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Research paper

Development of lipophilic ester prodrugs of dolutegravir for intestinal lymphatic transport

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ABSTRACT

The establishment of latent cellular and anatomical viral reservoirs is a major obstacle to achieving a cure for people infected by HIV. Mesenteric lymph nodes (MLNs) are one of the most important anatomical reservoirs of HIV. Suboptimal levels of antiretroviral (ARVs) drugs in these difficult-to-penetrate viral reservoirs is one of the limitations of current antiretroviral therapy (ART) regimens. This study aimed to design and assess highly lipophilic ester prodrugs of dolutegravir (DTG) formulated with long-chain triglyceride (LCT) for delivery of DTG to the viral reservoir in mesenteric lymph and MLNs. A number of alkyl ester prodrugs of DTG were designed based on the predicted affinity to chylomicrons (CM), and the six most promising prodrugs were selected and synthesised. The synthesised prodrugs were further assessed for their intestinal lymphatic transport potential and biotransformation in biorelevant media *in vitro* and *ex vivo*. DTG and the most promising prodrug (prodrug 5) were then assessed in pharmacokinetic and biodistribution studies in rats. Although oral administration of 5 mg/kg of unmodified DTG (an allometrically scaled dose from humans) with or without lipids achieved concentrations above protein binding-adjusted IC_{90} (PA-IC_{90}) (64 ng/mL) in most tissues, the drug was not selectively targeted to MLNs. The combination of lipophilic ester prodrug and LCT-based formulation approach improved the targeting selectivity of DTG to MLNs 4.8-fold compared to unmodified DTG. However, systemic exposure to DTG was limited, most likely due to poor intestinal absorption of the prodrug following oral administration. *In vitro* lipolysis showed a good correlation between micellar solubilisation of the prodrug and systemic exposure to DTG in rats *in vivo*. Thus, it is prudent to include *in vitro* lipolysis in the early assessment of orally administered drugs and prodrugs in lipidic formulations, even when intestinal lymphatic transport is involved in the absorption pathway. Further studies are needed to clarify the underlying mechanisms of low systemic bioavailability of DTG following oral administration of the prodrug and potential ways to overcome this limitation.

1. Introduction

The combination antiretroviral therapy (ART) of two or more antiretroviral (ARV) drugs is widely used for the treatment of HIV infection [1–12]. However, despite viral suppression in the blood, replicate-competent HIV is still found in patients receiving long-term ART [13,14]. The establishment of viral reservoirs during the early stages of infection has been reported in HIV-infected individuals and simian immunodeficiency virus (SIV) infected nonhuman primates [15–17]. These viral reservoirs constitute a substantial systemic viral burden [18], resulting in the recurrence of viremia after the cessation of ART [19–24]. Gut-associated lymphoid tissue (GALT), in particular mesenteric lymph nodes (MLNs), is an important site of immune response and one of the major anatomical HIV reservoirs [25–30]. In SIV-infected rhesus macaques on suppressive ARVs, latent viral reservoirs in MLNs were reported to be larger than other lymphoid tissues and lymph nodes [31,32]. However, suboptimal levels of ARVs in difficult-to-penetrate reservoirs, including MLNs, is one of the reasons of the persistence of

Abbreviations: ART, antiretroviral therapy; ARV, antiretroviral; SIV, simian immunodeficiency virus; GALT, Gut-associated lymphoid tissue; MLNs, mesenteric lymph nodes; LCT, long-chain triglyceride; CM, chylomicrons; PIs, protease inhibitors; DTG, dolutegravir; INSTI, integrase strand transfer inhibitor; CBD, Cannabidiol; FaSSIF, fasted state simulated intestinal fluid; IV, intravenous; PA-IC90, protein binding-adjusted IC90.

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HIV reservoirs and sustained viral replication [33–37]. Efficient targeting of ARVs to MLNs could reduce this important HIV reservoir and bring us closer to a functional cure.

Intestinal lymphatic transport is one of the approaches for targeting of lipophilic drugs or prodrugs to MLNs [38–43]. High lipophilicity (log $D_{7.4}$ greater than 5) [44] and long-chain triglyceride (LCT) solubility above 50 mg/mL are thought to be the most important physicochemical properties for intestinal lymphatic transport of drugs [45]. The affinity of drugs to chylomicrons (CM) was reported to exhibit a linear correlation with the *in vivo* intestinal lymphatic transport [46]. CM are the largest lipoproteins responsible for the absorption of dietary lipids *via* intestinal lymphatics. Since lipids can promote the production of CM and subsequent intestinal lymphatic transport of drugs, lipid-based formulations, especially LCT-based, have been used to target drugs to mesenteric lymph and MLNs [39–41,47–49]. We previously reported that efficient targeting of HIV protease inhibitors (PIs) to MLNs requires a combination approach of lipophilic prodrugs and LCT-based formulation [41] rather than an LCT-based formulation approach alone [48]. Therefore, a combination approach of chemical modifications and LCT-based formulation is needed to target not highly lipophilic ARVs to MLNs.

Dolutegravir (DTG) is a second-generation integrase strand transfer inhibitor (INSTI). It is currently the preferred option in most first-line ART regimens [6,9,50–52] due to its potent antiretroviral activity (protein-adjusted 90% inhibitory concentration (PA-IC_{90}) of 64 ng/mL) and high genetic barrier to drug resistance [53]. Since DTG is not a lipophilic compound (clog P is 0.05 [54]), it is unlikely to have substantial intestinal lymphatic transport following oral administration. Therefore, this study aims to develop a lipophilic ester prodrug system of DTG formulated with an LCT-based vehicle to target DTG to HIV reservoir in MLNs. To this end, a number of simple alkyl ester prodrugs were designed based on *in silico* prediction affinity to CM [44] and synthesised. Synthesised prodrugs were screened for their intestinal lymphatic transport potential by previously reported *in vitro* and *ex vivo* models [40,41,46,55,56]. The most promising prodrug candidate was then assessed *in vivo* for systemic pharmacokinetics and MLNs targeting of DTG in rats.

2. Materials and methods

2.1. Materials

Dolutegravir sodium (CAS: 1051375–19-9) was purchased from Chemshuttle (California, USA). Cannabidiol (CBD, CAS: 13956–29-1) was purchased from THC Pharm GmbH (Frankfurt, Germany). Acyl chlorides (lauroyl, myristoyl, palmitoyl, stearoyl and oleoyl chloride), DMSO-d_6, Intralipid®, Dulbecco's phosphate buffered saline (DPBS), serum triglyceride determination kit, porcine liver crude esterase, porcine pancreatin powder (8 × USP specifications), tris maleate, potassium bromide, phosphate-buffered saline tablets (PBS, P4417-100TAB), ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), sodium hydroxide (NaOH), sodium fluoride (NaF), anhydrous N, N-dimethylformamide (DMF), sesame oil and olive oil were all purchased from Merck Life Science (Gillingham, UK). Calcium chloride was purchased from Alfa Aesar (Lancashire, UK). Linoleoyl chloride was bought from Tokyo Chemical Industry (Oxford, UK). Sodium taurocholate hydrate (NaTc), L-alpha-phosphatidylcholine from egg yolk, sodium phosphate monobasic (NaH_2PO_4) were all purchased from Scientific Laboratory Supplies (Nottingham, UK). Costar Spin-X centrifuge tube filters, HPLC grade methyl tertiary butyl ether (MTBE), ethyl acetate, n-hexane, acetonitrile (ACN), ammonium acetate, ammonium formate crystal and formic acid were all purchased from Fisher Scientific (Loughborough, UK). Polyethylene glycol 400 (PEG-400) was purchased from VWR international LTD (Loughborough, UK). Rat plasma (pooled male Sprague Dawley rat plasma, K3EDTA), dog plasma (pooled male Beagle plasma, K3EDTA) and mouse plasma (pooled male CD-1 (ICR) mouse plasma, K3EDTA) were purchased from Sera Laboratories International Ltd (West Sussex, UK). HPLC-grade water was obtained from PURELAB® Ultra system (ELGA LabWater, UK). Other agents and solvents were obtained from commercial sources and were of HPLC grade or higher.

2.2. Chemistry

2.2.1. Design of dolutegravir ester prodrugs

The prodrugs of DTG were designed by substituting the 7-hydroxyl group with different carbon-length fatty acids to generate simple alkyl esters for increased lipophilicity, as well as predicted improved association with chylomicrons (CM). An established *in silico* model was used to predict the association of compounds with CM [44]. Prodrugs with moderate to high predicted affinity to CM (greater than20%) were selected for subsequent studies.

2.2.2. Synthesis of DTG ester prodrugs

The esterification of DTG was based on a previously reported methodology with slight modifications [57]. Briefly, corresponding acyl chloride (4.4 mmol) was mixed with DTG sodium (1.1 mmol) in anhydrous DMF (3 mL) and stirred under N_2 at 0 °C overnight. The crude product formation was monitored by thin layer chromatography (TLC) and LC-MS/MS. The mixture was filtered and purified by reverse phase chromatography (PuriFlash® PuriFlash 4100, Advion Interchim Scientific, Montluçon, France) using a 50 μm particle size C18 column (IR-50C18-F0012, Advion Interchim Scientific, Montluçon, France) withstand gradient acetonitrile/water from 50:50 to 90:10 (Table 1**)**. Fractions containing pure product were pooled and evaporated to dryness. Purified compound (10 mg) was dissolved in DMSO-d_6 for NMR characterization. Details of characterization methodologies of purified prodrugs are described in **Supplementary Material 1**. The schematic presentation of general synthesis principles and all synthesized prodrugs structures are shown in Fig. 1.

2.3. Assessment of association with CM

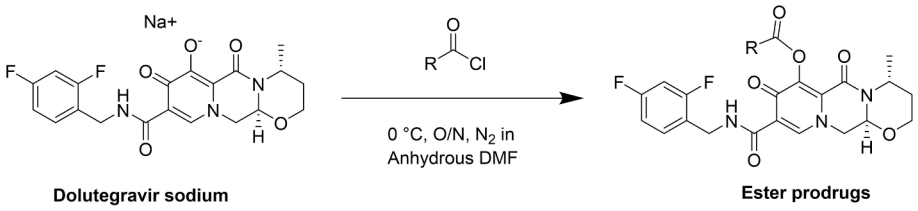
2.3.1. Preparation of artificial CM-like emulsion and rat plasma-derived CM

The artificial CM-like emulsion (Intralipid®) was prepared as previously described [40,41,44,48,55]. Rat plasma-derived CM were produced and isolated based on a previously described methodology with a slight modification [55]. Briefly, rats were fasted overnight, and then 0.5 mL of sesame oil was administered by oral gavage. Two additional doses of 0.3 mL of sesame oil were given each at one-hour intervals following the first dose. One hour after the last dose, rats were anaesthetized (terminal anaesthesia) with 2.5% isoflurane and approximate 10 mL of blood was withdrawn from the vena cava. Plasma was obtained by centrifugation (1,160 *g*, 10 °C, 10 min). The CM isolation was performed as previously described [40,41,55]. Rat plasma-derived CM were kept in 4 °C for up to 24 h until the association assay. The triglyceride

Table 1
Reverse phase gradient program.

Reverse phase gradient program [a]		
Column volume (CV) [b]	**Solvent A [c] (%)**	**Solvent B [d] (%)**
Pre-equilibration (3 CV)	**50**	**50**
0	**50**	**50**
3	**90**	**10**
30	**90**	**10**

[a] Flow rate: 20 mL/min.
[b] Column volume: 19 mL.
[c] Solvent A: acetonitrile.
[d] Solvent B: water.



Ester prodrugs

1: R = $(CH_2)_{10}CH_3$, MW = 601.68

2: R = $(CH_2)_{12}CH_3$, MW = 629.73

3: R = $(CH_2)_{14}CH_3$, MW = 657.79

4: R = $(CH_2)_{16}CH_3$, MW = 685.84

5: R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$, MW = 683.82

6: R = $(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$, MW = 681.81

Fig. 1. Chemical synthesis and structures of DTG and its ester prodrugs. MW, molecular weight.

concentration of artificial CM-like emulsion and rat plasma-derived CM were adjusted to 100 mg/dL with DPBS.

2.3.2. CM association assay

The stock solutions of tested compounds were prepared in 100% DMSO or a mixture of propylene glycol/ethanol (97:3, *v/v*) at a concentration of 0.1 mg/mL**.** The experiments for the uptake of tested compounds by the artificial CM-like emulsion and natural CM were performed by means of a previously described methodology [40,41,46,48,55].

2.3.3. Stability of DTG and its release from prodrugs in biorelevant conditions

The stability of DTG and its release from the prodrugs were assessed in plasma of mouse, rat and dog (surrogate for the environment of intestinal lymph) and fasted state simulated intestinal fluid (FaSSIF, pH = 6.5) with added esterase enzyme (20 IU/mL) [40,41], prepared as previously described [58]. FaSSIF and plasma were pre-heated at 37 °C for 5 min. The stability assay was initiated by spiking stock solution of tested compounds into these biorelevant medium to generate a final concentration of 10 μM or, in cases of poor solubility, 5 μM. The reaction mixtures were incubated at 37 °C and shaken at 200 rpm on a thermo-controlled orbital incubator (Thermo Scientific MaxQ4000, Waltham, MA, USA) for up to 2 h. A hundred microliters were sampled at pre-determined time points and spiked into 300 μL ice-cold acetonitrile to terminate the reaction. The samples were analysed for the levels of DTG and prodrugs by means of HPLC as described below. All experiments were performed in triplicates.

2.3.4. Long-chain triglyceride (LCT) solubility

The LCT solubilities of tested compounds were assessed by adding compound (more than 5 mg) to fresh sesame or olive oil (100 μL) stirred using a magnetic stirrer at 37 °C for 72 h. Following the incubation, the mixture was spin-filtered using Costar Spin-X Centrifuge Tube (Fisher Scientific, Loughborough, UK) at 2,400 *g* for 20 min at 37 °C. The filtrates were first diluted 10-fold with acetone, then further diluted 10-fold with ethanol, followed by a 100-fold dilution with acetonitrile. The diluted samples were analysed for compound concentrations by means of HPLC. All measurements were performed in triplicate.

2.4. Pharmacokinetic and biodistribution studies

2.4.1. Animals

The protocols for pharmacokinetic and biodistribution experiments were reviewed and approved by the University of Nottingham Ethical Committee in accordance with the Animals [Scientific Procedures] Act 1986. Male Sprague Dawley rats (Charles River Laboratories, UK) weighing 275–300 g were housed in Bio Support Unit, University of Nottingham in a controlled-temperature environment with 12 h light/dark cycles and were allowed free access to food (except fasting periods described below) and water.

2.4.2. Formulations of DTG and prodrug 5

Sodium salt form of DTG was used in pharmacokinetics and biodistribution studies. The administration doses of intravenous (IV) bolus and oral groups were 1.05 mg/kg and 5.25 mg/kg of DTG sodium (equivalent to 1 mg/kg and 5 mg/kg of DTG, respectively). Lipid-free formulations for IV and oral administration were solutions of DTG sodium in a mixture of polyethylene glycol 400 (PEG 400)/non pyrogenic water/ethanol (70:20:10, *v/v/v*) at concentrations of 1.05 mg/mL and 5.25 mg/mL, respectively. Sesame oil was used as LCT vehicle in this study. Due to limited solubility of DTG sodium in LCT, sesame oil (1 mL/kg) was administered immediately before drug administration in oral LCT-based group. The doses of prodrug 5 (Fig. 1) in pharmacokinetics and biodistribution studies were 1.63 mg/kg (1 mL/kg of 1.63 mg/mL solution in propylene glycol/ethanol/non pyrogenic water (80:10:10, *v/v/v*)) for IV administration (equivalent to 1 mg/kg of DTG), and 8.15 mg/kg (1 mL/kg of 8.15 mg/mL solution in sesame oil) for oral gavage (equivalent to 5 mg/kg of DTG).

2.4.3. Pharmacokinetic study

Right jugular vein cannulation surgery was performed under general gaseous anaesthesia (2.5% isoflurane in oxygen) as previously described [40,41,55]. Following the surgery, the animals were allowed to recover for 2 nights. Animals were then fasted for up to 16 h prior to the drug administration with free access to water. For the pharmacokinetic study of DTG, rats were divided into 3 groups: IV bolus *via* jugular vein cannula, and oral gavage administration with or without LCT. Following the administration, blood samples were collected from the jugular vein cannula at pre-determined time points (pre-administration, 5, 15, 30 min, 1, 2, 4, 6, 9, 14 and 24 h for IV group; and pre-administration, 30 min, 1, 2, 3, 4, 5, 6, 9, 14 and 24 h for oral lipid-free and LCT-based groups). In the pharmacokinetic study of prodrug 5, rats were divided

into 2 groups: IV bolus and oral gavage administration in LCT-formulation. Blood was sampled at pre-determined time points (pre-administration, 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 36 and 48 h for IV group; and pre-administration, 30 min, 1, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h for oral LCT-based group). EDTA (1.5 M) was used as an anticoagulant for DTG studies, and sodium fluoride (NaF, 10 mg/mL) was used as an anticoagulant and esterase inhibitor for prodrug experiments [40,59,60]. All blood samples were gently mixed and centrifuged (1,160 *g*, 10 °C, 10 min) to separate plasma, which was kept at −80 °C until analysis. At the end of the pharmacokinetic study, rats were euthanized by CO_2 inhalation and death was confirmed by cervical dislocation. Tissues were then harvested and stored at −80 °C until analysis. All biological samples were analysed for DTG and prodrug 5 levels by means of HPLC.

2.4.4. Biodistribution study

Rats were fasted overnight before the study for up to 16 h as described above. DTG sodium was administered by oral gavage to rats at a dose of 5.25 mg/kg (equivalent to 5 mg/kg of DTG) in lipid-free solution formulation with or without sesame oil (representing LCT-based and lipid-free formulation). Prodrug 5 was administered by oral gavage to rats at a dose of 8.15 mg/kg (equivalent to 5 mg/kg of DTG) solubilised in sesame oil. Rats were euthanized at pre-determined time points (2, 4 and 8 h following the administration) by CO_2 inhalation. The mesenteric lymph fluid samples were collected from the superior mesenteric lymph duct immediately after confirming the death of the animals. The mesenteric lymph nodes (MLNs), brain, testes, liver, spleen, thymus, duodenum and duodenum juice, ascending colon, small intestine contents and faeces in colon were harvested and kept in −80 °C until analysis.

2.4.5. In vitro lipolysis

Fasted state incomplete and complete digestion buffers and porcine pancreatin extract were prepared according to previous reports [42,55]. The *in vitro* lipolysis model used in this study was based on previously described methodology with minor modifications [42,43,55,61]. Briefly, 80 μL of freshly prepared prodrug 5 sesame oil solution was incubated with pre-warmed complete digestion buffer (35.5 mL) at 37 °C and stirred for 15 min, then 3.5 mL pancreatin extract was added to initiate the lipolysis reaction. The pH of experimental media was maintained at 6.8 by sodium hydroxide solution (1 M) titration throughout the whole reaction. The termination of lipolysis was determined by a rate of adding of 1 M NaOH falling below 3 μL/min. After the lipolysis reaction was completed, the mixture was transferred to a polyallomer ultracentrifuge tube and ultracentrifuged at 268,350 *g* for 90 min at 37 °C (SORVALL® TH-641 Rotor, Thermo Fisher Scientific, UK). The supernatant lipid fraction, middle micellar fraction and sediment were kept in −80 °C until analysis by means of HPLC.

2.5. Bioanalytical procedures

2.5.1. Sample preparation for HPLC analysis

The stock solution of DTG was prepared at a concentration of 1 mg/mL in DMSO. Prodrugs and CBD as an internal standard (IS) were dissolved in ACN to generate stock solutions at 1 mg/mL. All stock solutions were kept at −20 °C. Working solutions of DTG and prodrugs were prepared by diluting stock solutions with ACN to achieve concentrations of 100, 250, 500, 1000, 5000, 10000, 50000, 100000, 200,000 and 250000 ng/mL. CBD stock solution was diluted by ACN to 50 μg/mL to obtain IS working solution. Calibration curve samples were prepared by mixing 10 μL of working solutions of the DTG or prodrugs and 10 μL IS working solution with 100 μL blank biorelevant media. Three hundred microliters of ice-cold ACN was added and briefly vortex-mixed for protein precipitation. Liquid-liquid extraction was then initiated by adding 3 mL MTBE and vortex-mixing for 5 min. Samples were then centrifuged at 1,160 *g*, 10 °C for 10 min. The upper organic layer was collected and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted with 100 μL ACN-water mixture (1:1, *v/v*) and vortex-mixed for 5 min. Following a brief centrifugation, 60 μL of clear solution was injected into HPLC system. All biological samples (plasma, lymph, CM emulsion, FaSSIF and tissue homogenates) generated from *in vitro*, *ex vivo* and *in vivo* studies underwent the same sample processing procedure described above. The stability of prodrug 5 during sample processing in plasma and MLNs was assessed and reported in **Supplementary Material 2**.

2.5.2. Chromatography conditions

An HPLC-UV system consisting of a Waters Alliance 2695 separations module coupled with Waters 996 photodiode array detector was used for analysis. The autosampler was maintained at 5 °C and the column temperature was 40 °C. Chromatographic separation was achieved using a Waters XTerra MS C18 4.6 × 150 mm, 5 μm particle size column (Waters, Cheshire, UK) equipped with a 2 × 4 mm, 3 μm particle size guard column (Phenomenex, Macclesfield, UK). Data were collected and processed using Empower™ 2 software. Complete chromatography conditions for all tested compounds are described in **Supplementary Material Table S2**.

2.5.3. Statistical and pharmacokinetic analysis

One-way ANOVA followed by Tukey's or Dunnett's multiple-comparisons tests, or two-tailed unpaired *t*-test were used where appropriate. All values were expressed as mean ± standard deviation (SD). A significant difference was stated when a p value was below 0.05. The statistical analyses were performed using GraphPad Prism version 7.04 (GraphPad Software, Inc., San Diego, CA, USA). Pharmacokinetic parameters generated from plasma concentration–time profiles were calculated by non-compartmental analysis using Phoenix® WinNonlin® 6.3 software (Pharsight, Mountain View, CA, USA).

3. Results

3.1. Prodrug design, synthesis and structural characterisation

Physicochemical parameters of prodrugs used for *in silico* prediction of association with chylomicrons (CM) and predicted affinity of prodrugs to CM were calculated using ACD/I-Lab (Advanced Chemistry Development Inc., Toronto, ON, Canada) and are listed in Table 2. Prodrugs conjugated with 12-, 14-, 16- and 18-carbon length fatty acids were selected for synthesis due to their moderate to high predicted association with CM. The description of synthetic reactions and the chemical structures of prodrugs are shown in Fig. 1. The full characterisation of synthesised prodrugs can be found in **Supplementary Material 3**. Prodrugs 1–4 were conjugated with saturates fatty acids, while prodrugs 5 and 6 were conjugates with unsaturated oleic (C18:1) and linoleic acid (C18:2), respectively.

3.2. Association with artificial and natural CM

The affinity of DTG and prodrugs 1–6 to artificial and rat plasma-derived CM is summarised in Fig. 2. Unmodified DTG showed, as predicted, no affinity to CM, while all prodrugs showed moderate to high affinity to CM. Interestingly, as the saturated fatty acids chain length increased in compounds 1–4, the association of prodrugs with CM decreased. After introducing double bonds on the long-chain fatty acid in prodrugs 5 and 6, the association with CM increased with the number of double bonds introduced, suggesting the degree of unsaturation correlated with drug-CM association performance.

3.3. The stability of DTG and biotransformation of prodrugs in biorelevent media

The hydrolysis half-lives of DTG and its prodrugs in fasted state

Table 2
Physicochemical properties of DTG and its prodrugs, and in silico prediction of association with CM [44].

Compounds	cLog $D_{7.4}$	cLog P – cLog $D_{7.4}$	PSA	H-acceptors	FRB	Density (g/cm^3)	Molar volume (cm^3)	H-donors	Predicted association with CM (%)
DTG	0.05	0.18	99.18	8	3	1.53	273.7	2	0.034
1	5.2	0	105.25	9	15	1.27	472.2	1	22.88
2	6.08	0	105.25	9	17	1.24	504.2	1	51.63
3	6.82	0	105.25	9	19	1.22	536.3	1	78.31
4	7.96	0	105.25	9	21	1.2	568.3	1	94.14
5	6.76	0	105.25	9	20	1.21	561.6	1	84.71
6	6.37	0	105.25	9	19	1.22	554.9	1	76.97

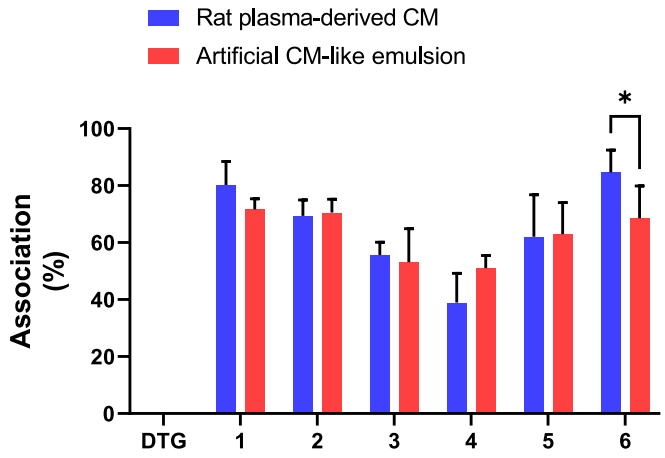


Fig. 2. Association of DTG and its prodrugs with rat plasma-derived CM and artificial CM-like emulsion, representing the potential for intestinal lymphatic transport (n = 5). Two-tailed unpaired *t* test was used for statistical analysis. All results are presented as mean ± SD, n = 5. *, $p < 0.05$.

simulated intestinal fluid (FaSSIF) plus esterase enzyme (20 IU/mL) and plasma (mouse, rat and dog) are shown in Fig. 3**A**. Due to high stabilities, the half-lives of catabolism of unmodified DTG in FaSSIF and plasma could not be calculated. The half-lives of prodrugs catabolism in FaSSIF increased with the extension of the length of fatty acids (prodrugs 1–4) and decreased when the number of double bonds increased (prodrugs 5–6). A similar trend can also be observed in the plasma of all tested species. All prodrugs were more stable in FaSSIF than in plasma. Parent drug DTG was efficiently released from all prodrugs in plasma (Fig. 3**B-D**). The half-lives of hydrolysis of prodrugs in FaSSIF and plasma are available in **Supplementary Material 4 Figure S1**.

3.4. Long-chain triglyceride (LCT) solubility

The solubilities of DTG and prodrugs in sesame and olive oils are summarised (Table 3). DTG had a very low solubility of 0.015 ± 0.002 mg/mL and 0.029 ± 0.004 mg/mL in sesame and olive oil, respectively. All saturated fatty acid prodrugs (1–4) exhibited solubility of no more than 5 mg/mL in both oils. However, unsaturated fatty acids ester prodrugs 5 and 6 showed significantly higher solubility of 49.1 ± 0.8 mg/mL and 40.4 ± 2.1 mg/mL in sesame oil, and 42.5 ± 1.5 mg/mL and 42.5 ± 5.4 mg/mL in olive oil, respectively. Taking together the results of CM affinity, the kinetics of DTG released in FaSSIF and plasma, and especially LCT solubility, prodrug 5 was selected as the most promising candidate for subsequent *in vivo* studies in rats.

3.5. Pharmacokinetics of DTG and prodrug candidate

The pharmacokinetic profiles of DTG and prodrug 5 in plasma were generated following intravenous (IV) bolus and oral gavage administration in lipid-free and LCT-based formulations in rats (Fig. 4**A-B**). Pharmacokinetic parameters of DTG and prodrug 5 were calculated based on the plasma concentration–time profiles and summarised in Table 4. The absolute oral bioavailability of DTG did not show a significant difference between lipid-free (57 ± 17%) and LCT-based (69 ± 19%) groups.

On the other hand, prodrug 5 showed a profile of released DTG following an IV bolus administration (Fig. 4**C**). Although prodrug 5 itself was only detectable in plasma up to 24 h after the IV bolus administration, the levels of released DTG were detectable in the systemic circulation for more than 48 h. The AUC_{inf} of DTG following IV bolus administration of prodrug 5 is comparable to the AUC_{inf} after IV bolus administration of unmodified DTG at an equivalent dose (Table 4). This suggests a complete or near complete biotransformation of prodrug 5 to active DTG *in vivo* in rats. After oral administration of prodrug 5 in LCT-based formulation, only DTG can be detected in systemic blood (Fig. 4**D**). This could be due to the rapid conversion of prodrug 5 in intestinal wall, liver or mesenteric lymph. Furthermore, the terminal half-life of released DTG was significantly increased following oral administration of prodrug 5 compared to oral administration of unmodified DTG with lipids (Table 4). However, the absolute oral bioavailability of released DTG following oral administration of prodrug 5 was substantially lower than after oral administration of unmodified DTG with or without lipids at an equivalent dose (Table 4).

3.6. Biodistribution of DTG and prodrug 5

The biodistribution profiles of DTG at 2, 4 and 8 h following oral administration of unmodified DTG with and without lipids are shown in Fig. 5**A-B**. In both lipid-free and LCT-based groups, the concentrations of DTG in mesenteric lymph and MLNs were lower than in serum at each time point. However, the levels of DTG in MLNs and other tissues were higher than protein binding-adjusted IC_{90} (PA-IC_{90}) (64 ng/mL). Following oral administration of prodrug 5 in LCT-based formulation, the distribution of released DTG and intact prodrug 5 in mesenteric lymph, MLNs and other tissues were analysed at the same time points as for unmodified DTG administration (Fig. 6**A-B**). At 2 h following oral administration of prodrug 5, the concentration of released DTG was two folds higher in mesenteric lymph than in serum, suggesting a mesenteric lymphatic targeting of prodrug 5 and release of active drug within the lymphatic system (Fig. 6**B**). High concentrations of prodrug 5 were found in small intestine contents and faeces in the colon following oral administration of prodrug 5 (Fig. 7). The drug concentration ratio in tissues to serum was previously used to estimate the efficiency of tissue distribution of drugs [62,63]. Orally administered prodrug 5 increased the lymph-to-serum and MLNs-to-serum ratio of DTG concentration by 8.4-fold and 4.8-fold, respectively, at 2 h in comparison to unmodified DTG (Fig. 8). This suggests that orally administered prodrug 5 increased the selectivity for targeting DTG to mesenteric lymphatics.

3.7. Intraluminal processing of prodrug 5

Following the results of unexpectedly low systemic exposure of DTG after oral administration of prodrug 5, the mechanism of processing of prodrug 5 formulated in an LCT-based vehicle in the small intestine was investigated using an *in vitro* lipolysis system. The results of the post-

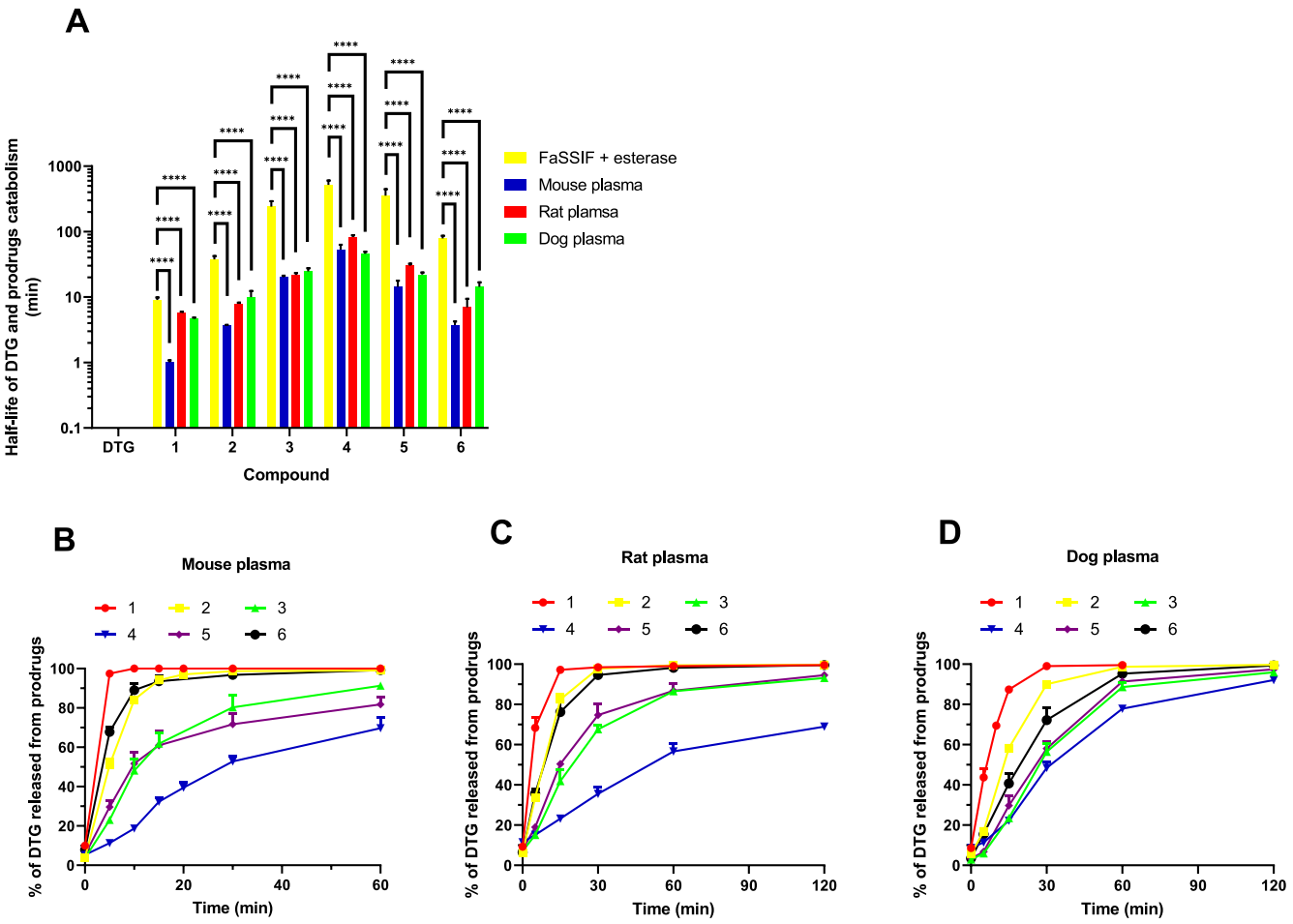


Fig. 3. The hydrolysis of DTG and its prodrugs in FaSSIF plus esterase enzyme (20 IU/mL) and plasma (mouse, rat and dog). (A) The half-lives of DTG and prodrugs catabolism in FaSSIF + esterase, mouse, rat and dog plasma. Due to the high stabilities in FaSSIF and plasma, the half-life of DTG cannot be calculated. (B)-(D) The release profiles of DTG from corresponding prodrugs in mouse, rat and dog plasma. All results are presented as mean ± SD, n = 3. One-way ANOVA followed by Dunnett's multiple comparison test was used for statistical analysis. FaSSIF + esterase was used as the control group to assess the stability of prodrugs in plasma ****, $p < 0.0001$. Additional statistical analysis for comparison of hydrolysis half-lives in FaSSIF and plasma between different prodrugs is available in Supplementary material Figure S2.

Table 3
The solubility of DTG and its prodrugs in sesame and olive oils.

	DTG	1	2	3	4	5	6
Sesame oil (mg/mL)	0.015 ± 0.002	1.2 ± 0.12	2.2 ± 0.09	2.2 ± 0.08	3.2 ± 0.3	49.1 ± 0.8[a]	40.4 ± 2.1[b]
Olive oil (mg/mL)	0.029 ± 0.004	1.1 ± 0.06	1.6 ± 0.07	4.5 ± 0.06	5.1 ± 0.05	42.5 ± 1.5[c]	42.5 ± 5.4[d,e]

One-way ANOVA followed by Dunnett's multiple comparison test was used for statistical analysis.

[a] ****, $p < 0.0001$ compared to DTG and other prodrugs in sesame oil group.
[b] ****, $p < 0.0001$ compared to DTG and other prodrugs in sesame oil group.
[c] ****, $p < 0.0001$ compared to DTG and other prodrugs in olive oil group.
[d] ****, $p < 0.0001$ compared to DTG and other prodrugs in olive oil group.
[e] No significant difference compared to prodrug 5 in olive oil group.

lipolysis distribution of prodrug 5 are shown in Fig. 9. Following the lipolysis of sesame oil formulation, around 5.8% of the prodrug 5 dose was found in the micellar layer (fraction available for absorption). More than 90% of prodrug 5 was distributed into unprocessed lipids or precipitated into sediment fraction (combined), indicating poor availability for absorption.

4. Discussion

The establishment of latent cellular and anatomical viral reservoirs is one of the major obstacles to achieving a cure from HIV infection. Mesenteric lymph nodes (MLNs) are one of the most important and largest reservoirs of HIV [26]. Poor penetration of drugs into these reservoirs is an important limitation of current ART regimens [33]. A combination approach of lipophilic prodrugs and long-chain triglyceride (LCT)-based formulation was reported to be efficient for targeting HIV protease inhibitors (PIs) to MLNs [41] rather than an LCT-based formulation-only approach [48]. This work aimed to develop a lipophilic ester prodrug system coupled with LCT-based formulation to achieve targeting of HIV integrase inhibitor dolutegravir (DTG) into HIV reservoirs in MLNs through intestinal lymphatic transport.

4.1. The affinity of DTG prodrugs to chylomicrons (CM)

The degree of intestinal lymphatic transport of drugs was reported in multiple studies to have a strong correlation with their affinity to CM [64,65]. DTG is not a highly lipophilic compound (cLog P = 0.5 [54]) and was no detectable affinity to CM (Table 2 and Fig. 2). We previously reported that increasing the lipophilicity of compounds by lipophilic ester prodrug approach can dramatically increase the affinity to CM [40,41]. Therefore, highly lipophilic alkyl ester prodrugs of DTG were

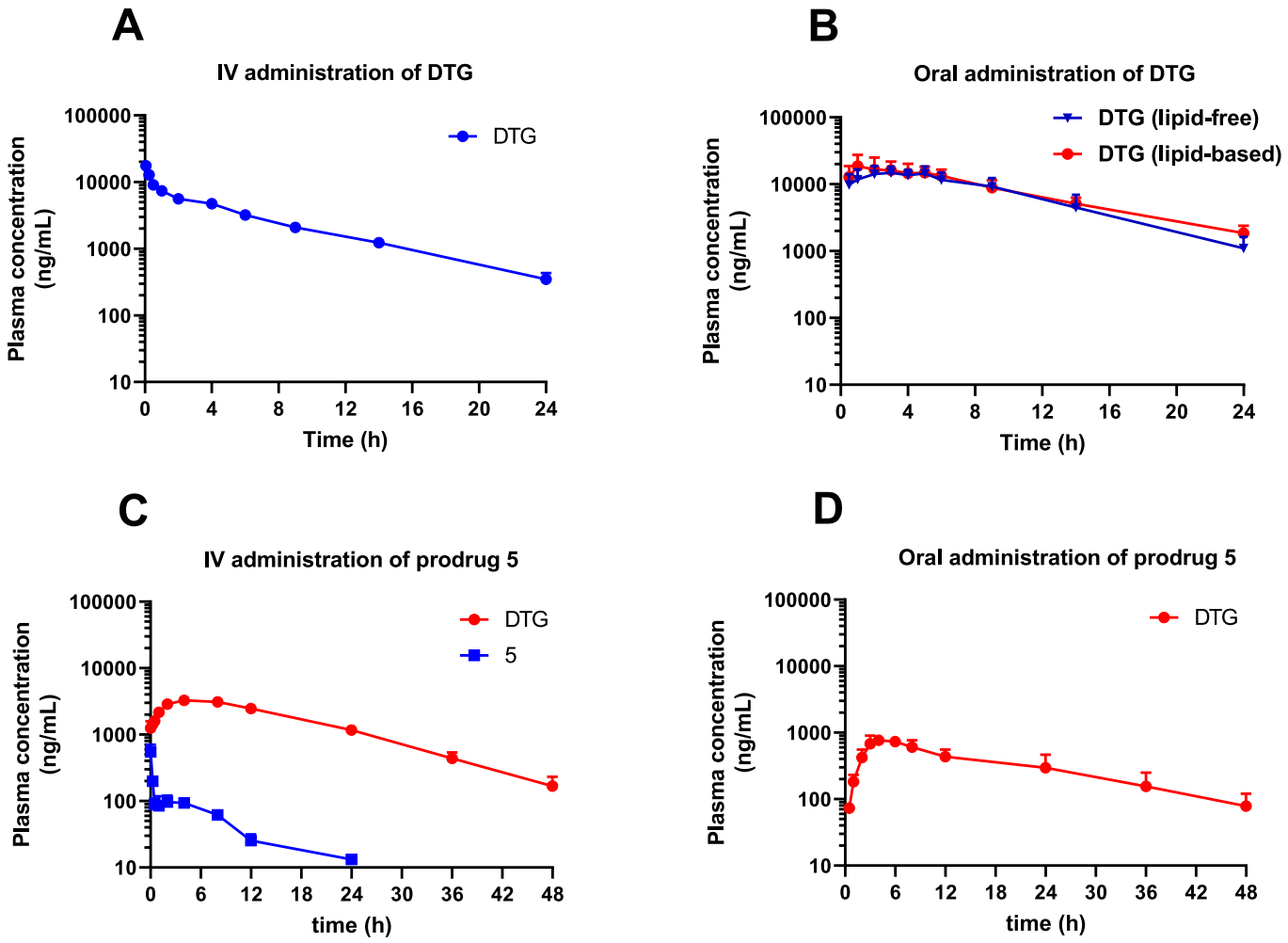


Fig. 4. Plasma concentration–time profiles of DTG and prodrug 5 in rats. (A) IV bolus of DTG sodium (1.05 mg/kg, n = 4); (B) oral gavage administration of DTG sodium (5.25 mg/kg) in lipid-free formulation (n = 4) and with lipids (n = 6); (C) IV bolus of prodrug 5 (1.63 mg/kg, n = 4); (D) oral gavage administration of prodrug 5 (8.15 mg/kg, n = 7) in LCT-based formulation. All values are presented as mean ± SD.

Table 4
Pharmacokinetic parameters of DTG following administration of DTG and prodrug 5 to rats (mean ± SD).

Route of administration	PK parameters of DTG following administration of DTG			PK parameters of DTG following administration of prodrug 5		PK parameters of prodrug 5 following administration of prodrug 5	
	i.v. (n = 4)	Oral		i.v. (n = 4)	Oral	i.v. (n = 4)	Oral
		Lipid-free (n = 4)	LCT-based (n = 6)		LCT-based (n = 7)		LCT-based (n = 7)
AUC_{inf} (h*ng/mL)	60730 ± 11348	172978 ± 50856	210804 ± 58839	69619 ± 7039	16357 ± 5482	1287 ± 145	–
C_0 (ng/mL)	17865 ± 1319	–	–	–	–	591 ± 172	–
C_{max} (ng/mL)	–	16519 ± 4196	21514 ± 7518	3296 ± 147	850 ± 142	–	–
$t_{1/2}$ (h)	5.37 ± 0.77	5.14 ± 0.98	6.45 ± 0.50	9.09 ± 1.10	13.71 ± 3.56***	7.3 ± 1.0	–
V_{ss} (mL/Kg)	120 ± 7	–	–	–	–	1292 ± 72	–
CL (mL/h/Kg)	17 ± 4	–	–	–	–	128 ± 14	–
F_{oral} (%)	–	57 ± 17	69 ± 19	–	5.4 ± 1.8****	–	–

AUC_{inf}, area under the curve from time zero to infinity; C_0, concentration extrapolated to time zero; C_{max}, maximum observed concentration; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state; CL, clearance; F_{oral}, absolute oral bioavailability.
Unpaired two-tailed test was used for statistical analysis, all values are presented as mean ± SD.
***, $p < 0.001$ compared to DTG oral LCT-based group; ****, $p < 0.0001$ compared to DTG oral LCT-based group.

designed. Six prodrugs were synthesised based on the predicted affinity value to CM for further experimental assessments (Table 2). All synthesised prodrugs showed moderate to high experimental association (30–70%) with artificial and natural CM (Fig. 2), indicating a high potential for intestinal lymphatic transport. Interestingly, there was a decrease in the affinity of prodrugs to CM when the saturated alkyl chain was elongated above C12. Similar results were observed in our previous studies [40,41]. It has been proposed that an interplay between specific physicochemical properties, such as lipophilicity and molecular weight, leads to a limited window favouring higher affinity to CM [41].

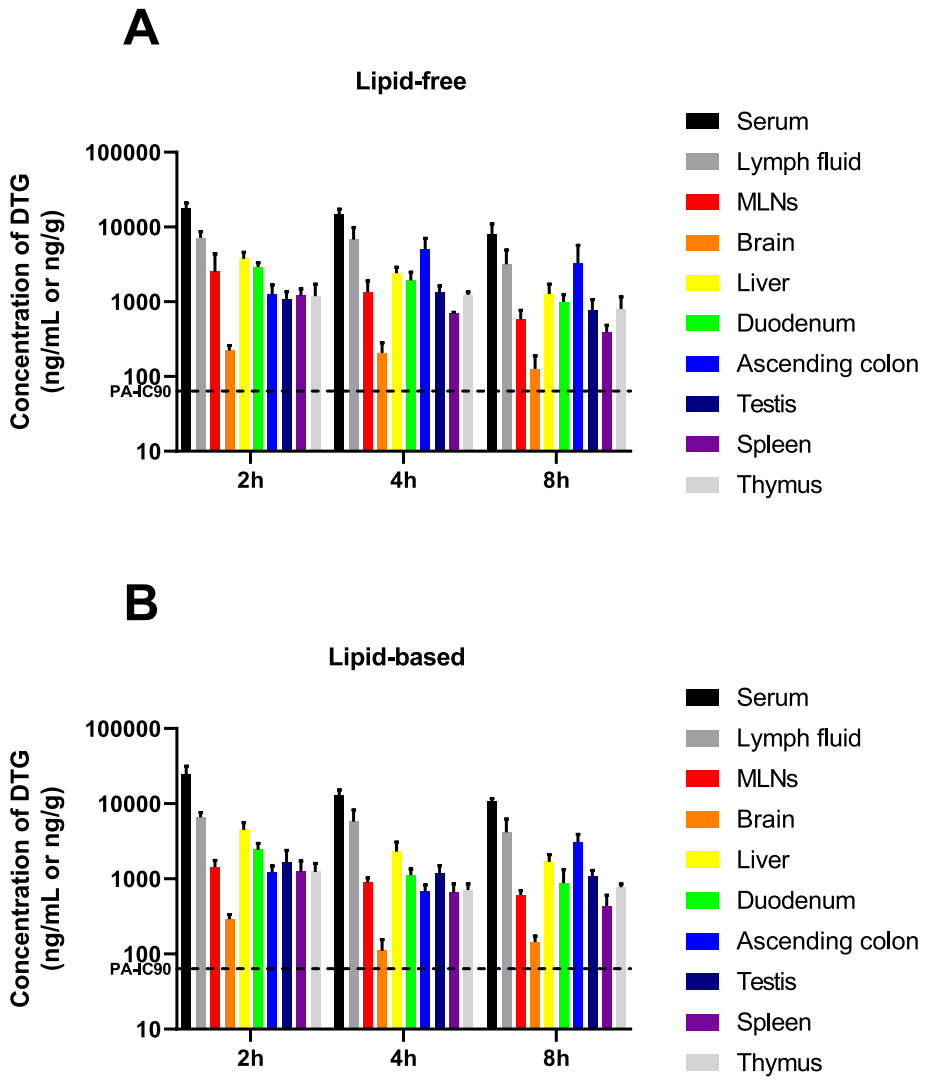


Fig. 5. Biodistribution of DTG at 2, 4 and 8 h following oral administration of DTG sodium (at an equivalent dose of 5 mg/kg of DTG) (A) in lipid-free formulation and (B) with lipids. All results are presented as mean ± SD, n = 4. PA-IC_{90} = 64 ng/mL.

4.2. The stability and biotransformation of DTG prodrugs in biorelevant media

In order to efficiently deliver active drugs to the intestinal lymph and MLNs, the ideal lipophilic prodrug has to be stable in the intestinal lumen, but rapidly enzymatically degraded in the lymph fluid following uptake into the intestinal lymphatic system. Therefore, prodrugs were assessed for their stability in a fasted state simulated intestinal fluid (FaSSIF) supplemented with esterase activity, representing the environment of the intestinal lumen, and plasma (a surrogate of lymph [40,41]). All prodrugs exhibited good stability in the conditions mimicking the intestinal environment (Fig. 3**A**) and rapid DTG release profiles in the plasma of all tested species (Fig. 3**B-D**), suggesting that the current prodrug system based on simple alkyl esters has the potential to deliver active DTG to intestinal lymph and MLNs efficiently. It is worth noting that only 'activated ester' prodrugs achieved the outcome of good stability in the intestine but rapid release in the plasma in our previous studies [40,41]. The 'activated ester' approach is to insert a heteroatom (O or S) at the beta or gamma position of the acyl moiety to increase the sensitivity of the carboxyl esters to the action of the plasma and lymph carboxylesterase [66]. However, in the case of DTG prodrugs, the 'activated ester' approach does not seem to be necessary as this outcome is achieved with simple alkyl esters.

4.3. The solubility of DTG prodrugs in LCT

The solubility of prodrug candidates in LCT was assessed in sesame and olive oils. As both sesame and olive oils are mainly composed of triglycerides containing unsaturated long-chain fatty acids, such as oleic and linoleic acids, they were reported to efficiently promote the intestinal lymphatic transport of lipophilic compounds [43]. The results indicate that the solubility of prodrugs (Table 3) was more related to the degree of saturation of conjugated alkyl esters than the chain length of the fatty acid (Fig. 1). Taken together, the results of CM affinity, stability and DTG released in FaSSIF and plasma, and especially LCT solubility, prodrug 5 was selected as the most promising candidate for subsequent *in vivo* studies in rats.

4.4. Pharmacokinetics of DTG and prodrug 5

A good oral bioavailability (75.6%) was previously reported following oral administration of DTG in a lipid-free formulation to rats [67]. Similar results were also observed in this study (Table 4). The oral bioavailability of DTG did not show significant differences between lipid-free (57 ± 17%) and LCT-based groups (69 ± 19%), which is expected due to its negligible affinity to CM (Fig. 2). On the other hand, the absolute systemic oral bioavailability of DTG was quite low following

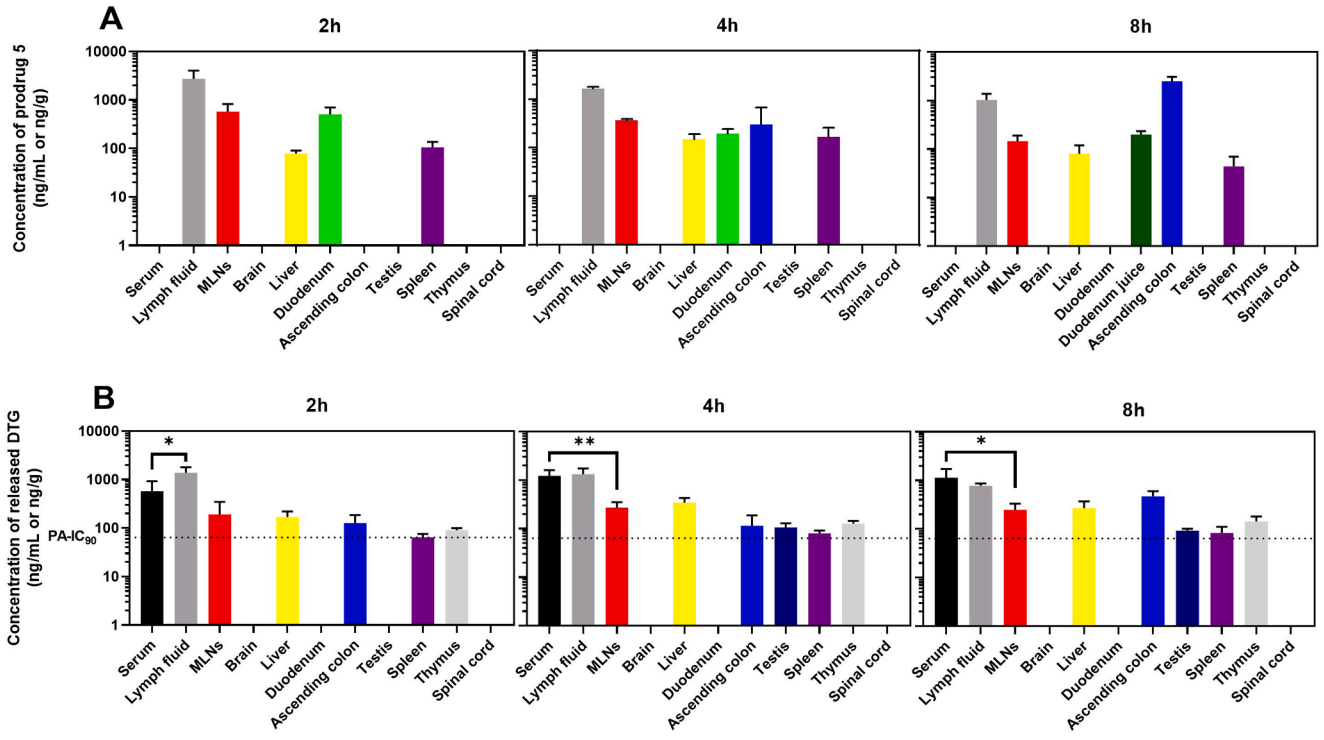


Fig. 6. Biodistribution of (A) prodrug 5 and (B) DTG at 2, 4 and 8 h following oral administration of prodrug 5 (at an equivalent dose of 5 mg/kg of DTG) in LCT-based formulation. All results are presented as mean ± SD, n = 4. PA-IC_{90} = 64 ng/mL. *, $p < 0.05$; **, $p < 0.01$.

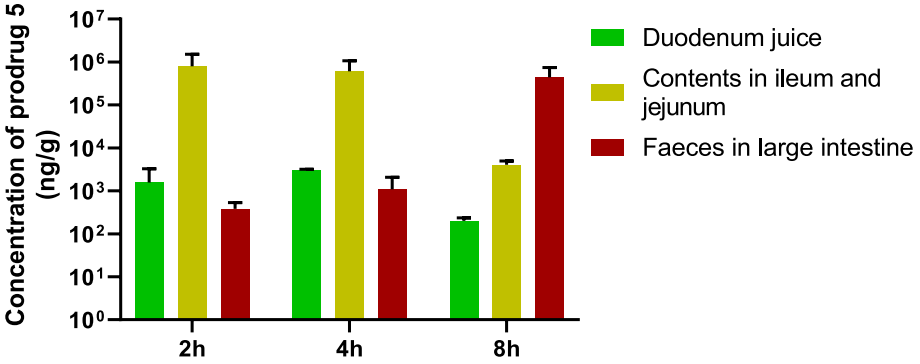


Fig. 7. Concentrations of prodrug 5 in duodenum juice, contents in ileum and jejunum and faeces in large intestine at 2, 4 and 8 h following oral administration of prodrug 5 (at an equivalent dose of 5 mg/kg of DTG) in LCT-based formulation. All results are presented as mean ± SD, n = 4.

the oral administration of prodrug 5 (Table 4). This result was surprising, as lipophilic prodrug systems designed for intestinal lymphatic targeting were previously reported to also improve the systemic oral bioavailability of the parent drug, or at least to be comparable to the parent drug administration [40,41,68,69]. Notably, prodrug 5 itself was not detected in plasma after oral administration. Furthermore, the prolonged terminal slope of released DTG in plasma was observed following oral administration of prodrug 5, suggesting flip-flop kinetics. It is likely that the prodrug was taken up into MLNs through intestinal lymphatic transport and then slowly released active DTG into the lymph and, subsequently, the systemic circulation.

4.5. Biodistribution of DTG and prodrug 5 in the mesenteric lymphatic system and other reservoirs

The results showed similar concentrations of DTG between lipid-free and LCT-based groups in systemic blood, mesenteric lymph, MLNs and other viral reservoirs following oral administration of unmodified DTG (Fig. 5**A-B**). This suggests that lipids do not affect the absorption and distribution of unmodified DTG. It should be noted that the levels of DTG were lower in MLNs and all other tissues in comparison to serum, indicating that MLNs and other reservoirs were not selectively targeted when unmodified DTG was orally administered (Fig. 5**A-B**). After oral administration of prodrug 5, it was indeed targeted to the intestinal lymphatic system and efficiently released active DTG within 2 h (Fig. 6**A-B**). The lymph-to-serum and MLNs-to-serum ratios of concentration of DTG were substantially increased up to 9.4-fold and 4.8-fold, respectively, following oral administration of prodrug 5 in comparison to oral administration of unmodified DTG (Fig. 8). This suggests that the lipophilic prodrug system can improve the selectivity of targeting of DTG to the mesenteric lymph and MLNs. However, although oral administration of prodrug 5 was shown to selectively target DTG to mesenteric lymphatics, the levels of prodrug 5 and released DTG in MLNs and other tissues were limited by low systemic bioavailability. It was hypothesised that the low oral bioavailability of DTG might be due to the low extent of absorption of the prodrug from the intestinal lumen.

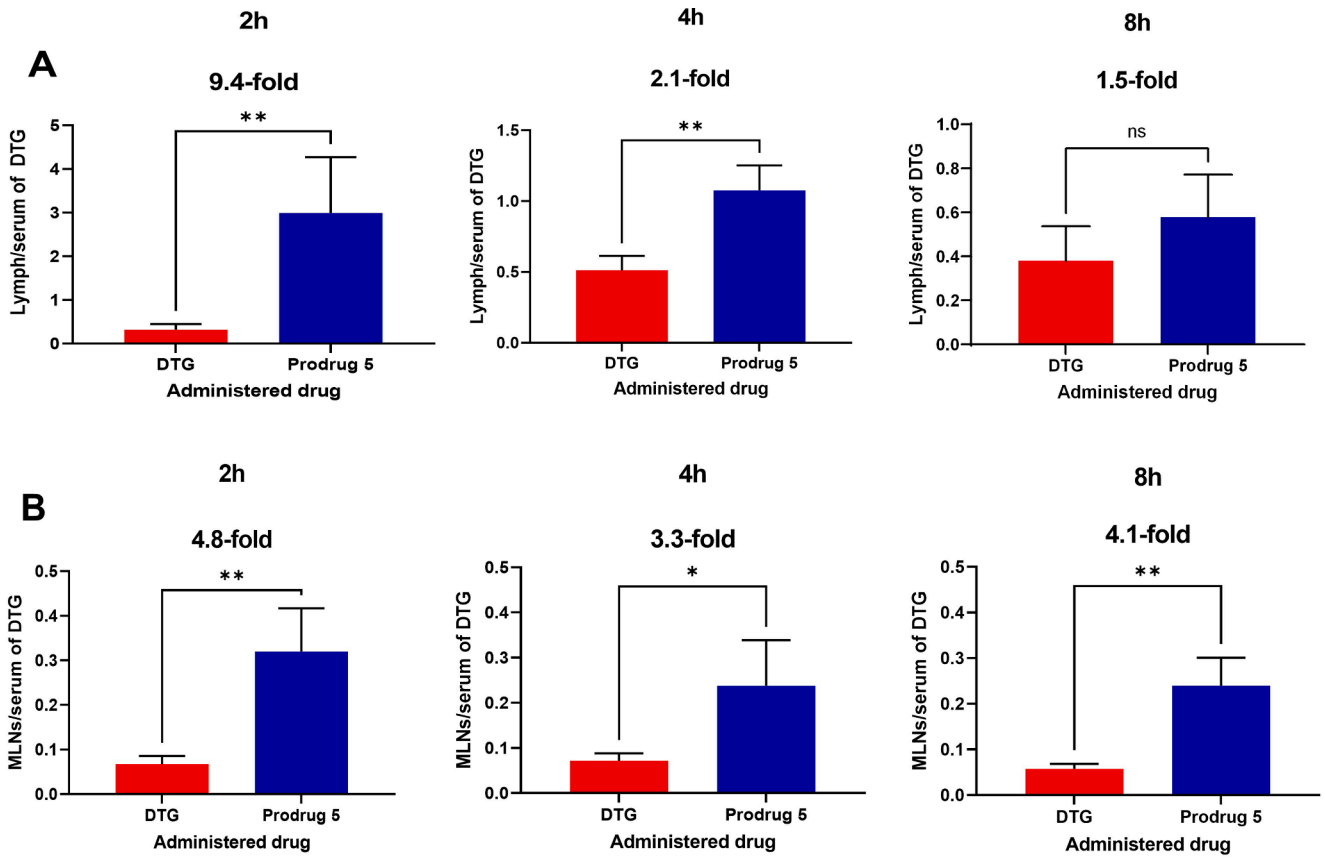


Fig. 8. (A) Lymph to serum and (B) MLNs to serum ratio of the concentration of DTG following oral administration of unmodified DTG or prodrug 5 in LCT-based formulation. All results are presented as mean ± SD, n = 4. Unpaired two-tailed *t* test was used for statistical analysis. ns, no significant; *, $p < 0.05$; **, $p < 0.01$.

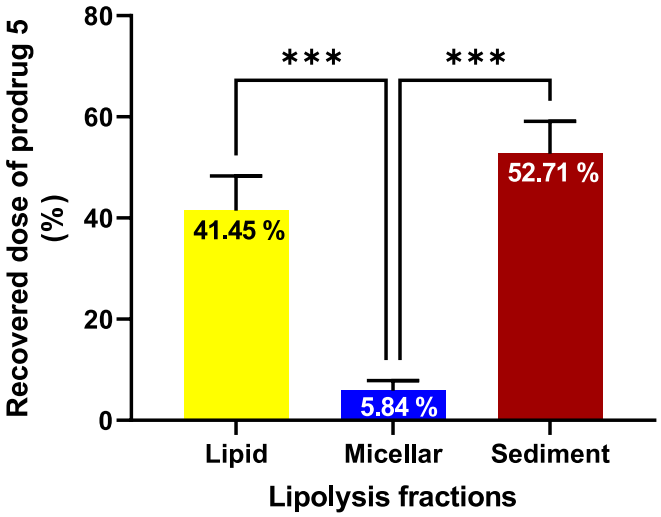


Fig. 9. Distribution of prodrug 5 in the lipid, micellar and sediment fractions after lipolysis of 80 μL of LCT-based formulation containing prodrug 5 at a concentration of 8.15 mg/mL. All results are presented as mean ± SD, n = 3. One-way ANOVA followed by Tukey multiple comparisons was used for statistical analysis. ***, $p < 0.001$. DTG levels were undetectable in all fractions.

This hypothesis was further tested by collecting and analysing the duodenum juice, contents in the ileum and jejunum, and faeces in the colon following oral administration of prodrug 5. Results showed extremely high concentrations of prodrug 5 in the upper and lower parts of the intestinal tract (Fig. 7). This was surprising, as in our and other groups previous reports, highly lipophilic drugs and prodrugs were well absorbed and exhibited enhanced systemic bioavailability with high concentrations in mesenteric lymph, MLNs and plasma following oral administration with LCT-based formulations [39–41,49,55,65,70–74]. To shed light on the intraluminal processing of prodrug 5 in the presence of LCT, *in vitro* lipolysis studies were conducted.

4.6. *In vitro lipolysis of LCT-based formulation of prodrug 5*

The LCT-based formulation containing prodrug 5 was evaluated using an *in vitro* lipolysis model. This model is commonly used to aid in designing and developing the lipidic delivery systems of oral drugs as it simulates the process of lipid digestion in the small intestine [42,55,72]. During lipolysis in the small intestine, triglycerides are hydrolysed into 2-monoglyceride and fatty acids by pancreatic lipase. The lipid breakdown products, bile salts and co-administered drugs form mixed micelles [75,76]. Following lipolysis, lipid and sediment phases are considered not readily absorbed fractions, while the aqueous micellar phase represents the fraction readily available for intestinal absorption [77]. The results showed that half of the dose of prodrug 5 precipitated during the lipids digestion process (Fig. 9). To note, similar rapid precipitation of lipophilic drugs during lipolysis has been previously reported [78,79]. It was previously suggested that the nature of the precipitated form (amorphous or crystalline) might depend on the physicochemical properties of the compound [77,80]. The high amount of precipitated prodrug 5 and limited distribution into micellar fraction could explain high levels of unabsorbed prodrug 5 in the intestinal tract contents (Fig. 7) and low oral systemic bioavailability of DTG following oral administration of prodrug 5 (Table 4), respectively. Around 5.8% of the prodrug 5 dose was recovered in micellar fraction available for absorption. This number, in fact, is very close to the absolute bioavailability of DTG following oral administration of prodrug 5 (5.4%).

Although the role of membrane permeability cannot be completely ruled out, the distribution of prodrug 5 into the micellar fraction seems to be a good predictor of the oral bioavailability of DTG when administered orally to rats in the form of prodrug 5. Interestingly, *in vitro* lipolysis has been reported not to predict well the oral bioavailability of drugs with substantial involvement of intestinal lymphatic transport in their mechanism of absorption. The model only simulates the intraluminal processing of drugs rather than the overall absorption and transport process [43,81–84]. The exact mechanism and reasons for the substantial precipitation of prodrug 5 during the *in vitro* lipolysis studies are unclear and warrant further investigation. However, these results indicate that despite previous reports of the non-predictability of *in vitro* lipolysis for systemic bioavailability of compounds prone to intestinal lymphatic transport, in some cases, *in vitro* lipolysis could be highly predictive. It could be useful to include *in vitro* lipolysis studies in screening routine for drugs or prodrug candidates for intestinal lymphatic targeting.

5. Conclusion

Oral administration of unmodified DTG in rats resulted in good absolute oral bioavailability, but with levels in tissues, including MLNs, below the levels in serum. A lipophilic prodrug system was designed and assessed for selective targeting of DTG to MLNs through intestinal lymphatic transport. Although the selected prodrug candidate showed limited systemic exposure to DTG, the tissue/serum ratio of DTG levels in MLNs, and thus targeting selectivity, was improved by this approach. Two unexpected phenomena were observed in this study. Firstly, poor oral bioavailability was observed for lipophilic prodrugs in the presence of LCT. Secondly, in this work *in vitro* lipolysis model predicted quite well the *in vivo* systemic bioavailability of a compound with substantial intestinal lymphatic transport. Both phenomena warrant further investigation, but it could be useful to include *in vitro* lipolysis assessment in studies aimed to select lipophilic drugs and prodrugs for targeting the MLNs.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2023.08.015.

References

[1] R.M. Gulick, J.W. Mellors, D. Havlir, J.J. Eron, C. Gonzalez, D. McMahon, D. D. Richman, F.T. Valentine, L. Jonas, A. Meibohm, E.A. Emini, J.A. Chodakewitz, Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy, N. Engl. J. Med. 337 (1997) 734–739, https://doi.org/10.1056/NEJM199709113371102.

[2] S.M. Hammer, K.E. Squires, M.D. Hughes, J.M. Grimes, L.M. Demeter, J.S. Currier, J.J. Eron Jr., J.E. Feinberg, H.H. Balfour Jr., L.R. Deyton, J.A. Chodakewitz, M. A. Fischl, A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team, N. Engl. J. Med. 337 (1997) 725–733, https://doi.org/10.1056/NEJM199709113371101.

[3] R.D. Moore, R.E. Chaisson, Natural history of HIV infection in the era of combination antiretroviral therapy, AIDS 13 (1999) 1933–1942, https://doi.org/10.1097/00002030-199910010-00017.

[4] R.W. Shafer, D.A. Vuitton, Highly active antiretroviral therapy (Haart) for the treatment of infection with human immunodeficiency virus type 1, Biomed. Pharmacother. 53 (1999) 73–86, https://doi.org/10.1016/s0753-3322(99)80063-8.

[5] J. Gallant, A. Lazzarin, A. Mills, C. Orkin, D. Podzamczer, P. Tebas, P.M. Girard, I. Brar, E.S. Daar, D. Wohl, J. Rockstroh, X. Wei, J. Custodio, K. White, H. Martin, A. Cheng, E. Quirk, Bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir, abacavir, and lamivudine for initial treatment of HIV-1 infection (GS-US-380-1489): a double-blind, multicentre, phase 3, randomised controlled non-inferiority trial, Lancet 390 (2017) 2063–2072, https://doi.org/10.1016/S0140-6736(17)32299-7.

[6] F. Maggiolo, R. Gulminetti, L. Pagnucco, M. Digaetano, S. Benatti, D. Valenti, A. Callegaro, D. Ripamonti, C. Mussini, Lamivudine/dolutegravir dual therapy in HIV-infected, virologically suppressed patients, BMC Infect. Dis. 17 (2017) 215, https://doi.org/10.1186/s12879-017-2311-2.

[7] N.D. Diaco, C. Strickler, S. Giezendanner, S.A. Wirz, P.E. Tarr, Systematic De-escalation of Successful Triple Antiretroviral Therapy to Dual Therapy with Dolutegravir plus Emtricitabine or Lamivudine in Swiss HIV-positive Persons, EClinicalMedicine 6 (2018) 21–25, https://doi.org/10.1016/j.eclinm.2018.11.005.

[8] M. Aboud, C. Orkin, D. Podzamczer, J.R. Bogner, D. Baker, M.A. Khuong-Josses, D. Parks, K. Angelis, L.P. Kahl, E.A. Blair, K. Adkison, M. Underwood, J. E. Matthews, B. Wynne, K. Vandermeulen, M. Gartland, K. Smith, Efficacy and safety of dolutegravir-rilpivirine for maintenance of virological suppression in adults with HIV-1: 100-week data from the randomised, open-label, phase 3 SWORD-1 and SWORD-2 studies, Lancet HIV 6 (2019) e576–e587, https://doi.org/10.1016/S2352-3018(19)30149-3.

[9] P. Cahn, J.S. Madero, J.R. Arribas, A. Antinori, R. Ortiz, A.E. Clarke, C.C. Hung, J. K. Rockstroh, P.M. Girard, J. Sievers, C.Y. Man, R. Urbaityte, D.J. Brandon, M. Underwood, A.R. Tenorio, K.A. Pappa, B. Wynne, M. Gartland, M. Aboud, J. van Wyk, K.Y. Smith, Durable Efficacy of Dolutegravir Plus Lamivudine in Antiretroviral Treatment-Naive Adults With HIV-1 Infection– 96-Week Results From the GEMINI-1 and GEMINI-2 Randomized Clinical Trials, J. Acquir. Immune Defic. Syndr. 83 (2020) 310–318, https://doi.org/10.1097/QAI.0000000000002275.

[10] P.E. Sax, J.K. Rockstroh, A.F. Luetkemeyer, Y. Yazdanpanah, D. Ward, B. Trottier, A. Rieger, H. Liu, R. Acosta, S.E. Collins, D.M. Brainard, H. Martin, Gs-Us-Investigators, Switching to Bictegravir, Emtricitabine, and Tenofovir Alafenamide in Virologically Suppressed Adults With Human Immunodeficiency Virus, Clin. Infect. Dis. 73 (2021) e485–e493, https://doi.org/10.1093/cid/ciaa988.

[11] O. Osiyemi, S. De Wit, F. Ajana, F. Bisshop, J. Portilla, J.P. Routy, C. Wyen, M. Ait-Khaled, P. Leone, K.A. Pappa, R. Wang, J. Wright, N. George, B. Wynne, M. Aboud, J. van Wyk, K.Y. Smith, Efficacy and Safety of Switching to Dolutegravir/Lamivudine Versus Continuing a Tenofovir Alafenamide-Based 3- or 4-Drug Regimen for Maintenance of Virologic Suppression in Adults Living With Human Immunodeficiency Virus Type 1: Results Through Week 144 From the Phase 3, Noninferiority TANGO Randomized Trial, Clin. Infect. Dis. 75 (2022) 975–986, https://doi.org/10.1093/cid/ciac036.

[12] S.G. Deeks, J. Overbaugh, A. Phillips, S. Buchbinder, HIV infection, Nat. Rev. Dis. Primers 1 (2015) 15035, https://doi.org/10.1038/nrdp.2015.35.

[13] T.W. Chun, L. Stuyver, S.B. Mizell, L.A. Ehler, J.A. Mican, M. Baseler, A.L. Lloyd, M.A. Nowak, A.S. Fauci, Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy, PNAS 94 (1997) 13193–13197, https://doi.org/10.1073/pnas.94.24.13193.

[14] J.K. Wong, M. Hezareh, H.F. Günthard, D.V. Havlir, C.C. Ignacio, C.A. Spina, D. D. Richman, Recovery of Replication-Competent HIV Despite Prolonged Suppression of Plasma Viremia, Science 278 (1997) 1291–1295, https://doi.org/10.1126/science.278.5341.1291.

[15] D. Finzi, M. Hermankova, T. Pierson, L.M. Carruth, C. Buck, R.E. Chaisson, T. C. Quinn, K. Chadwick, J. Margolick, R. Brookmeyer, J. Gallant, M. Markowitz, D. D. Ho, D.D. Richman, R.F. Siliciano, Identification of a Reservoir for HIV-1 in Patients on Highly Active Antiretroviral Therapy, Science 278 (1997) 1295–1300, https://doi.org/10.1126/science.278.5341.1295.

[16] T.W. Chun, D. Engel, M.M. Berrey, T. Shea, L. Corey, A.S. Fauci, Early establishment of a pool of latently infected, resting CD4+ T cells during primary HIV-1 infection, PNAS 95 (1998) 8869–8873, https://doi.org/10.1073/pnas.95.15.8869.

[17] J.B. Whitney, A.L. Hill, S. Sanisetty, P. Penaloza-MacMaster, J. Liu, M. Shetty, L. Parenteau, C. Cabral, J. Shields, S. Blackmore, J.Y. Smith, A.L. Brinkman, L. E. Peter, S.I. Mathew, K.M. Smith, E.N. Borducchi, D.I. Rosenbloom, M.G. Lewis, J. Hattersley, B. Li, J. Hesselgesser, R. Geleziunas, M.L. Robb, J.H. Kim, N. L. Michael, D.H. Barouch, Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys, Nature 512 (2014) 74–77, https://doi.org/10.1038/nature13594.

[18] J.D. Estes, C. Kityo, F. Ssali, L. Swainson, K.N. Makamdop, G.Q. Del Prete, S. G. Deeks, P.A. Luciw, J.G. Chipman, G.J. Beilman, T. Hoskuldsson, A. Khoruts, J. Anderson, C. Deleage, J. Jasurda, T.E. Schmidt, M. Hafertepe, S.P. Callisto, H. Pearson, T. Reimann, J. Schuster, J. Schoephoerster, P. Southern, K. Perkey, L. Shang, S.W. Wietgrefe, C.V. Fletcher, J.D. Lifson, D.C. Douek, J.M. McCune, A. T. Haase, T.W. Schacker, Defining total-body AIDS-virus burden with implications for curative strategies, Nat. Med. 23 (2017) 1271–1276, https://doi.org/10.1038/nm.4411.

[19] A.I. Hernández Cordero, C.X. Yang, J. Yang, X. Li, S. Horvath, T. Shaipanich, J. MacIsaac, D. Lin, L. McEwen, M.S. Kobor, S. Guillemi, M. Harris, W. Lam, S. Lam, M. Obeidat, R.M. Novak, F. Hudson, H. Klinker, N. Dharan, J. Montaner, S.F. P. Man, K. Kunisaki, D.D. Sin, J. Leung, M., INSIGHT START Pulmonary and Genomic Substudy Groups, HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression, EBioMedicine 83 (1999) 10246, https://doi.org/10.1016/j.ebiom.2022.104206.

[20] L. Ahdieh Grant, M.J. Silverberg, H. Palacio, H. Minkoff, K. Anastos, M.A. Young, M. Nowicki, A. Kovacs, M. Cohen, A. Muñoz, Discontinuation of potent antiretroviral therapy predictive value of and impact on CD4 cell counts and HIV RNA levels, AIDS 15 (2001) 2101–2108, https://doi.org/10.1097/00002030-200111090-00005.

[21] W. Stohr, S. Fidler, M. McClure, J. Weber, D. Cooper, G. Ramjee, P. Kaleebu, G. Tambussi, M. Schechter, A. Babiker, R.E. Phillips, K. Porter, J. Frater, Duration of HIV-1 viral suppression on cessation of antiretroviral therapy in primary infection correlates with time on therapy, PLoS One 8 (2013) e78287.

[22] D.J. Colby, L. Trautmann, S. Pinyakorn, L. Leyre, A. Pagliuzza, E. Kroon, M. Rolland, H. Takata, S. Buranapraditkun, J. Intasan, N. Chomchey, R. Muir, E. K. Haddad, S. Tovanabutra, S. Ubolyam, D.L. Bolton, B.A. Fullmer, R.J. Gorelick, L. Fox, T.A. Crowell, R. Trichavaroj, R. O'Connell, N. Chomont, J.H. Kim, N. L. Michael, M.L. Robb, N. Phanuphak, J. Ananworanich, R., V. study group., Rapid HIV RNA rebound after antiretroviral treatment interruption in persons durably suppressed in Fiebig I acute HIV infection, Nat. Med. 24 (2018) 923–926, https://doi.org/10.1038/s41591-018-0026-6.

[23] J.S.Y. Lau, M.Z. Smith, S.R. Lewin, J.H. McMahon, Clinical trials of antiretroviral treatment interruption in HIV-infected individuals, AIDS 33 (2019) 773–791, https://doi.org/10.1097/QAD.0000000000002113.

[24] J. Blazkova, F. Gao, M.H. Marichannegowda, J.S. Justement, V. Shi, E. J. Whitehead, R.F. Schneck, E.D. Huiting, K. Gittens, M. Cottrell, E. Benko, C. Kovacs, J. Lack, M.C. Sneller, S. Moir, A.S. Fauci, T.W. Chun, Distinct mechanisms of long-term virologic control in two HIV-infected individuals after treatment interruption of anti-retroviral therapy, Nat. Med. 27 (2021) 1893–1898, https://doi.org/10.1038/s41591-021-01503-6.

[25] A.P. Burke, W. Benson, J.L. Ribas, D. Anderson, W.S. Chu, J. Smialek, R. Virmani, Postmortem localization of HIV-1 RNA by in situ hybridization in lymphoid tissues of intravenous drug addicts who died unexpectedly, Am. J. Pathol. 142 (1993) 1701–1713.

[26] R.S. Veazey, M. DeMaria, L.V. Chalifoux, D.E. Shvetz, D.R. Pauley, H.L. Knight, M. Rosenzweig, R.P. Johnson, R.C. Desrosiers, A.A. Lackner, Gastrointestinal Tract as a Major Site of CD4+ T Cell Depletion and Viral Replication in SIV Infection, Science 280 (1998) 427–431, https://doi.org/10.1126/science.280.5362.427.

[27] T.W. Chun, D.C. Nickle, J.S. Justement, J.H. Meyers, G. Roby, C.W. Hallahan, S. Kottilil, S. Moir, J.M. Mican, J.I. Mullins, D.J. Ward, J.A. Kovacs, P.J. Mannon, A.S. Fauci, Persistence of HIV in gut-associated lymphoid tissue despite long-term antiretroviral therapy, J Infect Dis 197 (2008) 714–720, https://doi.org/10.1086/527324.

[28] T.W. North, J. Higgins, J.D. Deere, T.L. Hayes, A. Villalobos, L. Adamson, B. L. Shacklett, R.F. Schinazi, P.A. Luciw, Viral sanctuaries during highly active antiretroviral therapy in a nonhuman primate model for AIDS, J. Virol. 84 (2010) 2913–2922, https://doi.org/10.1128/JVI.02356-09.

[29] S. Siddiqui, S. Perez, Y. Gao, L. Doyle-Meyers, B.T. Foley, Q. Li, B. Ling, Persistent Viral Reservoirs in Lymphoid Tissues in SIV-Infected Rhesus Macaques of Chinese-Origin on Suppressive Antiretroviral Therapy, Viruses 11 (2019), https://doi.org/10.3390/v11020105.

[30] J. Musumali, P. Julius, S.N. Siyumbwa, D. Yalcin, G. Kang, S. Munsaka, J.T. West, C. Wood, Systematic post-mortem analysis of brain tissue from an HIV-1 subtype C viremic decedent revealed a paucity of infection and pathology, J. Neurovirol. 28 (2022) 527–536, https://doi.org/10.1007/s13365-022-01099-8.

[31] M. Horiike, S. Iwami, M. Kodama, A. Sato, Y. Watanabe, M. Yasui, Y. Ishida, T. Kobayashi, T. Miura, T. Igarashi, Lymph nodes harbor viral reservoirs that cause rebound of plasma viremia in SIV-infected macaques upon cessation of combined antiretroviral therapy, Virology 423 (2012) 107–118, https://doi.org/10.1016/j.virol.2011.11.024.

[32] S. Siddiqui, S. Perez, Y. Gao, L. Doyle-Meyers, B.T. Foley, Q. Li, B. Ling, Persistent Viral Reservoirs in Lymphoid Tissues in SIV-Infected Rhesus Macaques of Chines-Origin on Suppressive Antiretroviral Therapy, Viruses 11 (2019) 105, https://doi.org/10.3390/v11020105.

[33] C.V. Fletcher, K. Staskus, S.W. Wietgrefe, M. Rothenberger, C. Reilly, J. G. Chipman, G.J. Beilman, A. Khoruts, A. Thorkelson, T.E. Schmidt, J. Anderson, K. Perkey, M. Stevenson, A.S. Perelson, D.C. Douek, A.T. Haase, T.W. Schacker, Persistent HIV-1 replication is associated with lower antiretroviral drug concentrations in lymphatic tissues, PNAS 111 (2014) 2307–2312, https://doi.org/10.1073/pnas.1318249111.

[34] J.P. Freeling, R.J. Ho, Anti-HIV drug particles may overcome lymphatic drug insufficiency and associated HIV persistence, PNAS 111 (2014) E2512–E2513, https://doi.org/10.1073/pnas.1406554111.

[35] J. Stein, S.G. Bonsmann, M., Streeck, H.,, Barriers to HIV Cure, HLA 88 (2016) 155–163, https://doi.org/10.1111/tan.12867.

[36] C.V. Fletcher, Podany, A. T., Antiretroviral Drug Penetration into Lymphoid Tissue, in: T.J. Hope, Richman, D. D., Stevenson, M. (Ed.) Encyclopedia of AIDS, Springer, New York, 2018.

[37] A.S. Devanathan, M.L. Cottrell, Pharmacology of HIV Cure: Site of Action, Clin. Pharmacol. Ther. 109 (2021) 841–855, https://doi.org/10.1002/cpt.2187.

[38] J.A. Yanez, S.W. Wang, I.W. Knemeyer, M.A. Wirth, K.B. Alton, Intestinal lymphatic transport for drug delivery, Adv. Drug Deliv. Rev. 63 (2011) 923–942, https://doi.org/10.1016/j.addr.2011.05.019.

[39] A. Zgair, J.B. Lee, J.C.M. Wong, D.A. Taha, J. Aram, D. Di Virgilio, J.W. McArthur, Y.K. Cheng, I.M. Hennig, D.A. Barrett, P.M. Fischer, C.S. Constantinescu, P. Gershkovich, Oral administration of cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation, Sci. Rep. 7 (2017) 14542, https://doi.org/10.1038/s41598-017-15026-z.

[40] J.B. Lee, A. Zgair, J. Malec, T.H. Kim, M.G. Kim, J. Ali, C. Qin, W. Feng, M. Chiang, X. Gao, G. Voronin, A.E. Garces, C.L. Lau, T.H. Chan, A. Hume, T.M. McIntosh, F. Soukarieh, M. Al-Hayali, E. Cipolla, H.M. Collins, D.M. Heery, B.S. Shin, S. D. Yoo, L. Kagan, M.J. Stocks, T.D. Bradshaw, P.M. Fischer, P. Gershkovich, Lipophilic activated ester prodrug approach for drug delivery to the intestinal lymphatic system, J. Control. Release 286 (2018) 10–19, https://doi.org/10.1016/j.jconrel.2018.07.022.

[41] C. Qin, Y. Chu, W. Feng, C. Fromont, S. He, J. Ali, J.B. Lee, A. Zgair, M. Berton, S. Bettonte, R. Liu, L. Yang, T. Monmaturapoj, C. Medrano-Padial, A.A.R. Ugalde, D. Vetrugno, S.Y. Ee, C. Sheriston, Y. Wu, M.J. Stocks, P.M. Fischer, P. Gershkovich, Targeted delivery of lopinavir to HIV reservoirs in the mesenteric lymphatic system by lipophilic ester prodrug approach, J. Control. Release 329 (2021) 1077–1089, https://doi.org/10.1016/j.jconrel.2020.10.036.

[42] W. Feng, C. Qin, E. Cipolla, J.B. Lee, A. Zgair, Y. Chu, C.A. Ortori, M.J. Stocks, C. S. Constantinescu, D.A. Barrett, P.M. Fischer, P. Gershkovich, Inclusion of Medium-Chain Triglyceride in Lipid-Based Formulation of Cannabidiol Facilitates Micellar Solubilization In Vitro, but In Vivo Performance Remains Superior with Pure Sesame Oil Vehicle, Pharmaceutics 13 (2021), https://doi.org/10.3390/pharmaceutics13091349.

[43] W. Feng, C. Qin, S. Abdelrazig, Z. Bai, M. Raji, R. Darwish, Y. Chu, L. Ji, D.A. Gray, M.J. Stocks, C.S. Constantinescu, D.A. Barrett, P.M. Fischer, P. Gershkovich, Vegetable oils composition affects the intestinal lymphatic transport and systemic bioavailability of co-administered lipophilic drug cannabidiol, Int. J. Pharm. 624 (2022), 121947, https://doi.org/10.1016/j.ijpharm.2022.121947.

[44] P. Gershkovich, J. Fanous, B. Qadri, A. Yacovan, S. Amselem, A. Hoffman, The role of molecular physicochemical properties and apolipoproteins in association of drugs with triglyceride-rich lipoproteins: in-silico prediction of uptake by chylomicrons, J. Pharm. Pharmacol. 61 (2009) 31–39, https://doi.org/10.1211/jpp/61.01.0005.

[45] W.N.A. Charman, V.J. Stella, Estimating the maximal potential for lymphatic transport of lipophilic drug molecules, Int. J. Pharm. 34 (1986) 175–178, https://doi.org/10.1016/0378-5173(86)90027-X.

[46] P. Gershkovich, Hoffman, A., Uptake of lipophilic drugs by plasma derived isolated chylomicrons: linear correlation with intestinal lymphatic bioavailability, Eur. J. Pharm. Sci. 26 (2005) 394–404, https://doi.org/10.1016/j.ejps.2005.07.011.

[47] P. Gershkovich, B. Qadri, A. Yacovan, S. Amselem, A. Hoffman, Different impacts of intestinal lymphatic transport on the oral bioavailability of structurally similar synthetic lipophilic cannabinoids: dexanabinol and PRS-211,220, Eur. J. Pharm. Sci. 31 (2007) 298–305, https://doi.org/10.1016/j.ejps.2007.04.006.

[48] Y. Chu, C. Qin, W. Feng, C. Sheriston, Y. Jane Khor, C. Medrano-Padial, B. E. Watson, T. Chan, B. Ling, M.J. Stocks, P.M. Fischer, P. Gershkovich, Oral administration of tipranavir with long-chain triglyceride results in moderate intestinal lymph targeting but no efficient delivery to HIV-1 reservoir in mesenteric lymph nodes, Int. J. Pharm. 602 (2021), 120621, https://doi.org/10.1016/j.ijpharm.2021.120621.

[49] R. Kochappan, E. Cao, S. Han, L. Hu, T. Quach, D. Senyschyn, V.I. Ferreira, G. Lee, N. Leong, G. Sharma, S.F. Lim, C.J. Nowell, Z. Chen, U.H. von Andrian, D. Bonner, J.D. Mintern, J.S. Simpson, N.L. Trevaskis, C.J.H. Porter, Targeted delivery of mycophenolic acid to the mesenteric lymph node using a triglyceride mimetic prodrug approach enhances gut-specific immunomodulation in mice, J. Control. Release 332 (2021) 636–651, https://doi.org/10.1016/j.jconrel.2021.02.008.

[50] Updated recommendations on HIV prevention, infant diagnosis, antiretroviral initiation and monitoring: March 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO, in.

[51] V. Cento, C.F. Perno, Dolutegravir Plus Lamivudine Two-Drug Regimen: Safety, Efficacy and Diagnostic Considerations for Its Use in Real-Life Clinical Practice-A Refined Approach in the COVID-19 Era, Diagnostics (Basel) 11 (2021), https://doi.org/10.3390/diagnostics11050809.

[52] F. Maggiolo, R. Gulminetti, L. Pagnucco, M. Digaetano, A. Cervo, D. Valenti, A. Callegaro, C. Mussini, Long-term outcome of lamivudine/dolutegravir dual therapy in HIV-infected, virologically suppressed patients, BMC Infect. Dis. 22 (2022) 782, https://doi.org/10.1186/s12879-022-07769-6.

[53] R.C. Rathbun, S.M. Lockhart, M.M. Miller, M.D. Liedtke, Dolutegravir, a second-generation integrase inhibitor for the treatment of HIV-1 infection, Ann. Pharmacother. 48 (2014) 395–403, https://doi.org/10.1177/1060028013513558.

[54] ACD/ChemSketch, version 2020.1.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, www.acdlabs.com, 2020., in.

[55] A. Zgair, J.C. Wong, J.B. Lee, J. Mistry, O. Sivak, K.M. Wasan, I.M. Hennig, D. A. Barrett, C.S. Constantinescu, P.M. Fischer, P. Gershkovich, Dietary fats and

pharmaceutical lipid excipients increase systemic exposure to orally administered cannabis and cannabis-based medicines, Am. J. Transl. Res. 8 (2016) 3448–3459.
[56] J. Stappaerts, S. Geboers, J. Snoeys, J. Brouwers, J. Tack, P. Annaert, P. Augustijns, Rapid conversion of the ester prodrug abiraterone acetate results in intestinal supersaturation and enhanced absorption of abiraterone: in vitro, rat in situ and human in vivo studies, Eur. J. Pharm. Biopharm. 90 (2015) 1–7, https://doi.org/10.1016/j.ejpb.2015.01.001.
[57] B. Sillman, A.N. Bade, P.K. Dash, B. Bhargavan, T. Kocher, S. Mathews, H. Su, G. D. Kanmogne, L.Y. Poluektova, S. Gorantla, J. McMillan, N. Gautam, Y. Alnouti, B. Edagwa, H.E. Gendelman, Creation of a long-acting nanoformulated dolutegravir, Nat. Commun. 9 (2018) 443, https://doi.org/10.1038/s41467-018-02885-x.
[58] M. Margareth, Dissolution Media Simulating Fasted and Fed States, Dissolut. Technol. 11 (2004) 16, https://doi.org/10.14227/dt110204p16.
[59] W. Kromdijk, H. Rosing, M.P. van den Broek, J.H. Beijnen, A.D. Huitema, Quantitative determination of oseltamivir and oseltamivir carboxylate in human fluoride EDTA plasma including the ex vivo stability using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 891–892 (2012) 57–63, https://doi.org/10.1016/j.jchromb.2012.02.026.
[60] M.S. Kim, J.S. Song, H. Roh, J.S. Park, J.H. Ahn, S.H. Ahn, M.A. Bae, Determination of a peroxisome proliferator-activated receptor gamma agonist, 1-(trans-methylimino-N-oxy)-6-(2-morpholinoethoxy-3-phenyl-1H-indene-2-carboxylic acid ethyl ester (KR-62980) in rat plasma by liquid chromatography-tandem mass spectrometry, J. Pharm. Biomed. Anal. 54 (2011) 121–126, https://doi.org/10.1016/j.jpba.2010.07.033.
[61] P. Benito-Gallo, A. Franceschetto, J.C. Wong, M. Marlow, V. Zann, P. Scholes, P. Gershkovich, Chain length affects pancreatic lipase activity and the extent and pH-time profile of triglyceride lipolysis, Eur. J. Pharm. Biopharm. 93 (2015) 353–362, https://doi.org/10.1016/j.ejpb.2015.04.027.
[62] C.G. Thompson, E.P. Rosen, H.M.A. Prince, N. White, C. Sykes, G. de la Cruz, M. Mathews, C. Deleage, J.D. Estes, P. Charlins, L.R. Mulder, M. Kovarova, L. Adamson, S. Arora, E.S. Dellon, A.F. Peery, N.J. Shaheen, C. Gay, D. C. Muddiman, R. Akkina, J.V. Garcia, P. Luciw, A.D.M. Kashuba, Heterogeneous antiretroviral drug distribution and HIV/SHIV detection in the gut of three species, Sci. Transl. Med. 11 (2019), https://doi.org/10.1126/scitranslmed.aap8758.
[63] L. Labarthe, T. Gele, H. Gouget, M.S. Benzemrane, P. Le Calvez, N. Legrand, O. Lambotte, R. Le Grand, C. Bourgeois, A. Barrail-Tran, Pharmacokinetics and tissue distribution of tenofovir, emtricitabine and dolutegravir in mice, J. Antimicrob. Chemother. 77 (2022) 1094–1101, https://doi.org/10.1093/jac/dkab501.
[64] P. Gershkovich, Hoffman, A., Effect of a high-fat meal on absorption and disposition of lipophilic compounds: the importance of degree of association with triglyceride-rich lipoproteins, Eur. J. Pharm. Sci. 32 (2007) 24–32, https://doi.org/10.1016/j.ejps.2007.05.109.
[65] N.L. Trevaskis, R.M. Shanker, W.N. Charman, C.J. Porter, The mechanism of lymphatic access of two cholesteryl ester transfer protein inhibitors (CP524,515 and CP532,623) and evaluation of their impact on lymph lipoprotein profiles, Pharm. Res. 27 (2010) 1949–1964, https://doi.org/10.1007/s11095-010-0199-2.
[66] T.L. Huang, T. Shiotsuki, T. Uematsu, B. Borhan, Q.X. Li, B.D. Hammock, Structure-activity relationships for substrates and inhibitors of mammalian liver microsomal carboxylesterases, Pharm. Res. 13 (1996) 1495–1500, https://doi.org/10.1023/a:1016071311190.
[67] L. Moss, D. Wagner, E. Kanaoka, K. Olson, Y.L. Yueh, G.D. Bowers, The comparative disposition and metabolism of dolutegravir, a potent HIV-1 integrase inhibitor, in mice, rats, and monkeys, Xenobiotica 45 (2015) 60–70, https://doi.org/10.3109/00498254.2014.942409.
[68] E. Nieschlag, J. Mauss, A. Coert, P. Kićović, PLASMA ANDROGEN LEVELS IN MEN AFTER ORAL ADMINISTRATION OF TESTOSTERONE OR TESTOSTERONE UNDECANOATE, Acta Endocrinol (Copenh) 79 (1975) 366–374, https://doi.org/10.1530/acta.0.0790366.
[69] V. Bala, S. Rao, E. Bateman, D. Keefe, S. Wang, C.A. Prestidge, Enabling Oral SN38-Based Chemotherapy with a Combined Lipophilic Prodrug and Self-Microemulsifying Drug Delivery System, Mol. Pharm. 13 (2016) 3518–3525, https://doi.org/10.1021/acs.molpharmaceut.6b00591.
[70] C.T. Tian, J.J. Guo, Y.F. Miao, H.L. Wang, Q. Ye, C.L. Guo, M.Y. Zhang, Z.G. He, J. Sun, Long chain triglyceride-lipid formulation promotes the oral absorption of the lipidic prodrugs through coincident intestinal behaviors, Eur. J. Pharm. Biopharm. 176 (2022) 122–132, https://doi.org/10.1016/j.ejpb.2022.05.015.
[71] M. Grove, G.P. Pedersen, J.L. Nielsen, A. Mullertz, Bioavailability of seocalcitol I: Relating solubility in biorelevant media with oral bioavailability in rats–effect of medium and long chain triglycerides, J. Pharm. Sci. 94 (2005) 1830–1838, https://doi.org/10.1002/jps.20403.
[72] T. Quach, L. Hu, S. Han, S.F. Lim, D. Senyschyn, P. Yadav, N.L. Trevaksis, J. S. Simpson, C.J.H. Porter, Triglyceride-Mimetic Prodrugs of Buprenorphine Enhance Oral Bioavailability via Promotion of Lymphatic Transport, Front. Pharmacol. 13 (2022), 879660, https://doi.org/10.3389/fphar.2022.879660.
[73] S.M. Caliph, F.W. Faassen, C.J. Porter, The influence of intestinal lymphatic transport on the systemic exposure and brain deposition of a novel highly lipophilic compound with structural similarity to cholesterol, J. Pharm. Pharmacol. 66 (2014) 1377–1387, https://doi.org/10.1111/jphp.12268.
[74] G. Lee, S. Han, Z. Lu, J. Hong, A.R.J. Phillips, J.A. Windsor, C.J.H. Porter, N. L. Trevaskis, Intestinal delivery in a long-chain fatty acid formulation enables lymphatic transport and systemic exposure of orlistat, Int. J. Pharm. 596 (2021), 120247, https://doi.org/10.1016/j.ijpharm.2021.120247.
[75] H. Mu, C.E. Hoy, The digestion of dietary triacylglycerols, Prog. Lipid Res. 43 (2004) 105–133, https://doi.org/10.1016/s0163-7827(03)00050-x.
[76] Y. Huang, Q. Yu, Z. Chen, W. Wu, Q. Zhu, Y. Lu, In vitro and in vivo correlation for lipid-based formulations: Current status and future perspectives, Acta Pharm. Sin. B 11 (2021) 2469–2487, https://doi.org/10.1016/j.apsb.2021.03.025.
[77] A.T. Larsen, P. Sassene, A. Mullertz, In vitro lipolysis models as a tool for the characterization of oral lipid and surfactant based drug delivery systems, Int. J. Pharm. 417 (2011) 245–255, https://doi.org/10.1016/j.ijpharm.2011.03.002.
[78] N. Thomas, R. Holm, A. Mullertz, T. Rades, In vitro and in vivo performance of novel supersaturated self-nanoemulsifying drug delivery systems (super-SNEDDS), J. Control. Release 160 (2012) 25–32, https://doi.org/10.1016/j.jconrel.2012.02.027.
[79] A.T. Larsen, A.G. Ohlsson, B. Polentarutti, R.A. Barker, A.R. Phillips, R. Abu-Rmaileh, P.A. Dickinson, B. Abrahamsson, J. Ostergaard, A. Mullertz, Oral bioavailability of cinnarizine in dogs: relation to SNEDDS droplet size, drug solubility and in vitro precipitation, Eur. J. Pharm. Sci. 48 (2013) 339–350, https://doi.org/10.1016/j.ejps.2012.11.004.
[80] L.C. Alskar, J. Keemink, J. Johannesson, C.J.H. Porter, C.A.S. Bergstrom, Impact of Drug Physicochemical Properties on Lipolysis-Triggered Drug Supersaturation and Precipitation from Lipid-Based Formulations, Mol. Pharm. 15 (2018) 4733–4744, https://doi.org/10.1021/acs.molpharmaceut.8b00699.
[81] A. Dahan, Hoffman, A., The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats, Eur. J. Pharm. Biopharm. 67 (2007) 96–105, https://doi.org/10.1016/j.ejpb.2007.01.017.
[82] A. Dahan, Hoffman, A., Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats, Pharm. Res. 23 (2006) 2165–2174, https://doi.org/10.1007/s11095-006-9054-x.
[83] C.J. Porter, A.M. Kaukonen, A. Taillardat-Bertschinger, B.J. Boyd, J.M. O'Connor, G.A. Edwards, W.N. Charman, Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs– Studies with halofantrine, J. Pharm. Sci. 93 (2004) 1110–1121, https://doi.org/10.1002/jps.20039.
[84] A. Larsen, R. Holm, M.L. Pedersen, A. Mullertz, Lipid-based formulations for danazol containing a digestible surfactant, Labrafil M2125CS: in vivo bioavailability and dynamic in vitro lipolysis, Pharm. Res. 25 (2008) 2769–2777, https://doi.org/10.1007/s11095-008-9641-0.