade and used as received.

2.2. Analytical methods

2.2.1. HPLC

High performance liquid chromatography (HPLC) was used for the quantification of THC and CBD in phase-solubility and dissolution studies. The ranges of the HPLC methods were 0.1–50 μg/ml (0.2–200 μM) for CBD and 0.3–50 μg/ml (0.9–200 μM) for THC. The HPLC-system consisted of a Merck Hitachi L-7400 UV-detector (wavelength 205 nm), D-7000 interface module, L-7250 autosampler, L-7100 pump (Hitachi Ltd., Tokyo, Japan) and HPLC System Manager software (Hitachi, Ltd. 1996). Kromasil endcapped C_8-reverse-phase column (250 mm × 4.6 mm i.d., 5 μM) was purchased from Metachem Technologies Inc. (CA, USA). Chromatographic conditions were as follows: injection volume, 20 μl; isocratic flow rate, 1.0 ml/min and mobile phase, 85% (v/v) acetonitrile in water.

2.2.2. GC–MS

Gas chromatography–mass spectrometry (GC–MS), operating in the negative chemical ionization mode, was used to quantify THC in plasma. The range of the GC–MS detection method was 0.3–530 ng/ml, as previously been described (Mannila et al., 2004). The GC–MS system consisted of an Agilent 6890N gas chromatograph, 7683 autosampler and 5973N mass detector (Agilent Technologies, Palo Alto, CA). Data were processed by the Agilent Enhanced Chemstation software. A cross linked 5% phenyl methyl siloxane capillary column (HP-5MS; 30 m × 0.25 mm × 0.25 μM) (Agilent Technologies) was used with helium as the carrier gas, at a constant flow rate of 2.0 ml/min. The sample (1 μl) was injected in the pulsed splitless mode. The temperature program was as follows: an initial temperature of 50 °C was held for 1 min, then increased by 50 °C/min up to 205 °C, 3 °C/min up to 225 °C, and finally 60 °C/min up to 280 °C where it was maintained for 4 min. Temperatures of inlet, interface, MS source and quadrupole were 250, 280, 150 and 106 °C, respectively. Methane was used as the reagent gas. The area ratio of selected ions 410 (THC) and 413 (THC-d_3) was used for the quantification of THC.

2.2.3. Plasma sample preparation

On the day of analysis, plasma samples were thawed to ambient temperature. To 500 μl of plasma was added 50 μl of methanolic THC-d_3, 1 ml of urea solution (8 M) and 1 ml of methanol. The samples were purified by solid phase extrac-

tion using Oasis® HLB cartridges (Waters Ltd., MA, USA). The samples, once loaded onto the cartridges were washed with 3 ml of an acetic acid–methanol solution (60% (v/v) of methanol and 2% (v/v) of acetic acid) and then with 3 ml of ammonia–methanol solution (60% (v/v) of methanol and 0.8% (w/v) of ammonia at pH 10.0). The samples were eluted with methanol and evaporated under a stream of nitrogen at 40 °C, after which 100 μl of two derivatising solutions (A and B) was added to the residue. Solution A was a mixture of pyridine (30 μl), TFAA (100 μl) and hexane (4.87 ml). Solution B was a mixture of BSTFA (50 μl) and hexane (4.95 ml). Samples were analysed directly by GC–MS, as described above.

2.3. Phase-solubility studies

Effects of HP-β-CD and RM-β-CD on the aqueous solubilities of CBD and THC were studied using the phase-solubility method (Higuchi and Connors, 1965). Excess amounts of THC or CBD were added to phosphate buffer solutions (0.16 M, pH 7.4, ionic strength of 0.5) containing 0–0.08 M of HP-β-CD or RM-β-CD. The suspensions were shaken in the dark at room temperature for 72 h in order to reach equilibrium. The pH of the suspensions was held constant by adding aqueous solutions of either HCl or NaOH, when necessary. After equilibration, the suspensions were filtered (Millex HV 0,45 μM, Millipore, USA) and analysed by HPLC, as described in Section 2.2.1.

2.4. Preparation of solid formulations

The solid complexes of CBD and THC with RM-β-CD were prepared by adding CBD or THC to an aqueous 20% (w/v) RM-β-CD-solution. After 48 h of shaking the suspension was filtered (Millex HV 0.45 μM, Millipore, USA) and freeze-dried (FTS® Systems, Inc., NY, USA). The resulting powder was sieved (150 μM) and the content of CBD or THC was determined by HPLC. The complexation of CBD with RM-β-CD was confirmed by differential scanning calorimeter (DSC; data not shown). The complexation of THC with RM-β-CD could not be confirmed by DSC due to resin-like characteristics of pure THC.

The solid cannabinoid/RM-β-CD complex was measured into gelatine capsules (size 1) or compressed into tablets. The amount of THC or CBD in all formulations was equivalent to 1.0 mg. The amount of freeze-dried THC/RM-β-CD complex powder equivalent to 1 mg of THC was 25.7 mg. The respective amount of freeze-dried CBD/RM-β-CD complex powder was 13.6 mg. Lactose was used as an excipient (*ad* 100.0 mg) (Table 2). For the preparation of capsules containing either plain THC or a physical mixture of THC, a volume of ethanolic THC was measured into the capsules after which the ethanol was evaporated and lactose, or premixed RM-β-CD and lactose, respectively, were added. For capsules containing either plain CBD or a physical mixture of CBD, premixed solid CBD and lactose or premixed CBD, RM-β-CD and lactose, respectively, were packed into the capsules. In the case of capsules containing cannabinoid/RM-β-CD complexes, cannabinoid/RM-β-CD complex and lactose were weighed and mixed together before packing into capsules. For tablet formulations, a homogenous mixture of cannabinoid/RM-β-CD complex and lactose was prepared. Tablets were compressed by concave punches (7 mm in diameter) using a rotary tablet press (Fette Perfecta 1, Wilhelm Fette, Schwazebeck, Germany). Tablets were compressed to a radial breaking strength of 25–35N, measured by an Schleuniger 2E/205 Tablet hardness tester (Dr. K. Schleuniger & Co., Switzerland).

2.5. Dissolution studies

Dissolution studies were carried out in a 50 ml beaker (Schott, Mainz, Germany) containing 20 ml of dissolution medium (0.16 M phosphate buffer, pH 6.6, ionic strength 0.5). RM-β-CD (2%, w/v) was added to the dissolution medium in order to ensure the dissolution of studied cannabinoid. The beaker was placed into a shaking water bath (Certomat® WR, B. Braun, Melsungen, West-Germany) (140 agitations per minute, 37 °C). The capsule or tablet formulation was carefully inserted into a 30 mm × Ø 7 mm cylinder, prepared from a 0.5 mm steel mesh. This cylinder was placed in the beaker at the beginning of the experiment. Aliquots of 1 ml were carefully withdrawn between 0 and 180 min, and the cannabinoid concentration was measured by HPLC. The samples were immediately replaced with fresh dissolution medium of the same temperature after each aliquot was removed. The time required to reach 50% dissolution ($t_{50\%}$) was graphically determined for each experiment by a time versus percent dissolved curve. The difference in values of $t_{50\%}$ for different formulations were statistically compared by the Kruskall–Wallis test, followed by the Mann–Whitney test. A value of $p < 0.05$ was considered as statistically significant.

2.6. In vivo pharmacokinetic studies

Four New Zealand white rabbits (2.5–3.3 kg), one male and three females were purchased from National Laboratory Animal Center in Kuopio, Finland. The rabbits were allowed to eat commercial food pellets and drink water ad libitum, except during the first 5 h of each test, when they were under anaesthesia. All procedures with animals were reviewed and approved by the Animal Ethics Committee at the University of Kuopio.

Before each test the rabbits were given atropine (0.02 mg/kg; Atropin®, Leiras, Turku, Finland) to prevent excess salivation, and then anaesthetized with fentanyl citrate and fluanisone (0.1 and 3 mg/kg, respectively; Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) and midazolam (2 mg/kg; Dormicum®, Roche, Espoo, Finland). Anaesthetized rabbits were positioned on a table with the lower jaw supported in a horizontal position. THC was

given at a dose of 250 μg/kg intravenously, sublingually or orally.

The i.v. formulation was 20% (w/v) HP-β-CD solution containing 0.7 mg/ml of THC. The i.v. solution was filtered using a sterile membrane filter (pore size 0.22 μM). THC/HP-β-CD complex solution (0.4 ml/kg) was injected directly into a marginal ear vein. Sublingual and oral formulations were a 40% (w/v) RM-β-CD solution containing 13 mg/ml of THC and an ethanolic solution containing 12 mg/ml of THC. Sublingual administration was as follows: the rabbits' tongues were carefully lifted with tweezers and the appropriate volume of THC/RM-β-CD complex solution (20 μl/kg) or ethanolic solution of THC (20 μl/kg) was pipetted under the tongue with an autopipette. The average volume of the administered sublingual solution was 50 μl. This volume was observed to remain in the sublingual cavity without escaping down the GI-tract. For oral administration, the THC/RM-β-CD complex solution (20 μl/kg) or ethanolic THC solution (20 μl/kg) was administered to the GI-tract via catheterization, and rinsed with 3 ml of water.

Blood was withdrawn into Venoject® (Terumo, Leuven, Belgium) tubes from either a central artery or marginal vein of the ear prior to THC administration and over 2–300 min after administration. Blood samples were centrifuged at 3700 × *g* within 30 min, and the recovered plasma was immediately frozen to −20 °C. Samples were stored at −80 °C until analysis.

2.7. In vivo data analysis

The maximum plasma concentration of THC (C_{max}) and the time required to reach the maximum concentration (t_{max}) were obtained directly from the actual plasma concentration versus time data. $AUC_{0–300\,min}$ was calculated by linear trapezoidal method (0 min concentrations for the i.v. administration studies were extrapolated by the WinNonlin program (Version 4.0.1)). The elimination rate constants (k_{el}), elimination half-lives ($t_{1/2}$), clearances (CL) and volumes of distribution (V_{SS}) were determined from i.v. data for each rabbit using the WinNonlin program. $AUC_{300\,min–\infty}$ was determined by using Eq. (1):

$$AUC_{300\ min-\infty} = \frac{C_{300\ min}}{k_{el}} \quad (1)$$

where C(300 min) was the plasma concentration of THC at 300 min. $AUC_{0\,min–\infty}$ is a sum of $AUC_{0–300\,min}$ and $AUC_{300\,min–\infty}$. Absolute bioavailabilities (F, %) of orally and sublingually administered THC formulations were calculated according to Eq. (2):

$$F = \frac{AUC_{e.v.}}{AUC_{i.v.}} \times 100\% \quad (2)$$

where $AUC_{e.v.}$ is $AUC_{0\,min–\infty}$ for either sublingual or oral administration and $AUC_{i.v.}$ is $AUC_{0\,min–\infty}$ for i.v. administration.

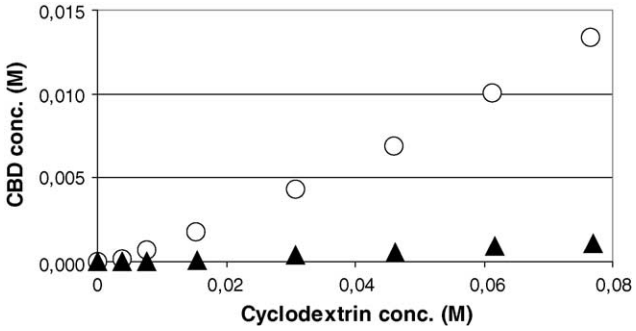

Fig. 1. The phase-solubility diagrams of CBD with RM-β-CD (◯) and HP-β-CD (▲).

3. Results and discussion

3.1. Phase-solubility studies

Figs. 1 and 2 show the phase-solubility diagrams of CBD and THC with HP-β-CD and RM-β-CD, respectively. These results show that both CBD and THC form A_L-type phase-solubility diagrams with HP-β-CD, which suggests the formation of 1:1 inclusion complexes between HP-β-CD and these cannabinoids (Higuchi and Connors, 1965). The complexation constants for THC and CBD were calculated by using Eq. (3):

$$K_{1:1} = \frac{\text{Slope}}{[S_0](1 - \text{Slope})} \quad (3)$$

where $K_{1:1}$ is the stability constant for the 1:1-complex and $[S_0]$ is the solubility of CBD (0.2 μM) or THC (0.9 μM) in the absence of HP-β-CD. The phase-solubility diagrams of CBD and THC with RM-β-CD, however, follow A_p-type phase-solubility behaviour, indicating the formation of 1:1 and 1:2 inclusion complexes (Higuchi and Connors, 1965). The binding constants for the 1:1- and 1:2-complexes were calculated according to Eq. (4):

$$\frac{[S_t] - [S_0]}{[L_t]} = K_{1:1}[S_0] + K_{1:1}K_{1:2}[S_0][L_t] \quad (4)$$

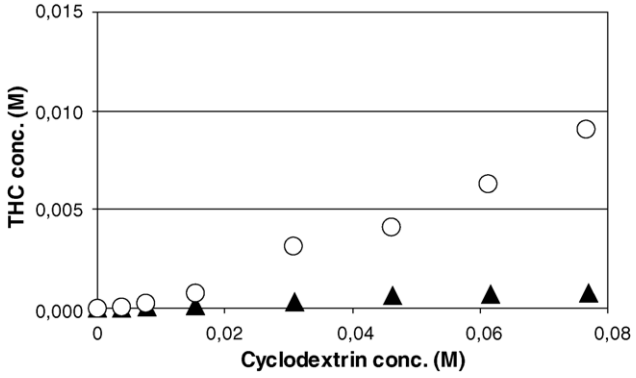

Fig. 2. The phase-solubility diagrams of THC with RM-β-CD (◯) and HP-β-CD (▲).

Table 1
The complexation constants of CBD and THC with HP-β-CD and RM-β-CD

Cannabinoid	Cyclodextrin	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-1})
CBD	HP-β-CD	13800	–
	RM-β-CD	484100	8
THC	HP-β-CD	4200	–
	RM-β-CD	19600	40

where $[S_t]$ is the total drug concentration at total RM-β-CD concentration $[L_t]$, $[S_0]$ is the solubility of CBD or THC in the absence of CD and $K_{1:1}$ and $K_{1:2}$ represent the binding constants for the 1:1- and 1:2-complex, respectively. The histogram of $([S_t] - [S_0])/[L_t]$ versus $[L_t]$ results in a linear plot with a slope of $K_{1:1}K_{1:2}[S_0]$ and an intercept of $K_{1:1}[S_0]$. The binding constants of CBD and THC with HP-β-CD and RM-β-CD are shown in Table 1.

THC is commercially available as an oral capsule containing 2.5–10 mg of THC in sesame seed oil (Marinol®). In sublingual formulations, even smaller dose of cannabinoids can be used when avoiding first-pass metabolism. Based on the phase-solubility studies, RM-β-CD was shown to offer superior characteristics compared to HP-β-CD. For example, 400 mg of HP-β-CD is needed to complex 1 mg of THC, and this amount is too much for sublingual applications. However, 1 mg of THC can be complexed with less than 26 mg of RM-β-CD. Besides, the complexation efficiency of THC and CBD increases at higher RM-β-CD concentrations, due to the A_P-type phase-solubility behaviour, which further reduces the amount of RM-β-CD required. Based on these calculations, RM-β-CD was selected for further dissolution studies and sublingual absorption studies.

3.2. Dissolution studies

Table 2 and Figs. 3 and 4 show that complexation with RM-β-CD significantly increase the dissolution rate of both

Table 2
The times required to achieve 50% dissolution ($t_{50\%}$) in dissolution studies

Formulations	$t_{50\%}$ (min)
Inclusion complexes	
CBD/RM-β-CD (13.6 mg)[a], capsule	3.2 ± 0.5*
THC/RM-β-CD (25.7 mg)[a], capsule	3.6 ± 0.8*
CBD/RM-β-CD (13.6 mg)[a], tablet	7.1 ± 0.8*
THC/RM-β-CD (25.7 mg)[a], tablet	5.4 ± 0.8*
Plain cannabinoids	
CBD[b], capsule	29.5 ± 9.6
THC[b], capsule	9.4 ± 3.6
Physical mixtures	
CBD and RM-β-CD (12.6 mg)[b], capsule	22.5 ± 5.5
THC and RM-β-CD (24.7 mg)[b], capsule	13.2 ± 4.5

All formulations contained 1.0 mg of either CBD or THC, and a required amount of lactose (the total sample weight was 100.0 mg).
[a] $n = 6$.
[b] $n = 4$.
* Significantly different from 'physical mixture' and 'plain cannabinoid' formulations ($p < 0.05$).

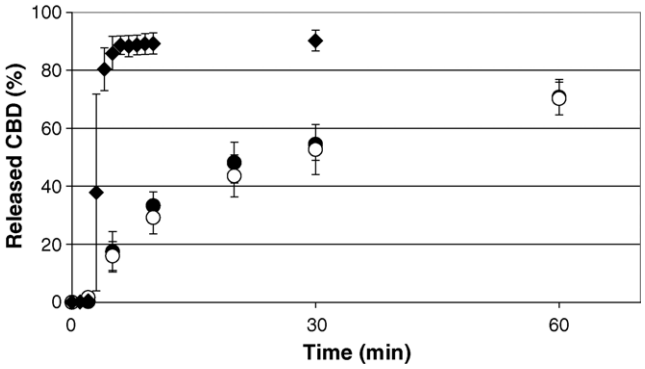

Fig. 3. The dissolution profiles of CBD capsule formulations, physical mixture of CBD and RM-β-CD (●), plain CBD (○) and CBD/RM-β-CD complex (♦)($n = 4$–6; mean ± S.D.).

THC and CBD. The values of $t_{50\%}$ for the dissolution of THC and CBD increased in following order: cannabinoid/RM-β-CD complex capsules < cannabinoid/RM-β-CD complex tablets < physical mixture in capsules ≈ plain cannabinoid in capsules.

Figs. 3 and 4 show the relatively large standard deviations at 3 and 4 min for cannabinoid/RM-β-CD complexes. This may be explained by the disintegration of gelatine capsules at slightly different rates over time. This explanation is supported by the fact that in cases of cannabinoid/RM-β-CD complex tablet formulations, such deviations were not so evident (data not shown).

3.3. In vivo pharmacokinetic studies

The rabbit was selected as an animal model for absorption studies due to its convenient size, which allows for sublingual administration and blood sample volumes that are sufficient for quantitative analysis. In addition, the rabbit has been described as one of the few laboratory animals that do not have keratinized mucosa, thus closely resembling human sublingual and buccal tissues (Squier and Wertz, 1996; Odou et al., 1999).

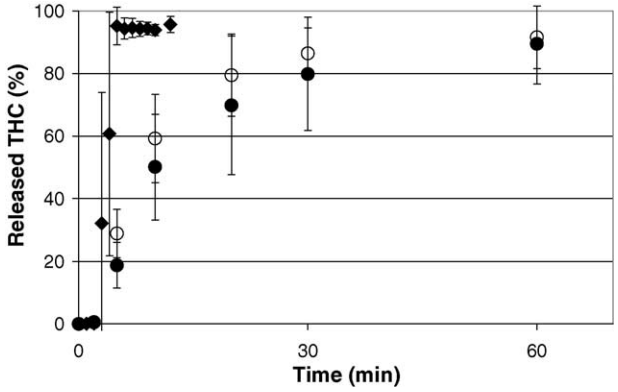

Fig. 4. The dissolution profiles of THC capsule formulations, physical mixture of THC and RM-β-CD (●), plain THC (○) and THC/RM-β-CD inclusion complex (♦)($n = 4$–6; mean ± S.D.).

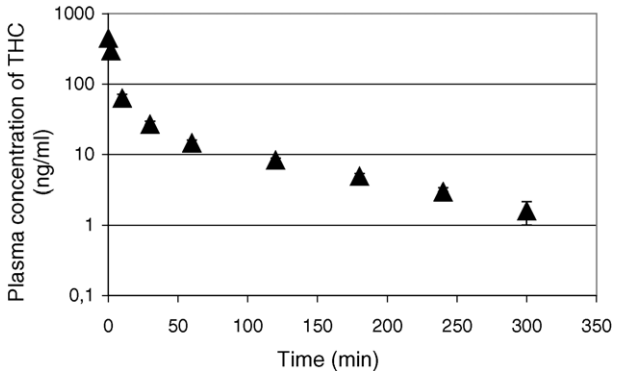

Fig. 5. The mean plasma concentrations of THC after i.v. administration (250 μg/kg) in rabbits (mean ± S.E.M.; $n = 4$).

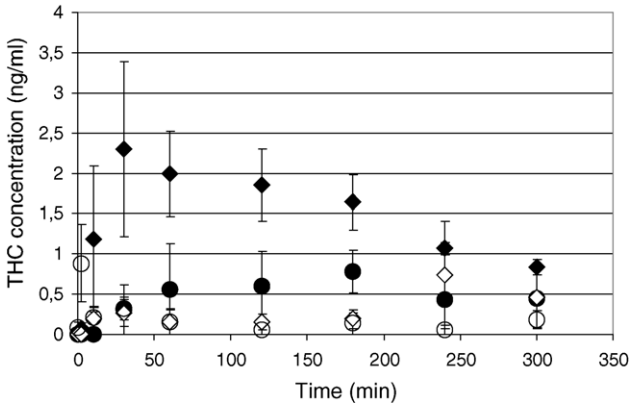

Fig. 6. The mean plasma concentrations of THC after extravascular administration (250 μg/kg) to rabbits, sublingual administration of THC/RM-β-CD complex (♦), sublingual administration of ethanolic THC (●), peroral administration of THC/RM-β-CD complex (◊) and peroral administration of ethanolic THC (○) (mean ± S.E.M.; $n = 4$).

Fig. 5 shows the mean plasma concentration after i.v. administration of THC in semilogarithmic scale. Appearance of the curve suggests biphasic kinetics with first order elimination phase. The representing kinetic values were determined for each rabbit individually by WinNonlin program, using a 2-compartment IV-bolus model with 1st order elimination. The values (mean ± S.D.) for k_{el}, $AUC_{0\,min-\infty}$, CL, V_{SS} and $t_{1/2}$ were $0.01 \pm 0.00\,min^{-1}$, 5300 ± 1200 min × ng/ml, 0.15 ± 0.03 l/min, 9.5 ± 1.9 l and 66.6 ± 3.1 min, respectively.

The pharmacokinetics of THC in rabbits has been reported after an i.v. dose of 1 mg/kg (Leuschner et al., 1986), and five elimination phases were observed. The terminal phase (the fifth phase), which was reported to commence 1–3 days after i.v. administration, was estimated to have a half-life of 31–66 h. The fourth elimination phase was reported to take place 4–8 h after administration of THC, and to have a half-life of 360–780 min. These elimination phases were not observed in the present study, probably due to the small dose of THC administered. The earlier study reported a third phase of elimination, which took place 10–30 min after administration and showed a half-life of 35–60 min, which is in agreement with our results. The first two elimination phases reported by Leuschner et al. (1986) could be interpreted as the distribution phase in the present study.

Fig. 6 shows the mean plasma concentrations of THC after administration of the studied extravascular formulations. C_{max}, t_{max}, $AUC_{0–300\,min}$, $AUC_{0\,min-\infty}$ and F values of THC after i.v., sublingual and oral administrations are summarized in Table 3.

The absolute bioavailability of the sublingual THC/RM-β-CD complex ($F = 12.1 \pm 1.4\%$; mean ± S.D.; $n = 4$) was higher than that of the oral THC/RM-β-CD complex ($F = 4.0 \pm 6.0\%$). These results indicate that the sublingual route is more efficient for THC than oral, most probably due to the avoidance of hepatic first-pass metabolism. The absolute bioavailability of the sublingual THC/RM-β-CD complex was also higher than that of the sublingual ethanolic THC ($F = 3.8 \pm 2.8\%$). This may be due to the increased water solubility of complexed THC, and on the other hand due to precipitation of THC from ethanolic THC after rapid dilution of ethanol in the GI-tract. The outcome can also be related to suggested permeation enhancing effect of RM-β-CD (Senel and Hincal, 2001). These explanations are further supported by the fact that the absolute bioavailability of oral THC/RM-β-CD complex was also higher ($F = 4.0 \pm 6.0\%$) than that of oral ethanolic THC ($F = 1.3 \pm 1.4\%$).

The mean t_{max} value was reached after 120 min with sublingual administration of the THC/RM-β-CD complex (Table 3), and individual t_{max} values varied from 30 to 240 min. The present t_{max} is much smaller than earlier

Table 3
C_{max}, t_{max}, $AUC_{0–300\,min}$, $AUC_{0\,min-\infty}$ and F values (mean ± S.D.) of THC after intravenous, sublingual and peroral administration of THC/CD inclusion complex and ethanolic THC in rabbits ($n = 4$)

Route of administration	Formulation	C_{max} (ng/ml)	t_{max} (min)	$AUC_{0–300\,min}$ (ng/ml × min)	$AUC_{0\,min-\infty}$ (ng/ml × min)	F (%)
Intravenous	THC/HP-β-CD inclusion complex	440 ± 150[a]	0[a]	5100 ± 1200	5300 ± 1200	100
Sublingual	Ethanolic THC	1.0 ± 0.9	180 ± 98	160 ± 170	190 ± 140	3.8 ± 2.8
	THC/RM-β-CD inclusion complex	3.1 ± 1.2	120 ± 110	480 ± 23	640 ± 120	12 ± 1.4
Peroral	Ethanolic THC	0.9 ± 1.0	31 ± 29	44 ± 68	74 ± 82	1.3 ± 1.4
	THC/RM-β-CD inclusion complex	1.1 ± 1.6	140 ± 120	120 ± 180	170 ± 250	4.0 ± 6.0

The THC dose was 250 μg/kg.
[a] Extrapolated value.

reported values of t_{max} at 4 h, which was reported after the sublingual administration of THC in a sesame seed oil vehicle in humans (Mattes et al., 1993). Oral administration of the THC/RM-β-CD complex in the present study resulted in a t_{max} value at 140 min after administration. This result is in good agreement with studies that have reported a t_{max} of THC to be 2–5 h after the oral administration of Marinol® in humans (Mattes et al., 1993; Brenneisen et al., 1996). The present study also suggests that when compared to the oral administration of THC, sublingual administration of the THC/RM-β-CD complex not only leads to higher bioavailability, but also the possibility of a faster therapeutic effect for THC.

4. Conclusions

These results demonstrate that RM-β-CD increases both the aqueous solubility and dissolution rate of THC and CBD, thus making the development of novel sublingual formulation possible. The present study also shows that the sublingual administration of THC/RM-β-CD complex solution substantially increases the bioavailability and absorption rate of THC, compared to either the oral administration of THC or the sublingual administration of ethanolic THC.

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