# Constitutive Triglyceride Turnover into the Mesenteric Lymph Is Unable to Support Efficient Lymphatic Transport of a Biomimetic Triglyceride Prodrug

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**ABSTRACT:** The triglyceride (TG) mimetic prodrug (1,3-dipalmitoyl-2-mycophenoloyl glycerol, 2-MPA-TG) biochemically integrates into intestinal lipid transport and lipoprotein assembly pathways and thereby promotes the delivery of mycophenolic acid (MPA) into the lymphatic system. As lipoprotein (LP) formation occurs constitutively, even in the fasted state, the current study aimed to determine whether lymphatic transport of 2-MPA-TG was dependent on coadministered exogenous lipid. *In vitro* incubation of the prodrug with rat digestive fluid and *in situ* intestinal perfusion experiments revealed that hydrolysis and absorption of the prodrug were relatively unaffected by the quantity of lipid in formulations. *In vivo* studies in rats, however, showed that the lymphatic transport of TG and 2-MPA-TG was significantly higher following administration with higher quantities of lipid and that oleic acid (C18:1) was more effective in promoting prodrug transport than lipids with higher degrees of unsaturation. The recovery of 2-MPA-TG and TG in lymph correlated strongly ($R^2$ = 0.99) and more than 97% of the prodrug was associated with chylomicrons. Inhibition of LP assembly by Pluronic L81 simultaneously inhibited the lymphatic transport of 2-MPA-TG and TG. In conclusion, although the TG mimetic prodrug effectively incorporates into TG resynthetic pathways, lipid coadministration is still required to support efficient lymphatic transport. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

**Keywords:** lymphatic transport; prodrugs; lipids/lipoproteins; intestinal absorption; site-specific delivery

## INTRODUCTION

Following oral administration, promotion of drug transport to the systemic circulation via the intestinal lymphatic system, rather than the portal blood, has the potential to confer several pharmacokinetic and pharmacodynamic advantages. First, lymphatic drug transport provides a route to enhanced oral bioavailability via avoidance of hepatic first-pass metabolism and/or a reduction in enterocyte-based metabolism.[1–3] Second, the lymphatic system plays key roles in immune function,[4,5] tumor metastasis,[6] metabolic syndrome,[7] and virus (e.g., HIV) replication,[8] suggesting that targeted delivery of drugs into and through the lymphatics (realizing that delivery into the intestinal lymph will target mainly the mesenteric and thoracic lymph) may improve drug treatment in a range of pathologies. Indeed, enhancing lymphatic drug transport has been shown to improve the efficacy of vaccines,[9,10] immunomodulators,[11] antitumor,[10] and antiviral[12] agents.

The majority of small-molecule drugs are absorbed and transported to the systemic circulation via the mesenteric blood capillaries and portal vein following oral delivery. Drug access into the intestinal lymphatics, however, can be achieved by utilizing lipoproteins (LP) as a delivery chaperone. LP are assembled in enterocytes from both ingested (exogenous) and endogenous lipids and are preferentially transported from the intestine to the systemic circulation via the lymphatics. Selective lymphatic access of LP occurs, at least in part, because the blood endothelium is relatively impermeable to large colloidal particles such as LP, whereas the lymphatic endothelium is more permeable.[13,14] Emerging evidence also suggests that active transport of LP across the lymphatic endothelium is possible via transcellular routes.[15,16] Following oral absorption, drugs with physicochemical properties that promote partitioning into developing LP in the enterocyte therefore concentrate in intestinal LP and in doing so gain access to the lymphatic system. For molecules that access the lymph in this way, coadministration with lipids (either from the formulation or diet) is required to promote LP assembly, and drug molecules must have high LP affinity [typically high lipophilicity—log $D > 5$ at physiologically relevant pH, solubility in long-chain triglyceride (TG) $> 50$ mg/g[1]] to promote partitioning into lymph LP. Most drugs, however, are not sufficiently lipophilic to enable significant lymphatic transport, as contemporary lead optimization programs commonly seek to avoid high lipophilicity where possible, in order to promote aqueous drug solubility and to reduce the risk of nonspecific off-target toxicity.

**Abbreviations used:** ADA, arachidonic acid; BPF, bile and pancreatic fluid; CM, chylomicron; DGAT, diacylglycerol acyltransferease; FA, fatty acid; G3P, glycerol-3-phosphate; LA, linoleic acid; LNA, linolenic acid; LP, lipoprotein; MG, monoglyceride; MPA, mycophenolic acid; OA, oleic acid; PL81, Pluronic L81; TG, triglyceride; VLDL, very low-density lipoprotein.

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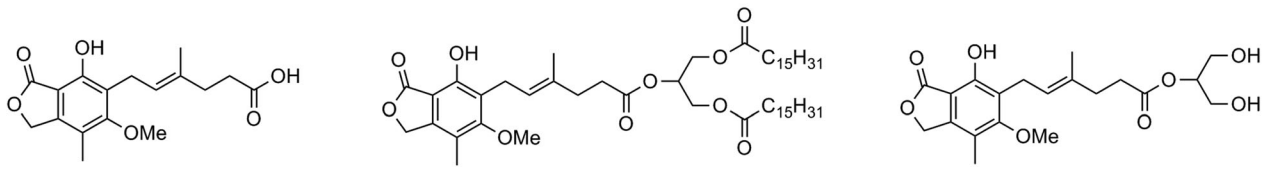


**Figure 1.** Chemical structures of MPA, 2-MPA-TG, and the hydrolysis product (2-MPA-MG) of 2-MPA-TG with molecular weight and cLog $D_{pH7.4}$ values calculated using ACD/Labs Release Software (version 9.12).

To promote lymphatic access for a broader range of compounds (i.e., those with medium or low lipophilicity), a TG mimetic prodrug strategy has been explored in our laboratory and shown to significantly enhance the lymphatic transport of a widely used immunosuppressant, mycophenolic acid (MPA; Fig. 1).[17] In these studies, after intraduodenal administration to rats, only 0.17% of the dose of (parent) MPA (cLog $D_{pH7.4}$ 0.26) was recovered in the intestinal lymph, whereas the bioavailability of MPA in the systemic circulation was 39% (because of absorption via the portal blood). In contrast, administration of 1,3-dipalmitoyl-2-mycophenoloyl glycerol (2-MPA-TG; Fig. 1), a prodrug where MPA is conjugated to the 2′ position of a TG backbone, enhanced the lymphatic recovery of total MPA-related species by 80-fold when compared with administration of MPA alone. Lymphatic transport of the prodrug provides potential opportunities to enhance the therapeutic efficacy of MPA, as the principle site of action of MPA is within lymphocytes and lymphocytes are present in much higher concentration within the lymphatic system than in the general vascular circulation.[18] Previous studies have confirmed that 2-MPA-TG gains access to the lymph by mimicking the catabolic/anabolic pathway of dietary TG absorption and transport.[19] The first step in this pathway is the luminal hydrolysis of 2-MPA-TG that results in cleavage of the two fatty acids (FAs) in the sn-1 and sn-3 position of the glyceride backbone and generation of the monoglyceride (MG) equivalent of the prodrug (2-MPA-MG; Fig. 1). 2-MPA-MG is subsequently absorbed and re-esterified with available FA to form TG derivatives of MPA. In this way, the prodrug catabolic/anabolic pathway mimics the pathway for constitutive lipid turnover into lymph in the fasted state (as even in the fasted state, TG resynthesis occurs, and basal levels of LP turnover through the enterocyte into lymphatics[20]) and also for exogenous lipid absorption postprandially or after administration of a lipid-based formulation. As the mass of prodrug that passes through the enterocyte during absorption (~1 mg/h in the current study) is lower than typical TG turnover in the fasted state (~2–3 mg/h[21,22]), and as the prodrug biochemically integrates into lipid resynthetic pathways, the working hypothesis underpinning the current work was that significant lymphatic transport of the prodrug may be possible even in the absence of coadministered exogenous lipid. This would remove the potential for food effects and variability in lymphatic transport with different dosing strategies.

The current study has therefore examined whether coadministered lipid is required to support the resynthesis process, and whether this is dependent on the type of lipid employed. The role of LP assembly and lipid coadministration in the lymphatic transport of 2-MPA-TG was determined via coadministration of 2-MPA-TG with differing quantities of oleic acid (OA) to mesenteric lymph-duct-cannulated rats. Experiments were repeated in the presence of the chylomicron (CM) assembly inhibitor Pluronic L81 (PL81). The data suggest that contrary to our initial suggestion, MPA access to the lymph after administration of 2-MPA-TG is dependent on lymphatic lipid transport and assembly into CM. In parallel, *in vitro* hydrolysis and *in situ* intestinal perfusion studies were conducted to confirm that the extent of lymphatic transport in the presence of varying lipid loads did not reflect factors other than LP incorporation, for example, luminal prodrug hydrolysis, absorption, or resynthesis. An *in vivo* study using formulations containing long-chain FA with different degrees of unsaturation was also conducted to examine the selectivity of coadministered lipids in promoting lymphatic transport of the TG mimetic prodrug.

## MATERIALS AND METHODS

### Chemicals and Prodrug

Mycophenolic acid (>98%) was purchased from AK Scientific (Palo Alto, California). The TG mimetic prodrug 2-MPA-TG was synthesized as previously described.[17] OA, linoleic acid (LA), linolenic acid (LNA), arachidonic acid (ADA), and Tween 80 were purchased from Sigma–Aldrich (St. Louis, Missouri). PL81 was purchased from BASF (Florham Park, New Jersey). A922500 was purchased from AdooQ BioScience (Irvine, California). Sodium hydroxide, hydrochloric acid, and acetonitrile (ACN; for liquid chromatography) were purchased from Merck Pty. Ltd. (Bayswater, Victoria, Australia). Ultrapure water was obtained from a Milli-Q™ system (EMD Millipore Corporation, Billerica, Massachusetts). All other chemicals were analytical grade or above.

### Preparation of Lipid Formulations for *In Vitro* and *In Vivo* Experiments

Lipid-based formulations containing 2-MPA-TG were prepared as described previously.[21] Briefly, approximately 2 mg of 2-MPA-TG, 25 mg Tween 80, and FA (0, 4, or 40 mg of OA; or 40 mg of LA, LNA, or ADA) were mixed in a glass vial as a lipid phase (for PL81 containing formulations, 2 mg of PL81 was also added to the lipid phase) and incubated at 37°C for 12–18 h to equilibrate and allow the prodrug to dissolve. An aqueous phase consisting of 5.6 mL phosphate-buffered saline (PBS; pH 7.4) was subsequently added to the glass vial and the formulation emulsified by ultrasonication with a Misonix XL 2020 ultrasonic processor (Misonix, Farmingdale, New York) equipped with a 3.2-mm microprobe tip running at an amplitude of 240 μm and a frequency of 20 kHz for 2 min at room temperature. The mass of prodrug solubilized in the formulation was verified on the day of dosing using HPLC-MS as described previously.[17]

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