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Different impacts of intestinal lymphatic transport on the oral bioavailability of structurally similar synthetic lipophilic cannabinoids: Dexanabinol and PRS-211,220

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ABSTRACT

The aim of this article was to investigate the role of intestinal lymphatic transport in the oral bioavailability of two structurally similar synthetic lipophilic cannabinoids: dexanabinol and PRS-211,220. For this purpose, the long chain triglyceride (LCT) solubility and affinity to chylomicrons *ex vivo* of both cannabinoids were evaluated. Their oral bioavailability was assessed in rats following administration in a lipid-free and a LCT-based formulation. The intestinal lymphatic transport of these two molecules was also directly measured in a freely moving rat model. LCT solubility of dexanabinol and PRS-211,220 was 7.9 ± 0.2 and 95.8 ± 5.3 mg/g, respectively. The uptake by chylomicrons was moderate ($31.6 \pm 5.2\%$) and high ($66.1 \pm 2.4\%$), respectively. The bioavailability of dexanabinol (37%) was not affected by LCT solution, whereas administration of PRS-211,220 in LCT improved the absolute oral bioavailability three-fold (from 13 to 35%) in comparison to the lipid-free formulation. The intestinal lymphatic transport of dexanabinol and PRS-211,220 was 7.5 ± 0.8 and $60.7 \pm 6.8\%$ of the absorbed dose, respectively. In conclusion, despite structural similarity and similar lipophilicity, dexanabinol and PRS-211,220 exhibited a very diverse pattern of oral absorption, and the lymphatic system played quite a different role in the oral bioavailability of these molecules. The low lymphatic transport of dexanabinol is likely driven by relatively lower affinity to chylomicrons and lower LCT solubility.

1. Introduction

The oral route is the most convenient method of drug administration for both the patient and medical care professionals. Following oral administration, most drugs are absorbed directly into portal blood, but lipophilic molecules can be also transported to the systemic circulation by the intestinal lymphatic system (Charman and Porter, 1996; Edwards et al., 2001). Intestinal lymphatic transport is a complex process consisting of several consecutive events in intestinal lumen and enterocyte. The key mechanism of the intestinal lymphatic transfer is the association of the lipophilic drug with chylomicrons (CM) in the enterocyte and subsequent uptake of these drug-containing large lipoproteins by the intesti-

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Abbreviations: LCT, long chain triglycerides; TG, triglycerides; CM, chylomicrons; THF, tetrahydrofuran; clog P, calculated log P; TFA, trifluoroacetic acid; NMDA, N-methyl D-aspartate; DDW, double distilled water

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nal lymphatic system (Charman and Stella, 1986; Porter and Charman, 2001; O'Driscoll, 2002). The degree of association with CM was previously proposed by our group to be indicative of the intestinal lymphatic transport potential of lipohilic drugs (Gershkovich and Hoffman, 2005). The physicochemical properties of molecules required for lymphatic transport of drugs are thought to be high lipophilicity ($\log P > 5$) and reasonable solubility in long chain triglycerides (LCT) (Charman and Stella, 1986; Charman, 2000). However, high $\log P$ value and high LCT solubility does not always lead to significant intestinal lymphatic transport (Myers and Stella, 1992; Hauss et al., 1994; Grove et al., 2006).

Although lymphatic transport and association with CM and other triglyceride-rich lipoproteins may lead to many pharmacokinetic and pharmacodynamic consequences (Eder, 1982; Gupta and Benet, 1990; Humberstone et al., 1998a,b; Wasan and Cassidy, 1998; Wasan et al., 2002, 2006; McIntosh et al., 2004; Shayeganpour et al., 2005; Brocks et al., 2006; Gershkovich et al., 2007), the foremost reason for the broad interest in lymphatic transport is the potential to utilize this pathway to increase the oral bioavailability of highly lipophilic drugs. Lymphatic transport may contribute to the increase in the oral bioavailability of lipophilic compounds by a number of potential mechanisms: additional pathway of transfer of lipophilic compound from the enterocyte to the blood (Dahan and Hoffman, 2005), bypass of hepatic first-pass metabolism (Charman and Porter, 1996) and reduction in intestinal first-pass metabolism (Trevaskis et al., 2006a). However, there is scarce knowledge regarding the impact of lymphatic transport on bioavailability, since the number of compounds for which lymphatic transport is quantified is very limited due to the complicated surgical procedures required. The most extensive research in this area has been performed on halofantrine, a highly lipophilic drug. It was found that intestinal lymphatic transport accounted for a very high portion of the total oral bioavailability of this compound in rat (Porter et al., 1996; Caliph et al., 2000) and even more in dog (Khoo et al., 2001, 2003; Holm et al., 2003).

The aim of the current work was to assess the ability of the intestinal lymphatic transport to improve the oral bioavailability of two structurally similar synthetic cannabinoids, dexanabinol and PRS-211,220 (Fig. 1). Despite very encouraging pharmacological properties (Mattes et al., 1993), cannabinoids are barely used in clinical practice, primary due to their low bioavailability following oral administration. The low oral bioavailability of natural and synthetic cannabinoids is explained mainly by their high lipophilicity and extensive hepatic first pass metabolism (Agurell et al., 1986; Mattes et al., 1993; Bland et al., 2005). Dexanabinol (HU-211) is a synthetic cannabinoid analogue which acts as a non-competitive N-methyl D-aspartate (NMDA) receptor antagonist and antioxidant. This compound was shown to be highly neuroprotective in an animal model of brain injury (Shohami et al., 1995), but recently completed phase III clinical trials have shown less promising results (Maas et al., 2006). PRS-211,220 is another synthetic cannabinoid with high neuroprotective potential as demonstrated in a mid-cerebral artery occlusion rat model (Lavie et al., 2002). These two compounds have similar structure and similar calculated octanol/water partition (i.e. clog P) values, but quite different solubility in LCT and affinity to CM.

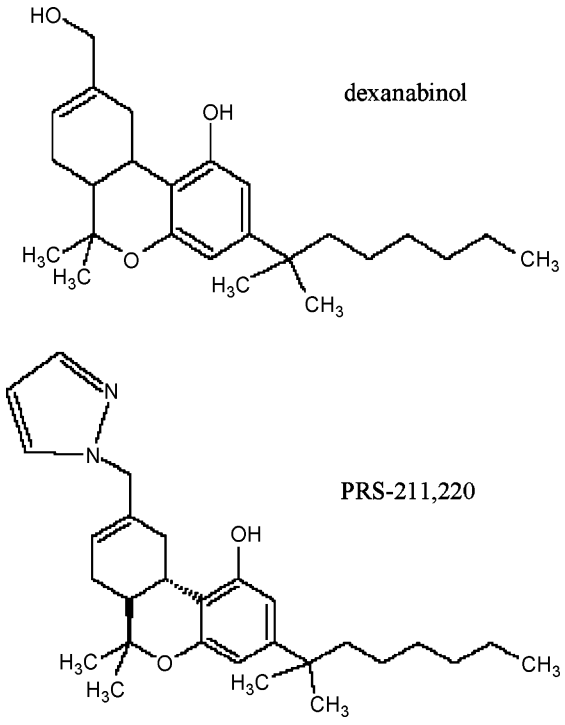

Fig. 1 – Chemical structure of dexanabinol and PRS-211,220.

The experimental design that was used in this work entailed two stages:

1. Assessments of bioavailability of two lipophilic cannabinoids following their oral administration to rats in lipid-free and LCT-based formulations, as LCT have been shown to facilitate intestinal lymphatic transport of lipophilic drugs (O'Driscoll, 2002).
2. Quantification of intestinal lymphatic versus portal transport of the synthetic cannabinoids in a freely moving rat model following their administration in LCT-based formulation and direct measurement of the tested drug concentrations in lymph fluid and plasma concurrently.

2. Materials and methods

2.1. *Materials*

Dexanabinol and PRS-211,220 were kindly provided by Pharmos Ltd. (Rehovot, Israel). All other chemicals were of analytical reagent grade, and solvents were of HPLC grade.

2.2. *Calculation of physicochemical properties*

The physicochemical properties of the tested compounds were calculated by ACD/Lab version 10.0 (Advanced Chemistry Development Inc., Toronto, Canada).

2.3. Solubility of the tested compounds in long chain triglycerides

Excess amounts of tested compounds were added to peanut oil. Following incubation for 72 h at 37 °C with constant mixing using magnetic stirrer, samples were centrifuged at 1050 × *g* for 15 min at 37 °C and the concentration of the drugs, following appropriate dilution with ethanol, was determined by HPLC.

2.4. Uptake of dexanabinol and PRS-211,220 by isolated CM

The studies of uptake of molecules by isolated CM (triglyceride (TG) concentration 100 mg/dL) were performed as previously published (Gershkovich and Hoffman, 2005). The stock solutions of dexanabinol and PRS-211,220 (0.1 mg/mL) were prepared in propylene glycol (v/v). An appropriate volume of stock solution of tested drug was added to aliquots of 2 mL of CM emulsion to reach final molar concentration of 1.75×10^{-6} M. The 2 mL aliquots of the CM emulsion were incubated with the tested drug at 37 °C for 60 min with constant mixing by magnetic stirrer and subjected to density gradient ultracentrifugation as previously described (Gershkovich and Hoffman, 2005). The top 1 mL, representing CM fraction, was collected using Pasteur pipette and kept at −20 °C pending analysis.

2.5. Animal studies

All research protocols adhered to the "Principles of Laboratory Animal Care" (NIH publication 85-23, revised in 1985) and were approved by the Animal Experimentation Ethics Committee of the Hebrew University Hadassah Medical School in Jerusalem. Male Wistar rats weighting 350–450 g (Harlan, Israel) were used in these studies.

2.5.1. Pharmacokinetic analysis of dexanabinol and PRS-211,220 following IV bolus administration

The right jugular vein was cannulated using PE50 tubing. After the surgery, rats were housed individually in metabolic cages and fasted overnight with free access to drinking water. The next morning, rats were allocated into two groups: dexanabinol and PRS-211,220. In the case of dexanabinol, a dose of 4.5 mg/kg (6 mg/mL solution in propylene glycol 80%, ethanol 10%, double distilled water (DDW) 10%) was administered as slow (over 30 s) IV bolus injection. Systemic blood (0.3 mL) was sampled at 10 min pre-dose and 5 min, 0.5, 1, 2, 4, 7, 10, 14 and 19 h post-dose. In the case of PRS-211,220, a dose of 5 mg/kg (10 mg/mL solution in propylene glycol 80%, ethanol 10%, DDW 10%) was injected intravenously over 30 s. Systemic blood (0.3 mL) was sampled at 5 min pre-dose and 5 min, 0.25, 0.5, 1, 2, 4, 6, 9, 14 and 24 h post-dose. Plasma was separated by centrifugation (800 × *g*, 5 min, 15 °C) and stored at −20 °C until analysis.

2.5.2. Oral administration of dexanabinol and PRS-211,220 in lipid-free and lipid-based formulation

The rats were treated as described for the IV bolus study. The next morning after the surgery rats were allocated into four groups: dexanabinol in lipid-free formulation (6 mg/mL solution in propylene glycol 80%, ethanol 10%, DDW 10%), dexanabinol in lipid-based formulation (6 mg/mL solution in peanut oil), PRS-211,220 in lipid-free formulation (10 mg/mL solution in propylene glycol 80%, ethanol 10%, DDW 10%) and PRS-211,220 in lipid-based formulation (10 mg/mL solution in peanut oil).

A dose of 4.5 mg/kg dexanabinol in lipid-free and lipid-based formulations was administered by oral gavage. Systemic blood (0.3 mL) was sampled at 10 min pre-dose and 1, 2, 3, 4, 5, 7, 10, 15 and 19 h post-dose.

A dose of 5 mg/kg PRS-211,220 in lipid-free and lipid-based formulations was administered by oral gavage. Systemic blood (0.3 mL) was sampled at 10 min pre-dose and 0.5, 1, 2, 3, 4, 6, 9, 14 and 24 h post-dose.

Plasma was separated by centrifugation (800 × *g*, 5 min, 15 °C) and stored at −20 °C until analysis.

2.5.3. Intestinal lymphatic transport of dexanabinol and PRS-211,220 in freely moving rat model

2.5.3.1. Surgical procedures. Male Wistar rats weighting 400–450 g were used for these studies. Each animal received twice 0.5 mL of peanut oil by oral gavage 1 and 2 h prior to the surgery to aid in visualization of the mesenteric lymph duct. Anesthesia was induced by intraperitoneal (IP) injection of 1 mL/kg of ketamine 20 mg/mL:xylazine 100 mg/mL (90:10, v/v). During the surgery additional IP doses of 0.5 mL/kg of ketamine were administered every 40 min.

The cannulation of the mesenteric lymph duct was performed by a modification of previously described methods (Bollman et al., 1948; Warshaw, 1972). The cannula was secured in place by 5–0 silk. In addition, the right jugular vein and the stomach were cannulated using PE50 tubing.

All cannulas were tunneled subcutaneously to the dorsal part of the neck. The venous and lymphatic cannulas were connected by 2 cm of Tygon® tube (Thomas Scientific, ID 0.7 mm; OD 2.4 mm) to form a shunt of the lymph flow during the post-surgical recovery period. The connection enabled direct drainage of the lymph into the jugular vein, mimicking the normal physiology.

2.5.3.2. Experimental procedure. Following the surgery, animals were transferred to metabolic cages and stabilized overnight (16–24 h), during which the animals fasted, but were infused intragastrically by a solution consisting of 277 mM glucose and 3.4 mM KCl (2.5 mL/h). This solution was also available as a drinking medium *ad libitum*. Five hours after the surgery each animal was administered 200 IU of heparin subcutaneously to prevent occlusion of the lymphatic cannula. One hour prior to the administration of the tested compounds, the intragastric infusion was stopped and drinking solution was changed to water. One hour following the administration of the tested drug, the intragastric infusion of 277 mM glucose and 3.4 mM KCl solution was renewed (2 mL/h) and the solution was also available as a drinking medium. The shunt between the lymphatic and venous cannulas was disconnected following the drug administration and throughout the study to enable collection of lymph fluid and blood samples.

Dexanabinol solution in peanut oil (6 mg/mL) was administered by slow (1 min) intragastric bolus via intragastric cannula in a dose of 4.5 mg/kg. Lymph was collected continuously into

12 mL tubes containing 6 μL of heparin per 1 h for 19 h. Lymph collection tubes were changed 2, 4, 7, 10, 15 and 19 h after dexanabinol administration. Systemic blood (0.3 mL) was sampled at 10 min pre-dose and 1, 2, 3, 4, 5, 7, 10, 15 and 19 h post-dose.

PRS-211,220 solution in peanut oil (10 mg/mL) was administered by slow intragastric bolus via intragastric cannula in a dose of 5 mg/kg. Lymph was collected continuously for 24 h as described above. Lymph collection tubes were changed 2, 4, 6, 9, 14 and 24 h after PRS-211,220 administration. Systemic blood (0.3 mL) was sampled at 10 min pre-dose and 0.5, 1, 2, 3, 4, 6, 9, 14 and 24 h post-dose.

Plasma was separated by centrifugation (800 × *g*, 5 min, 15 °C) and lymph and plasma samples were stored at −20 °C until analysis.

2.6. *Analytical procedures*

Dexanabinol and PRS-211,220 were analysed using LC–MS system comprised of Waters pump (600 controller), Waters auto-sampler (717_{plus} Auto-sampler) and Waters Micro-mass ZQ mass spectrometer (Waters Co., Milford, MA). The compounds served as each other's internal standard. Plasma or lymph samples of 100 μL were mixed with ethanolic solution of internal standard (10 μg/mL) and vortex-mixed with 300 μL of THF for 1 min. After vortex mixing with 3.5 mL of hexane for 2 min and centrifugation (1050 × *g*, 7 min), the upper organic layer was decanted to a fresh test tube and evaporated to dryness by vacuum evaporator. The residue was reconstituted with 100 μL of ethanol and 15 μL were injected into LC–MS system. The mobile phase consisted of methanol: acetonitrile: water (1:7:2) containing 0.1% (v/v) formic acid and 0.05% (v/v) TFA. Flow rate was set at 0.3 mL/min. Retention times for dexanabinol and PRS-211,220 were 4.5 and 5.5 min, respectively. The separation was achieved by XTerra MS, RP_{18}, 3.5 μm, 2.1 mm × 100 mm column (Waters Co., Milford, MA) that was kept at 35 °C. The detection masses (M/Z) were 387 and 437 for dexanabinol and PRS-211,220, respectively. The limit of quantification was 5 ng/mL for both compounds. Calibration curves were linear in the range of 5 ng/mL to 100 μg/mL. The precisions were <2%, expressed as a percentage coefficient of variation.

TG concentrations in plasma were measured using a TG enzymatic kit (Roche, Mannheim, Germany) and Uvikon 933 Double Beam UV/VIS Spectrophotometer (Kontron, Zurich, Switzerland).

2.7. *Statistical analysis*

The data in this paper is presented as mean ± S.E.M., if not specified otherwise. A p-value of less than 0.05 was considered statistically significant. Differences were assessed for significance using Student's *t*-test or ANOVA followed by Tukey–Kramer test, when appropriate.

3. Results

3.1. *Physicochemical properties of tested compounds*

The physicochemical properties of dexanabinol and PRS-211,220, including the experimental solubility in LCT and affinity to CM are summarized in Table 1. Interestingly, despite very similar clog *P* values, PRS-211,220 has shown significantly higher experimental solubility in LCT and significantly lower calculated water solubility than dexanabinol. For both compounds the calculated log *P* was identical to the corresponding $\log D_{(7.4)}$ value, indicating that neither dexanabinol nor PRS-211,220 are ionized at the physiological pH. Although both compounds have shown notable affinity to plasma derived isolated CM *ex vivo*, the association of PRS-211,220 to CM was significantly more efficient.

Table 1 – Summary of experimental and calculated physicochemical properties of dexanabinol and PRS-211,220

Physicochemical property	Dexanabinol	PRS-211,220
MW[a] (g/mol)	386.6	436.6
log P[a]	8.2	8.5
$\log D_{(7.4)}$[a]	8.2	8.5
Water solubility$_{(7.4)}$[a] (μg/mL)	0.38	0.02
LCT solubility[b] (mg/g)	7.9 ± 0.2*	95.8 ± 5.3
Affinity to CM[b] (%)	31.6 ± 5.2*	66.1 ± 2.4

[a] Calculated value (ACD/PhysChem).
[b] Experimental value (see Section 2).
* Statistically significantly different from PRS-211,220.

3.2. *Oral bioavailability of dexanabinol and PRS-211,220 following administration in lipid-free and lipid-based formulation*

The plasma concentration–time plots of dexanabinol following intravenous bolus administration, and following oral gavage administration in lipid-free formulation and LCT-based formulation are shown in Fig. 2. The same data for PRS-211,220 is presented in Fig. 3. The pharmacokinetic parameters of this concentration–time data are summarized in Table 2. It can be clearly seen that there was no difference in the oral bioavailability of dexanabinol when administered as lipid-free solution and when administered with LCT. Both lipid-free and lipid-based formulations provided relatively good oral bioavailability of 36–37%. On the other hand, the oral bioavailability of PRS-211,220 was relatively poor (13%)

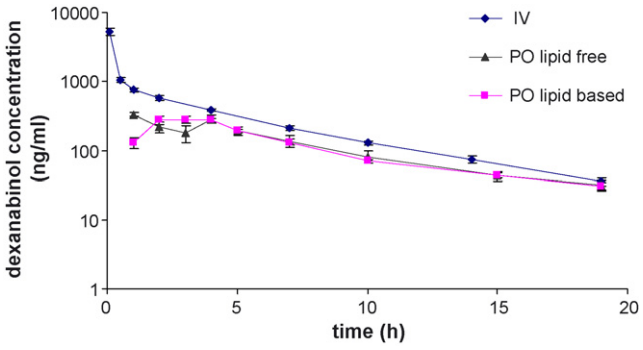

Fig. 2 – Plasma dexanabinol concentration–time semi-logarithmic plot (mean ± S.E.M.) following IV bolus administration (*n* = 5), PO administration in lipid-free formulation (*n* = 5) and PO administration in LCT solution (*n* = 6) of dexanabinol in the dose of 4.5 mg/kg.

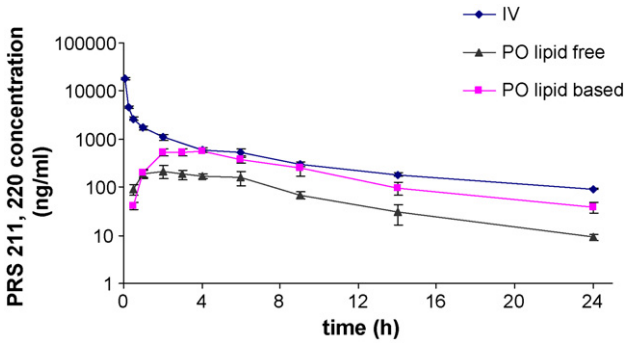

Fig. 3 – Plasma PRS-211,220 concentration–time semi-logarithmic plot (mean ± S.E.M.) following IV bolus administration ($n = 5$), PO administration in lipid-free formulation ($n = 4$) and PO administration in LCT solution ($n = 6$) of PRS-211,220 in the dose of 5 mg/kg.

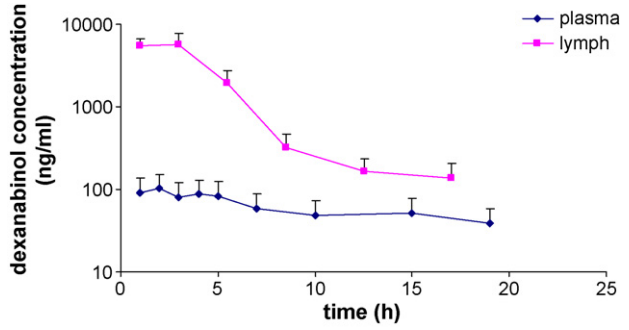

Fig. 4 – Plasma and lymph (middle interval) dexanabinol concentration–time plot (mean ± S.E.M.) following intragastric bolus administration of dexanabinol (4.5 mg/kg) in LCT solution to lymph-cannulated rats ($n = 4$).

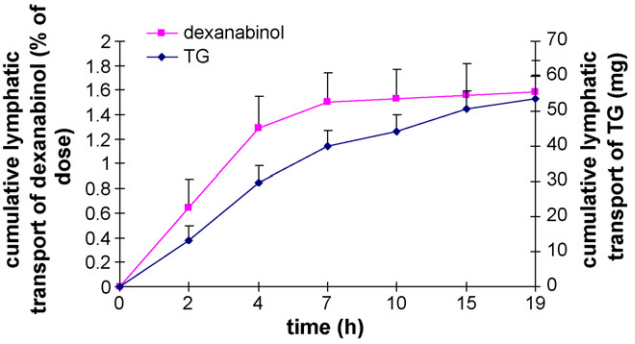

Fig. 5 – Cumulative intestinal lymphatic transport of dexanabinol and TG (mean ± S.E.M.) following intragastric bolus administration of dexanabinol (4.5 mg/kg) in LCT solution to lymph-cannulated rats ($n = 4$).

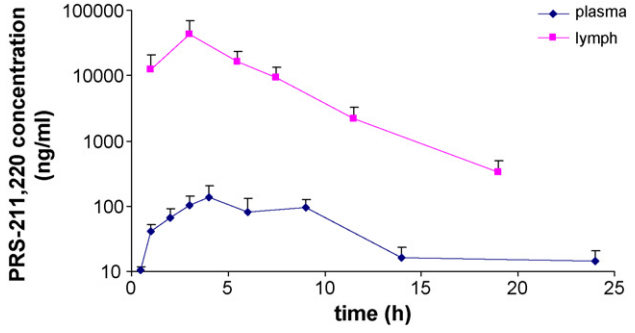

Fig. 6 – Plasma and lymph (middle interval) PRS-211,220 concentration–time plot (mean ± S.E.M.) following intragastric bolus administration of PRS-211,220 (5 mg/kg) in LCT solution to lymph-cannulated rats ($n = 5$).

when administered in lipid-free formulation. However, the oral bioavailability of PRS-211,220 increased by almost three-fold when given in LCT-based formulation.

3.3. *Intestinal lymphatic transport of dexanabinol and PRS-211,220*

The plasma and lymph concentration–time plots of dexanabinol in lymph cannulated rats are shown in Fig. 4. The cumulative percent of the absorbed dose of dexanabinol recovered in lymph and the cumulative transport of TG in lymph following intragastric administration of dexanabinol in peanut oil solution are shown in Fig. 5. Although a relatively small absolute amount of dexanabinol was recovered in lymph (Fig. 5), it can be seen from the difference between the concentrations in lymph and plasma, that the intestinal lymphatic system plays a certain role in the oral absorption of this compound (Fig. 4). Fig. 6 presents the concentration–time plots of PRS-211,220 in plasma and lymph, and the cumulative transport of PRS-211,220 and TG via the intestinal lymph is shown in Fig. 7. The pharmacokinetic parameters derived from

Table 2 – Summary of pharmacokinetic parameters of dexanabinol and PRS-211,220 derived from intravenous bolus administration and oral gavage administration in lipid-free and in peanut oil solution

Compound	Experiment	Dose (mg/kg)	AUC_{all} (h ng/mL)	$T_{1/2}$ (h)	V_{ss} (mL/kg)	CL (mL/h kg)	F (%)
Dexanabinol	IV_{bolus}	4.5	5,902 ± 283*	4.5 ± 0.2	3180 ± 278	724 ± 32	–
	$PO_{lipid\text{-}free}$	4.5	2,193 ± 219	6.3 ± 0.9	–	–	37 ± 5
	PO_{oil}	4.5	2,117 ± 135	6.8 ± 1.4	–	–	36 ± 4
PRS-211,220	IV_{bolus}	5	14,019 ± 384*	9.2 ± 0.9	2127 ± 150	316 ± 7	–
	$PO_{lipid\text{-}free}$	5	1,776 ± 368**	6.7 ± 1.1	–	–	13 ± 3**
	PO_{oil}	5	4,935 ± 688	6.9 ± 0.7			35 ± 6

* Statistically significantly different from both PO administration experiments.
** Statistically significantly different from PO administration in lipid-based formulation.

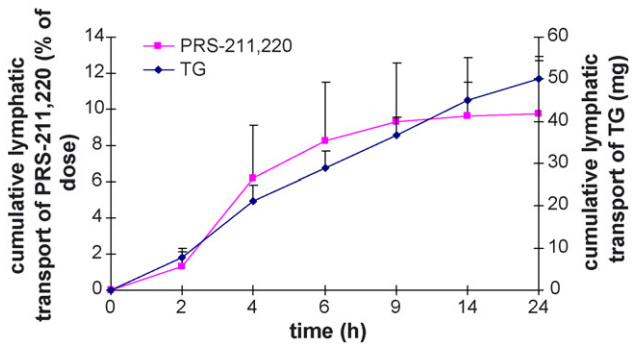

Fig. 7 – Cumulative intestinal lymphatic transport of PRS-211,220 and TG (mean ± S.E.M.) following intragastric bolus administration of PRS-211,220 (5 mg/kg) in LCT solution to lymph-cannulated rats ($n = 5$).

Table 3 – Summary of pharmacokinetic parameters of dexanabinol and PRS-211,220 derived from intestinal lymphatic transport study

PK parameter	Dexanabinol	PRS-211,220
AUC_{all} (h ng/mL)	1139 ± 58	1210 ± 595
% of administered dose absorbed via non-lymphatic pathway	19.3 ± 1	8.6 ± 4.2
% of administered dose recovered in lymph	1.6 ± 0.3	9.7 ± 3.2
Overall intestinal *F* (% of administered dose)	20.9 ± 1.2	18.4 ± 6.8
% of bioavailable dose recovered in lymph	7.5 ± 0.8	60.7 ± 6.8

the intestinal lymphatic transport studies of dexanabinol and PRS-211,220 are summarized in Table 3. It can be clearly seen that when conditions favoring intestinal lymphatic absorption are provided (i.e. LCT-based vehicle), dexanabinol has significantly lower intestinal lymphatic transport component in its total oral bioavailability than does PRS-211,220.

4. Discussion

While the number of highly lipophilic compounds under biopharmaceutical development is constantly increasing (Lipinski et al., 2001), these molecules frequently have poor and erratic oral absorption and consequently are not selected to be leading compounds despite their promising pharmacological properties. There are a few techniques to improve the oral bioavailability of lipophilic compounds, including optimization of intestinal dissolution profile by choosing a proper lipid-based formulation, reduction in intraluminal degradation and metabolism, inhibition of intestinal and hepatic first-pass metabolism and inhibition of intestinal efflux pumps (Dahan and Hoffman, 2006a). However, for highly lipophilic compounds that have significant affinity to CM, lymphatic transport frequently remains the most reliable method to efficiently improve the poor oral bioavailability. The enhancement of the intestinal lymphatic transport of these molecules can be achieved by administration with an LCT-based formulation (O'Driscoll, 2002). LCT-based formulations increase the amount and size of chylomicrons assembled in the enterocyte by increasing the intra-enterocyte synthesis of triglycerides using exogenous and endogenous long chain fatty acids (Trevaskis et al., 2005, 2006b).

There is a long-time dispute in the literature regarding which molecules are candidates for intestinal lymphatic transport (Charman and Stella, 1986; Charman, 2000; O'Driscoll, 2002; Holm and Hoest, 2004; Grove et al., 2006). Some *in vivo* (Dahan and Hoffman, 2006b), *ex vivo* (Gershkovich and Hoffman, 2005) and *in silico* (Holm and Hoest, 2004) methods have been proposed for prediction of the intestinal lymphatic transfer of lipophilic molecules. However no one of these methods gives a reliable quantitative answer. In this work we selected two lipophilic synthetic cannabinoid molecules with intestinal lymphatic transport potential using an *ex vivo* model based on affinity to CM (Gershkovich and Hoffman, 2005). This model accurately indicated that dexanabinol would have less extent of intestinal lymphatic transport than PRS-211,220. Following assessment of the rank order of the intestinal lymphatic transport potential of lipophilic compounds, quantification of the intestinal lymphatic transport by direct measurement of the lipophilic molecules in lymph fluid in an *in vivo* freely moving rat model was performed.

The results of this work demonstrate the interrelationship between intestinal lymphatic transport and the total oral bioavailability of two structurally similar synthetic cannabinoids. It was found that despite similar lipophilicity (Table 1), the lymphatic system played a completely different role in the overall oral bioavailability of these two compounds (Table 3).

PRS-211,220 obeys the widely accepted rules for a compound with significant lymphatic transport potential, which are good TG solubility, $\log P > 5$ (Charman and Stella, 1986; Charman, 2000) and significant association with CM (Gershkovich and Hoffman, 2005). Furthermore, its bioavailability, which is low when given in lipid-free solution, was significantly improved when the drug was administered in LCT solution (Fig. 3, Table 2). Since the overall intestinal absorption was reduced two-fold in the lymphatic transport study (Tables 2 and 3), it can be speculated that the intestinal lymphatic transfer of PRS-211,220 in naive rats (i.e. with no indwelling cannulas) would be 20% of a given dose. It should be noted, that facilitation of the intestinal lymphatic transport by LCT formulation did not affect the degree of transfer to the portal blood, which confirms previously published finding about the lymphatic transport of vitamin D_3 (Dahan and Hoffman, 2005).

On the other hand, dexanabinol has $\log P > 5$, but low TG solubility and only a moderate degree of uptake by CM (Table 1). This compound has relatively good oral bioavailability when given in lipid-free formulation (Fig. 2, Table 2). Taking into consideration similar lipophilicity of these compounds, the dissimilar magnitude of oral bioavailability from water-based formulation is probably not attributed to differences in intestinal permeability. Thus, it is more likely attributed to different solubility in water and perhaps different first-pass metabolism profile.

The LCT formulation did not increase the bioavailability of dexanabinol. Furthermore, when this drug was given in LCT formulation, only a relatively minor portion of the administered (and the absorbed) compound was transferred by the

lymphatic pathway, as measured directly in the lymph fluid (Table 3). In addition to the lower (relatively to the PRS-211,220) affinity to the CM of dexanabinol, its high bioavailability when given in lipid-free formulation (Fig. 2, Table 2) can be an additional explanation for the limited lymphatic transport. It can be speculated, that the efficient removal of the drug from the enterocyte to the portal blood creates "sink" conditions in the enterocyte, i.e. at any given time, the intra-enterocyte concentration of the compound is low, and thus the degree of association with chylomicrons in the enterocyte is reduced.

Quantification of intestinal lymphatic transport by direct measurement of tested drug recovered in lymph in animal models is highly complicated, expensive and a time consuming process. Lipophilic compounds will need an assessment of lymphatic transport potential at some stage in the process of biopharmaceutical development only if they have low oral absorption when given in a lipid-free formulation (like PRS-211,220), unless the lymphatic system is a likely target organ of the tested molecule. It should be noted also, that even for lipophilic molecules with poor oral bioavailability like PRS-211,220, the needed information regarding the potential of intestinal lymphatic transport to increase the oral bioavailability can be frequently achieved by simple oral administration in lipid-free versus LCT solution.

When the final aim is the lymphatic targeting of the lipophilic compound, rather than an increase in its total oral bioavailability, even small absolute amounts of drug transferred via the lymphatic system may create a very high concentration of the drug in the lymph fluid. For example, when lymph versus plasma concentrations are assessed, it can be seen that both PRS-211,220 and dexanabinol are absorbed via the intestinal lymphatic system and lymph concentrations are several fold higher than plasma concentrations for both compounds (Figs. 4 and 6). Thus, if lymphatic system targeting would be an aim for a compound like dexanabinol, its lymphatic transport could not be considered insignificant.

There are very few reports concerning the role of the intestinal lymphatic system in total oral bioavailability. The current work includes the *in vitro* assessment and the *in vivo* evaluation of two compounds which are similar in structure and lipophilicity but very different in other physicochemical properties. Therefore, it provides valuable information about the interrelation between the physicochemical properties of lipophilic drugs and the impact of intestinal lymphatic transport on oral bioavailability.

5. Conclusion

It was demonstrated that despite structural similarity and similar lipophilicity, dexanabinol and PRS-211,220 exhibit a very diverse pattern of oral absorption, and the lymphatic system played quite a different role in the oral bioavailability of these molecules. Dexanabinol, which has moderate affinity to chylomicrons, low solubility in LCT and relatively high predicted solubility in water, shows relatively good oral bioavailability when administered in lipid-free formulation, and intestinal lymphatic transport does not improve further its oral bioavailability. On the other hand, PRS-211,220, which has high affinity to chylomicrons, high LCT solubility and low predicted water solubility, shows low oral bioavailability when administered in lipid-free formulation which can be significantly improved by facilitation of intestinal lymphatic transport using an LCT formulation. High affinity to CM (which may be assessed *ex vivo*) seems to be an important prerequisite for the significant intestinal lymphatic transport and thus for the substantial improvement in oral bioavailability. It seems unlikely that the oral bioavailability of compounds with moderate affinity to CM and relatively good bioavailability when given in lipid-free formulation may be further increased by conditions favoring the intestinal lymphatic transfer.

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