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Review

Understanding lymphatic drug delivery through chylomicron blockade: A retrospective and prospective analysis

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ABSTRACT

Scientists have developed and employed various models to investigate intestinal lymphatic uptake. One approach involves using specific blocking agents to influence the chylomicron-mediated lymphatic absorption of drugs. Currently utilized models include pluronic L-81, puromycin, vinca alkaloids, colchicine, and cycloheximide. This review offers a thorough analysis of the diverse models utilized, evaluating existing reports while delineating the gaps in current research. It also explores pharmacokinetic related aspects of intestinal lymphatic uptake pathway and its blockage through the discussed models. Pluronic L-81 has a reversible effect, minimal toxicity, and unique mode of action. Yet, it lacks clinical reports on chylomicron pathway blockage, likely due to low concentrations used. Puromycin and vinca alkaloids, though documented for toxicity, lack information on their application in drug intestinal lymphatic uptake. Other vinca alkaloids show promise in affecting triglyceride profiles and represent possible agents to test as blockers. Colchicine and cycloheximide, widely used in pharmaceutical development, have demonstrated efficacy, with cycloheximide preferred for lower toxicity. However, further investigation into effective and toxic doses of colchicine in humans is needed to understand its clinical impact. The review additionally followed the complete journey of oral lymphatic targeting drugs from intake to excretion, provided a pharmacokinetic equation considering the intestinal lymphatic pathway for assessing bioavailability. Moreover, the possible application of urinary data as a non-invasive way to measure the uptake of drugs through intestinal lymphatics was illustrated, and the likelihood of drug interactions when specific blockers are employed in human subjects was underscored.

1. Background

1.1. Overview of the lymphatic system

The lymphatic system, an integral component of the circulatory system, comprises a complex network of lymphatic vessels, tissues, and organs (Yousef et al., 2021). Its organs encompass the bone marrow, thymus, lymph nodes, and spleen (Trevaskis, Hu, et al., 2015; Trevaskis, Kaminskas, & Porter, 2015). Within the lymphatic tissues are lymphatic follicles situated in the mucous membranes lining the respiratory, gastrointestinal, and urinary tracts, collectively termed mucosa-associated lymphatic tissues (MALTs) (Cesta, 2006). These nodules form aggregates in structures like the tonsils and the ileum of the small intestine, known as Peyer's patches (Zhang et al., 2021).

The lymphatic system serves several key functions, including maintaining fluid balance, transporting dietary lipids and fat-soluble vitamins, and regulating the immune responses (Trevaskis, Hu, et al., 2015; Trevaskis, Kaminskas, & Porter, 2015; Zhang et al., 2021). Unlike the closed cardiovascular system, where the heart pumps blood throughout the body, the lymphatic system operates as an open-ended network without a central pumping organ (Yousef et al., 2021). Lymph, a clear to white fluid, flows through lymphatic vessels, carrying excess fluid and other substances from tissues back to the bloodstream, thus, serving as an intermediate between the tissues and the vasculature (Yousef et al., 2022).

Each day, approximately 90% of the plasma propelled into the interstitial space by arterioles is reabsorbed into venules (Hansen et al., 2015). The remaining fluid is subsequently drained through the lymphatic system. The entrance into the lymphatic vasculature is facilitated through initial lymphatic capillaries featuring flap-like junctions, aiding in the removal of excess fluid, along with macromolecules, cells and waste products, from the interstitium (Breslin et al., 2018).

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Subsequently, the lymph travels through various lymphatic vessels, including lymphatic collectors that merge into larger trunks, and ultimately emptying into lymphatic ducts. These ducts then return lymph to the venous blood circulation (Breslin et al., 2018; Hansen et al., 2015).

Within the intestinal region, the lymphatic capillaries known as lacteals exhibit distinctive structure and function (Bernier-Latmani & Petrova, 2017). These specialized vessels play a key role in absorbing dietary lipids, fat-soluble vitamins, and other xenobiotics (Hokkanen et al., 2019; Yousef et al., 2022). Situated in the intestinal villi, lacteals channel into pre-collecting and collecting lymphatic vessels found in the mesentery. These vessels, in turn, drain into the cisterna chyli located at the posterior end of the lymphatic duct (thoracic duct) that connects to the venous blood (Cifarelli & Eichmann, 2019).

1.2. Lymphatic drug delivery

Before, the significance of the lymphatic system was primarily functional; and related to its role related to immunity, maintaining fluid balance and transporting lipids. However, recent progress in understanding the lymphatic system, along with its crucial involvement in numerous diseases, immune regulation, and cancer spread, has led to new insights. These advancements have prompted the exploration of novel approaches to develop vaccines, drugs, and drug delivery systems tailored to enhance delivery into or through the lymphatic system (Trevaskis, Hu, et al., 2015; Trevaskis, Kaminskas, & Porter, 2015; Yousef et al., 2021; Zhang et al., 2021).

Lymphatic targeted delivery offers many advantages, such as increased drug exposure, especially following oral administration, and an increased concentration of the drug in the lymphatic system (Porter & Charman, 2001; Trevaskis, Hu, et al., 2015; Trevaskis, Kaminskas, & Porter, 2015). When a drug is orally administered, it has been presumed to predominantly absorb in the small intestine and enter the venous circulation through the hepatic portal vein, potentially leading to losses of highly metabolized drugs in the liver. However, drugs entering intestinal lymphatics bypass the liver and its first-pass effect, potentially leading to increased oral bioavailability (Cifarelli & Eichmann, 2019;

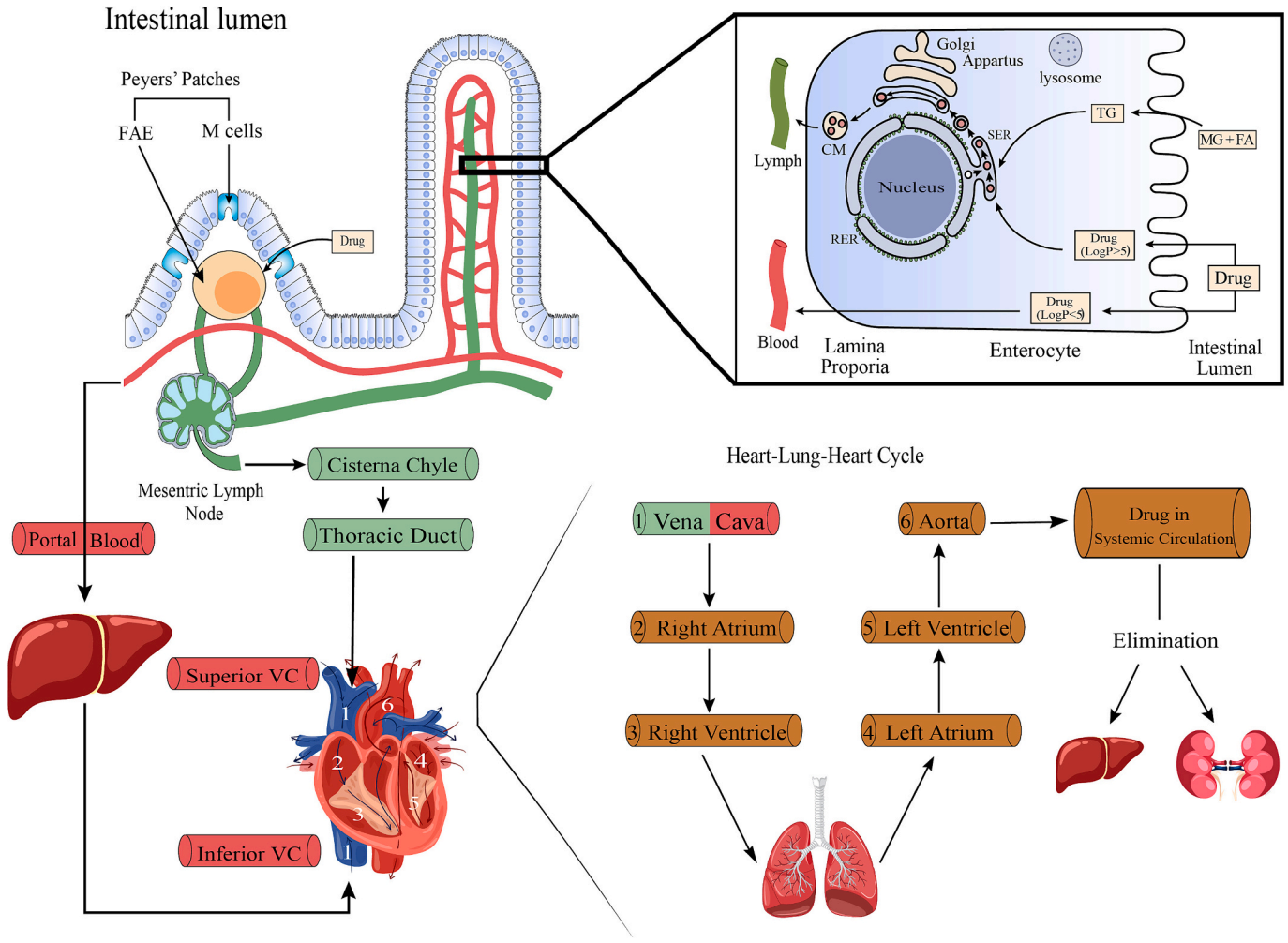

Fig. 1. Journey of drugs from the intestinal lumen to the systemic circulation. Transcellular drug absorption can occur through both portal and lymphatic pathways. Drugs with low lipophilicity (log $P < 5$) are absorbed into enterocytes before entering the portal blood circulation. These drugs then travel to the liver, after which they get into the right atrium through the inferior vena cava, and complete the heart-lung-heart cycle, ultimately entering the systemic blood. Lipophilic drugs (log $P > 5$) are typically packaged into chylomicrons within enterocytes. As these chylomicrons enter the lamina propria, they are taken up by intestinal lymphatic vessels (lacteals) that funnel into lymphatic vessels in the mesentery. This flow drains into the cisterna chyli at the posterior end of the thoracic duct, leading to the junction of the left subclavian and left internal jugular veins. Drugs then enter the right atrium through the anterior vena cava, undergoing the heart-lung-heart cycle before reaching systemic blood. From the 1. vena cava, blood gets the 2. right atrium, then it moves into the 3. right ventricle before making its initial journey to the lungs for pulmonary oxygenation. After being oxygenated in the lungs, the blood, along with any accompanying medication, flows back to the 4. left atrium. It is then propelled into the 5. left ventricle, which in turn pumps the oxygen-rich blood and medication though the 6. aorta to the entire body for various actions, disposition, and eventual elimination. Additionally, another main intestinal lymphatic pathway involves drugs passing through M cells of Peyer's patches in the intestinal lumen, reaching the mesenteric lymph and joining the previously described flow until entering the general systemic vascular circulation.

Porter & Charman, 2001). Moreover, targeting lymphatics can boost therapeutic efficacy by concentrating the drug in the lymphatics, minimizing potential non-specific tissue uptake and off-target toxicities to other tissues (Chaturvedi et al., 2020; Punjabi et al., 2021).

Drugs accessing the intestinal lymph utilize three main pathways: firstly, absorption occurs through a transcellular route, closely associated with the triglyceride core of chylomicrons; secondly, drugs can pass through microfold cells (M cells) located in the lymphatic Peyer's patches; and thirdly, there's a paracellular route, potentially facilitated by absorption enhancers (Managuli et al., 2018; Yáñez et al., 2011). These pathways converge, guiding drugs into the mesenteric lymph, which then progresses to empty into the thoracic duct. From there, it enters the junction of the left subclavian and left internal jugular veins, merging with the venous blood supply in the superior vena cava before finally reaching the right atrium of the heart (Hansen et al., 2015; Moore Jr & Bertram, 2018). In contrast, drugs entering the bloodstream from the small intestine *via* the portal blood undergo hepatic first-pass metabolism in the liver before joining the venous circulation through the inferior vena cava (DeSesso & Jacobson, 2001). Subsequently, all blood follows the same route in the well-known heart-lung-heart cycle. Starting from the right atrium, the blood is propelled to the right ventricle, then in the first pass to the lungs for pulmonary oxygenation. From the lungs, the oxygenated blood and drug within it returns to the left atrium and is subsequently pumped to the left ventricle. Finally, the left ventricle propels the oxygen-rich blood and drug throughout the entire body for action, disposition and subsequent elimination (Kroeker, 2018) as seen in Fig. 1.

Considering all the pathways through which orally administered drugs access the systemic circulation, the following general bioavailability (F) equation, describing the amount reaching the systemic circulation, falls short in clearly accounting for and incorporating the lymphatic component (Fedi et al., 2021).

$$F = F_{absorbed} \times F_{gut} \times F_{liver}$$

Where:

F = Fraction of the drug reaching the systemic circulation (bioavailability).

$F_{absorbed}$ = Fraction of the drug absorbed into the intestinal cells.

F_{gut} = Fraction of the drug escaping gut decomposition, metabolism, or loss into feces.

F_{liver} = Fraction of the drug escaping first-pass metabolism by the liver.

For a drug with multiple absorption pathways, the bioavailability equation becomes more complex. F is the total bioavailability, which is the fraction of the administered drug that reaches the systemic circulation from all relevant pathway inputs. It must be expressed as the sum

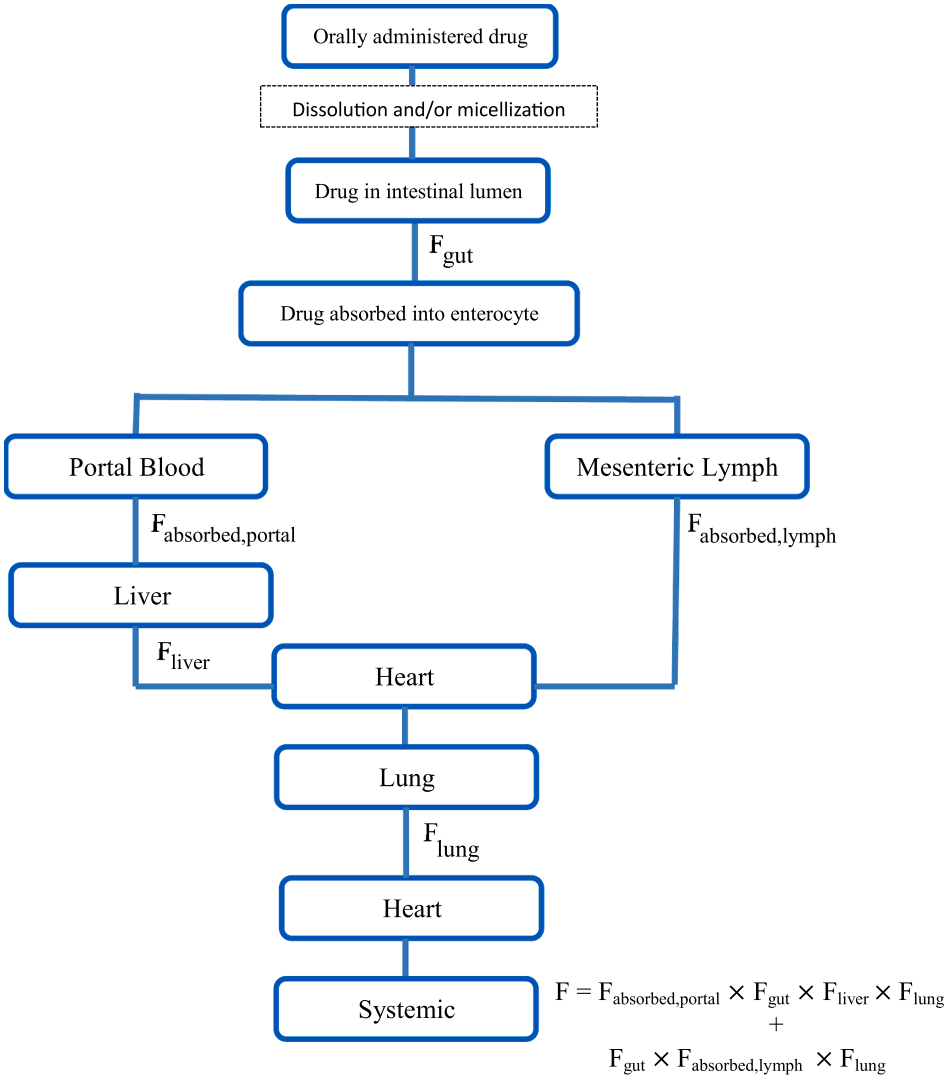

Fig. 2. Illustration of movement of orally administered drugs until reaching the systemic circulation and the impact of that on the description and quantification of bioavailability.

of the individual bioavailabilities through each pathway (Fig. 2).

A simplified version of the equation would be:

$$F = F_1 + F_2$$

Where:

F_1 and F_2 are the individual bioavailabilities through each pathway and each is a fraction of the drug absorbed through a specific pathway (portal and lymph, respectively).

The lung is a first-pass organ that may or may not extract drugs before reaching the systemic circulation, therefore, fraction of the drug escaping the first-pass metabolism by the lung (F_{lung}) should be considered and recognized as well and may not be negligible and should not be overlooked.

Consequently, the bioavailability of the drug through the hepatic portal system (F_1) can be represented by the equation:

$$F_1 = F_{absorbed,portal} \times F_{gut} \times F_{liver} \times F_{lung}$$

Where:

$F_{absorbed,portal}$ = Fraction of the drug absorbed from the enterocyte into the hepatic portal blood.

Additionally, the bioavailability of drug through the lymphatic system (F_2) can be represented by the equation:

$$F_2 = F_{gut} \times F_{absorbed,lymph} \times F_{lung}$$

Where:

$F_{absorbed,lymph}$ = Fraction of the drug absorbed through the intestinal lymphatics. This encompasses the various mechanisms of intestinal lymph absorption, such as chylomicron uptake, passage through M cells, or the paracellular pathway.

When both absorption pathways (portal and lymph) are combined together, the equation will be:

$$F = F_{absorbed,portal} \times F_{gut} \times F_{liver} \times F_{lung} + F_{gut} \times F_{absorbed,lymph} \times F_{lung}$$

If negligible lung extraction on the first pass is assumed, then the equation reduces to:

$$F = F_{absorbed,portal} \times F_{gut} \times F_{liver} + F_{gut} \times F_{absorbed,lymph}$$

This equation assumes independence between the pathways meaning the absorption through one pathway does not affect the absorption through the other. Thus, if there is negligible lymph absorption the equation could be reduced to:

$$F = F_{absorbed} \times F_{gut} \times F_{liver}$$

Conversely, if a drug is targeted to be absorbed solely through the lymphatic pathway and not able to be absorbed through the hepatic portal system:

$$F = F_{gut} \times F_{absorbed,lymph} \times F_{lung}$$

In light of the above discussion and taking into consideration presystemic losses in the gut, liver, and lungs, another comprehensive general equation would be as follows:

$$F = \text{Drug Input x } F_A \times (1 - E)$$

$$F = \text{Drug In x } F_A \times (1 - E)$$

Where by:

Drug in = Sum of drug input into the body.

F_A = Drug absorption into the body through all pathways.

E = Extraction or loss across all first-pass organs and metabolically active tissue or fluid including gut, liver and, lung *etc.*

The primary route for the absorption of lipophilic drugs (LogP >5 and a solubility of >50 mg per g in long-chain triglycerides) - is through the intestinal lymphatics *via* the chylomicron pathway. These drugs are likely to be preferentially absorbed into the intestinal lymphatics because of their ability to incorporate with chylomicrons (Elz et al., 2022; Feng et al., 2022; Trevaskis et al., 2008). If the desired drug fails to meet these criteria, there are alternative strategies to get it into the chylomicrons. This can be achieved by adjusting its lipophilicity, employing a lipid-based drug delivery system, or creating a lipophilic prodrug (Khan et al., 2013; Markovic et al., 2020; Trevaskis et al., 2008). For a lipophilic prodrug, the original drug is chemically bonded to a lipophilic component through a linker that can be readily broken down within the body (Markovic et al., 2020). Additionally various drug delivery systems have been utilized to target the lymphatic system (Table 5) including numerous nano-formulations (Feeney et al., 2016; Khan et al., 2013; Kim et al., 2013; Porter & Charman, 2001; Trevaskis et al., 2008; Zhang et al., 2021).

1.3. Lymph blockage models

Scientists have used diverse methods to explore and quantify intestinal lymphatic uptake. *In-vitro* models, such as the $CaCO_2$ cellular model and other cellular models, along with models involving chylomicron association, have been reported (Gershkovich & Hoffman, 2005; Lu et al., 2015). *In-vivo* methods include the lymphatic cannulation method and the lymph blocking method (Chaturvedi et al., 2020; Trevaskis, Hu, et al., 2015; Trevaskis, Kaminskas, & Porter, 2015).

This review specifically delves into lymph-blocking models, where the focus is on studying the transport of drugs through the lymphatic system by impeding the production or secretion of chylomicrons from enterocytes. The study of the pharmacokinetics of a drug can demonstrate reduced systemic exposure of a substrate, typically a drug, following the administration of a chylomicron inhibitor compared to when a chylomicron inhibitor is not administered and this provides experimental evidence that suggests absorption, may be at least partially, through the lymphatic route. A typical pharmacokinetic profile will demonstrate that without chylomicron blockade a drug is absorbed over time and reaches a maximum concentration (C_{max}) and its pharmacokinetics can be followed with serial concentration-time points (Fig. 3, solid line). Upon the administration of a chylomicron blocker before the drug and formulation in question a reduction in both maximum concentration (C_{max}) and the area under the concentration-time curve (AUC) and a shorter time to maximum concentration (T_{max}) will also be apparent depending on the extent of input through the slower lymphatic route to the systemic circulation with the reduction between the two experimental conditions over time representing lymphatic absorption of the drug (Fig. 3, dashed line).

As previously demonstrated in *in-silico* simulations the total concentrations of drug in the circulating blood are the sum of the drug entering from each of the two pathways (i,e. lymphatic and portal). The drug absorption constant into the intestinal lymphatics (ka_{lymph}) is assumed to be slower and as there is additional processing required within the enterocytes for drug uptake into the mesenteric blood. Contrarily, the drug absorption constant into the portal circulation (ka_{portal}) is of a higher value (Brocks & Davies, 2018). For low, moderate and high E drugs, there are increases in T_{max} with increases in $F_{absorbed,lymph}$. It should follow that with lymphatic blocking and decreases in $F_{absorbed,lymph}$a corresponding reduction in T_{max} should also be apparent in comparative pharmacokinetic studies.

We identified 48 studies in the literature that provide such data to determine an effect on T_{max} with a chylomicron inhibitor. In all of these studies whether the drug was high medium or low E (extraction) in the model was never mentioned. Furthermore, the effect on T_{max} was not reported nor discussed in 14 of these studies (Dahan & Hoffman, 2006; Iwanaga et al., 2006; Tang et al., 2013; Sun et al., 2014; Arzani et al., 2015; Garg et al., 2016; Ravi & Vats, 2017; Wang et al., 2017; Liao et al., 2019; Patel & Sawant, 2019; Patel, Mundada, & Sawant, 2019, Patel, Shah, et al., 2019; Rizk et al., 2021; De Souza et al., 2024, Zheng et al., 2024). Consistent with the *in-silico* study, findings and pharmacokinetic theory it was reported but not clearly discussed that a reduction in T_{max} occurred with the use of chylomicron blockers was apparent in six

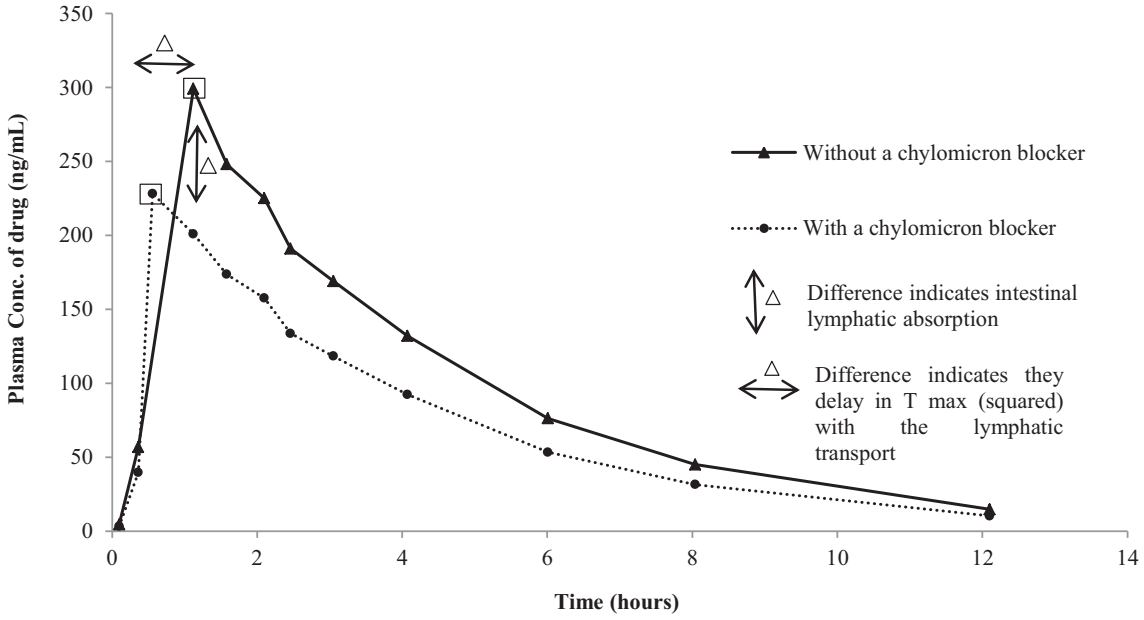

Fig. 3. Hypothetical graph of drug plasma concentration time profile with and without chylomicron blocker for a drug subjected to intestinal lymphatic absorption.

studies (Valicherla et al., 2016; Meher et al., 2020; Gao et al., 2011; Shrivastava et al., 2021; Jitta et al., 2024; Fuentes et al., 2024). Moreover, it was noted that the T_{max} following administration of a blocker fell within the same range as the animals that did not receive the blockers in some of these reports. There was no apparent effect on this pharmacokinetic index (T_{max}) observed in 24 of the different drugs examined in these studies (Bao et al., 2021; Bhalekar et al., 2016; El-Laithy et al., 2015; Elsheikh et al., 2021; Gausuzzaman et al., 2022; Goo et al., 2022; Gurumukhi & Bari, 2022; Harisa et al., 2023; Li, Hu, et al., 2017; Li, Zhuang, et al., 2017; Lind et al., 2008; Makwana et al., 2015; Mishra et al., 2014; Muheem et al., 2024; Mundada et al., 2020; Patel & Patel, 2021; Qiao et al., 2018; Rangaraj et al., 2020; Ryšánek et al., 2021; Sun et al., 2011; Tang et al., 2013; Wu et al., 2018; Xing et al., 2016; Xu et al., 2019; Ye et al., 2020). Contrary to pharmacokinetic theory it was evident that treatment with a chylomicron blocker led to a longer T_{max} in at least four studies (Fu et al., 2013; Li et al., 2020; Lin et al., 2023; Liu et al., 2024), however, no discussion, or explanation was put forth for these findings. There was a discussion regarding the T_{max} of cinacalcet where an unfamiliar rise in the levels of cinacalcet in both serum and tissues was observed in animals pre-treated with cycloheximide (chylomicron blocker), approximately 20 h after administering the drug. The late increase in cinacalcet concentrations was suggested to be due to either delayed absorption from the gastrointestinal lumen, because of significantly reduced gastrointestinal motility, or impaired elimination imparted by cycloheximide (Ryšánek et al., 2021). The toxicological manifestations of cycloheximide and its route of administration and dose are likely to be factors responsible for augmented T_{max} effect findings in several studies (Al Nebaihi et al., 2023).

Chylomicrons are spherical particles that consist mainly of a core comprising about 84% triglycerides and around 7% cholesterol, enveloped by a monolayer composed of nearly 7% phospholipids, ~2% cholesterol, and 2% proteins (Yousef et al., 2022). Typically, dietary lipids undergo encapsulation into chylomicrons within the cytoplasm of enterocytes before being absorbed by lacteals (Desmarchelier et al., 2019). The triglycerides from ingested lipids undergo hydrolysis by lipases, breaking down into monoglycerides and fatty acids prior to reaching the duodenum. Once inside the enterocytes, long-chain fatty acids ($C \geq 12$) and monoglycerides are re-esterified in the rough endoplasmic reticulum forming primordial lipoprotein particles (Fig. 4). These particles include a protein component (apo B) connected with a phospholipid monolayer. Simultaneously, the smooth endoplasmic reticulum facilitates the synthesis of large triglyceride-rich droplets. The final step in chylomicron biosynthesis is core expansion, characterized by the merging of the primal lipoproteins with the big triglyceride-rich droplets. Subsequently, the chylomicron is transported to the Golgi apparatus for packaging and secretion which occurs through an exocytosis process (Hussain, 2000; Williams et al., 2004).

To investigate intestinal lymphatic uptake *via* the chylomicron flow blockade approach, various models have been developed and implemented to target the various steps of chylomicron formation and secretion. The data collection method is outlined below, and the various models are presented in Fig. 5, with detailed explanations provided in the subsequent sections.

2. Methods

The data were gathered through a thorough literature review using the PubMed, Web of Science, and Scopus databases. The utilized search strategy included keywords such as “intestinal lymphatic uptake,” “chylomicron blocking agents,” “pharmacokinetics,” “Pluronic L-81,” “puromycin,” “vinca alkaloids,” “colchicine,” and “cycloheximide.” Boolean operators were employed to combine these terms for a comprehensive search. Articles were selected based on their relevance to the topic, particularly those providing data on the effectiveness and toxicity of blocking agents on chylomicron-mediated lymphatic absorption. The inclusion criteria also focused on studies offering pharmacokinetic data and discussing experimental and clinical implications.

2.1. Pluronic L-81 model

Pluronic L-81 (PL-81) is a liquid non-ionic surfactant with a molecular weight of 2750 g/mol, and it remains in a liquid state at room temperature. Comprising polyoxyethylene-polyoxypropylene-polyoxyethylene block copolymers, PL-81 features hydrophilic moieties (polyoxyethylene) at both ends of the hydrophobic chain (polyoxypropylene). The composition includes 10% hydrophilic residues and 90% hydrophobic ones (Fatma et al., 2006; Krupka et al., 2010). Synthesized through the controlled addition of propylene oxide to the two hydroxyl groups of propylene glycol, PL-81 finds applications as a defoaming agent in dishwashing, metal cleansing, water treatment, and

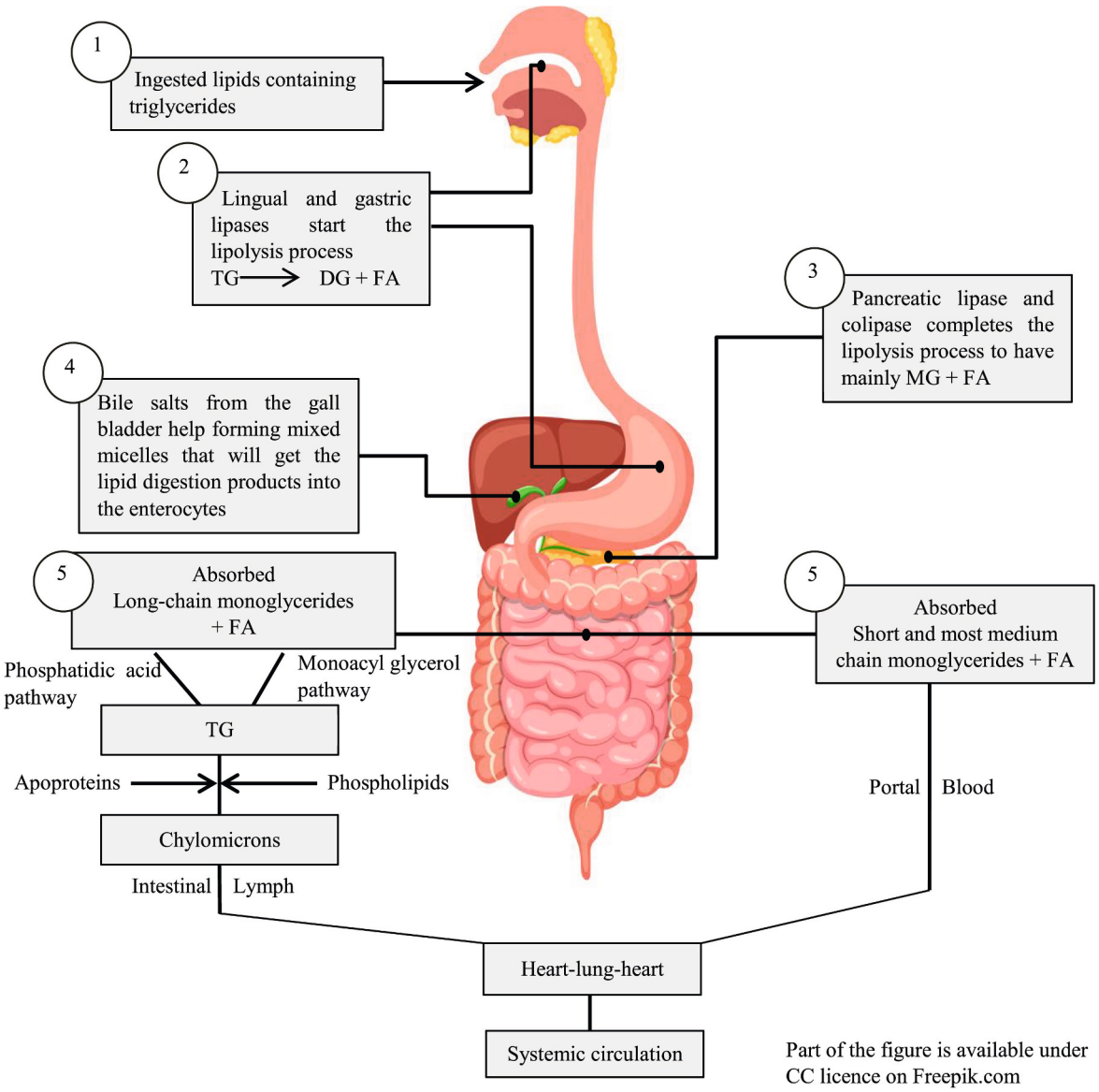

Fig. 4. Demonstration of the steps involved in the digestion and absorption of ingested triglycerides.

paper processing (Tso et al., 1981).

In the late seventies of the last century, Bochenek *et al* discovered that certain non-ionic detergents had an impact on the absorption of lipids and cholesterol. The influence of these agents was found to be connected to their physicochemical properties. Among these detergents, those containing 90% hydrophobic components, such as PL-81, were the most effective in inhibiting lipid absorption. The study demonstrated that adding a hydrophobic detergent to a high-fat, high-cholesterol diet resulted in reduced levels of cholesterol and triglyceride in the serum. Additionally, this effect was correlated with a decrease in body weight. The observation that 0.5% PL-81 lowered plasma cholesterol and triglyceride levels, liver cholesterol, and body weight in rats on a high-fat, high-cholesterol diet sparked considerable attention to it (Bochenek & Rodgers, 1977).

Support for the impact of PL-81 was evident in further investigations. In a particular study, male Sprague-Dawley rats (200–250 g) were administered lipid emulsions containing labeled triolein [^{3}H]-triolein and labeled [^{14}C]-cholesterol, with or without PL-81 for the experimental and control groups, respectively. The observed accumulation of absorbed lipids in the enterocytes of the experimental group suggested that PL-81 hindered the assembly and/or secretion of lipoproteins from intestinal mucosal cells. The study also demonstrated the reversible nature of the effect of PL-81, as the animals regained their lipid transport ability 24 h after discontinuation of PL-81 input (Tso et al., 1980).

In another study conducted by the same group, the rate of intestinal transport of absorbed lipid into lymph was investigated using a lipid emulsion containing [^{3}H]-triolein. The study employed intraduodenal infusions of various doses of PL-81 to evaluate its potential inhibitory effect on lipid transport, and the kinetics of this inhibition were determined. Infusing PL-81 at 0.25 mg/h showed no effect, while infusions at 0.5 and 1 mg/h led to a decrease in intestinal lipid transport. The inhibition occurred rapidly, with a half-life of 69 min for the 0.5 mg/h dosage and 35 min for the 1 mg/h dosage. This hindrance of lipid transportation resulted in the accumulation of lipids in the mucosa, as evidenced by both radiochemical and morphological observations (Tso et al., 1981).

Consistent with earlier discoveries, the intraduodenal infusion of four categories of intestinal lymph fistula rats with [^{3}H]-triolein, and 1 mg/h of PL-81 resulted in a decrease in lipid transport by the small intestine. The study also determined that the position of the hydrophilic

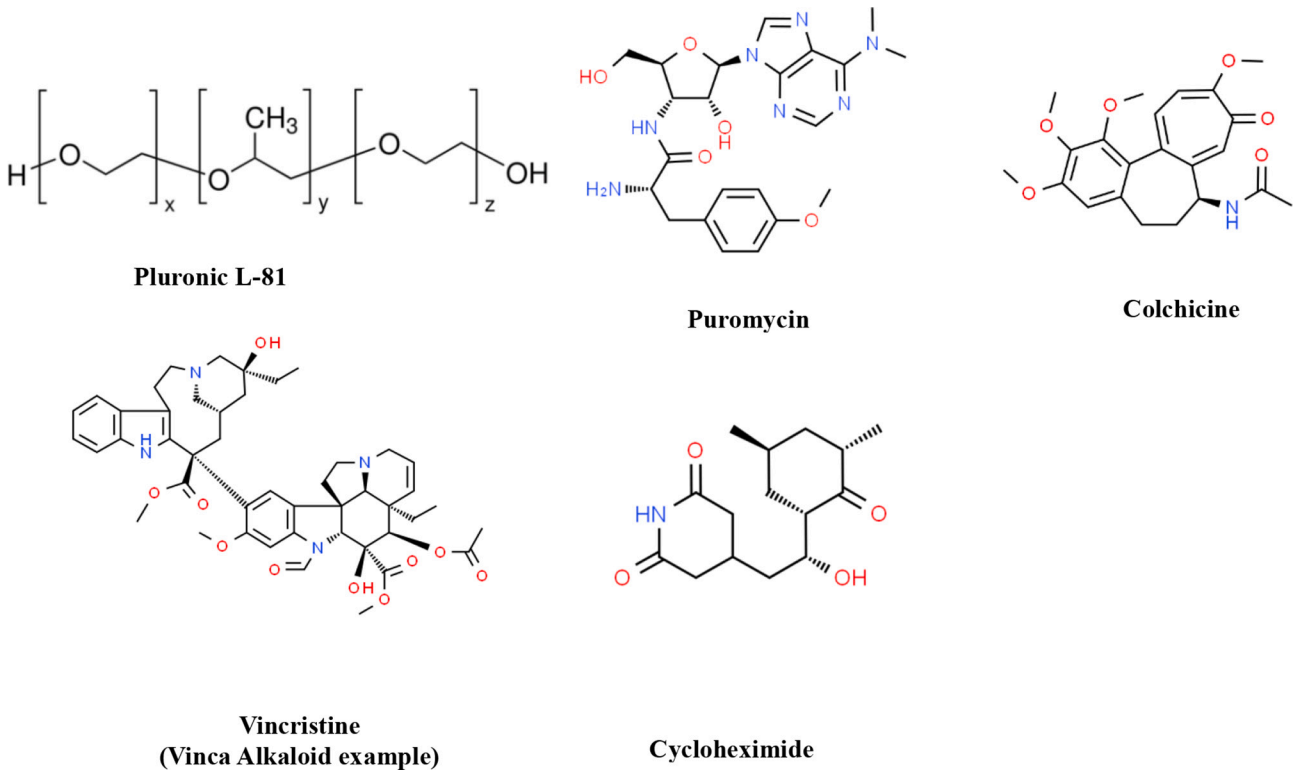

Fig. 5. Structure of the different chemical substances used in chylomicron blocking models to study intestinal lymphatic transport of drugs. Pluronic L-81 structure shows the polyoxyethylene-polyoxypropylene-polyoxyethylene (EO_3-PO_{43}-EO_3) triblock structure. The image is available under Creative Commons licence through the link: https://en.m.wikipedia.org/wiki/File:Pluronic_P-123_structure.png. The link was accessed December 1, 2023. Structures of puromycin, colchicine, vincristine and cycloheximide were obtained from Chemspider available under the IDs: 388623, 5933, 5758, and 5962, respectively. The website (https://www.chemspider.com) was accessed on December 1, 2023.

moiety and the hydrophilic-to-hydrophobic ratio in PL-81 play a crucial role in its action, as evidenced by a comparison with two other pluronics (PL-25R1 and PL-84) (Tso & Gollamudi, 1984).

Other evidence regarding the impact of PL-81 was gathered through the infusion of intestinal lymph fistula rats with a lipid emulsion containing [1-^{14}C]-oleic acid. In this experiment, rats received a dose of 1 mg/h of PL-81. Both chemically and radioactively assessed lymphatic triacylglycerol output showed suppression in the experimental rats compared to the controls. In the following stage of the investigation, the time required for the appearance of very low-density lipoproteins in control rats and chylomicrons in PL-81-treated rats was determined in the central lacteal. In control rats, the average appearance time was 10.8 min, whereas in PL-81-treated experimental rats, it was 16.2 min. This difference in appearance time supported the theory that chylomicron and very low-density lipoprotein are segregated during packaging in enterocytes, and PL-81 selectively inhibits the formation of chylomicrons (Nutting et al., 1989).

Based on the findings reported in the studies until the late 1980s, it was revealed that PL-81 disrupted the secretion of chylomicrons and could potentially reduce the formation and stability of luminal triglyceride-rich lipid droplets (Nutting et al., 1989; Tso & Gollamudi, 1984). In the early 1990s, PL-81 was identified as a hydrophobic surfactant that impedes intestinal chylomicron secretion at the pre-Golgi level, while leaving triacylglycerol uptake and re-esterification unaffected (Black, 1992; Hayashi et al., 1990). To explore the impact of this inhibition, Black *et al* conducted a study on newborn female piglets. They subjected the piglets to 24-h intraduodenal infusions of either low-triacylglycerol or high-triacylglycerol diets, with or without the addition of PL-81. Subsequent evaluations included the assessment of apolipoproteins (apo B-48, A-I, and A-IV) synthesis and content, along with apo B-48 and A-IV mRNA levels in the small intestine. The introduction of PL-81 to the high-triacylglycerol infusion led to a decrease in jejunal apo B-48 content, synthesis, and mRNA levels below basal levels. The typical increase in apo A-I synthesis caused by triacylglycerol absorption was negated in both the jejunum and ileum. Although dampened, the expected elevation in jejunal apo A-IV synthesis and mRNA levels with triacylglycerol absorption persisted with PL-81 treatment (Black, 1992). Upon further investigation, it was discovered that the stimulation of apo-lipoprotein (apo A-IV) synthesis and secretion is not coordinated with lipid uptake into the enterocytes or cellular triglyceride content. Rather, it is probable that the processes associated with the packaging and release of chylomicrons are responsible for the elevation in apo A-IV synthesis and secretion. This aligns with the results obtained with PL-81, suggesting that the inhibition of chylomicrons would not impact apo A-IV (Hayashi et al., 1990). Instead, its impact seems to be selective, primarily affecting apo B-48 (Black, 1992).

In another study, the impact of PL-81 on chylomicron composition was examined. The experiment involved intraduodenal infusion of PL-81 at a constant rate of 1.0 mg/h, in combination with mixed micellar solutions or saline, in mesenteric lymph fistula male Sprague Dawley rats (250–350 g). The interference disrupted trans-epithelial lipid flux during fat absorption, causing the entrapment of exported lipids within enterocytes and resulting in cytosolic and endoplasmic reticulum lipid accumulation, with the Golgi region remaining unaffected. It reduced mesenteric triglyceride, phospholipid, and total cholesterol secretion. Although there were only negligible changes in chylomicron composition, a slightly higher phospholipid/triglyceride ratio was noted. The chylomicron apo-lipoprotein pattern showed almost no alterations. As a result, the study concluded that PL-81 led to a substantial decrease in chylomicron formation without major compositional alterations (Pidlich et al., 1996).

PL-81, employed as a chylomicron blocker, has found application in numerous studies. Some have delved into the intestinal lymphatic transport of endogenous substances (Nauli et al., 2003; Wollin et al., 1998). In the studies relevant to this work, PL-81 served as a blocking agent to investigate *in-vivo* xenobiotics lymphatic transport (Table 1). In one study, a lipophilic model molecule-vitamin D3- was utilized for this purpose. Using this model, male Wistar rats (300–325 g) were given an intraduodenal infusion of Lipofundin® emulsion, consisting of 20% *w/v* medium and long-chain triglycerides in a 1:1 ratio, with or without PL-81. The PL-81 concentration in the Lipofundin® emulsion was 1 mg/mL, and the infusion rate was set at 1 mL/h. Four hours after initiating the infusion, the animals received an oral gavage of vitamin D3 (0.5 mg/kg). The chosen PL-81 dose demonstrated efficacy in inhibiting the intracellular transport of chylomicrons. Nonetheless, it was noteworthy that the intraduodenal infusion of the emulsion without PL-81 also marginally decreased the area under the concentration-time curve of vitamin D3. This reduction was attributed to the additional lipid load provided by the emulsion, leading to an overall decrease in vitamin absorption. Moreover, vitamin D3 absorption in the PL-81 model showed a good correlation with the mesenteric lymph duct cannulation model, emphasizing the crucial role of packaging the lipophilic molecule into the chylomicron in the cascade of lymphatic absorption (Dahan & Hoffman, 2005).

Also, in male Wistar rats weighing 200–250 g, the administration of 5 mL/kg PL-81 resulted in decreased concentrations of ß-carotene and its cleavage product, vitamin A, in both plasma and liver. The observed effect was hypothesized to occur through the modulation of ß-carotene uptake into enterocytes and/or its secretion into the lymph. That modulation was attributed to the known inhibition of chylomicron synthesis by PL-81 and the prevention of the conversion of ß-carotene into vitamin A within enterocytes (Schweigert et al., 2002).

In 2016, a study utilizing PL-81 demonstrated that co-administering lipids can enhance intestinal lymphatic uptake through chylomicrons. The investigation focused on determining whether the lymphatic uptake of the triglyceride (TG) mimetic pro-drug (1,3-dipalmitoyl-2-mycophenoloyl glycerol, 2-MPA-TG) depended on the presence of exogenous lipids. Conducted in male Sprague-Dawley rats (280–320 g), the study revealed an increase in the lymphatic transport of 2-MPA-TG (2 mg dispersions) when administered with higher quantities of lipids. A comparison between groups with and without the chylomicron blocker (2 mg of PL-81) demonstrated a strong correlation ($R^2 = 0.99$) in the recovery of 2-MPA-TG in the lymph. Over 97% of the pro-drug was associated with chylomicrons, as evidenced by the inhibition of lymphatic transport when PL-81 was present, highlighting its inhibitory effect on the transportation of 2-MPA-TG through the lymphatic system (Han et al., 2016).

In contrast to certain models and inhibitors that may carry the risk of systemic side effects and irreversible tissue damage, as will be discussed in detail next, PL-81 appeared to be devoid of visible side effects. Consequently, the PL-81 model has proven to be an effective approach to hinder the secretion of chylomicrons from the enterocyte. This interference is achieved by a conformational change of apo B and disrupting droplets through the destabilization of their surface, thereby affecting the core expansion process (Morita et al., 2003; Nutting et al., 1989; Tso & Gollamudi, 1984).

The absence of observed adverse effects aligns with the characteristics of PL-81. It selectively disrupts the chylomicron assembly while leaving the digestion, and absorption of triglycerides and cholesterol unaffected. This was demonstrated by the oral d-xylose loading test (Dahan & Hoffman, 2005). The absorption of d-xylose involves both active and passive processes and has been used to evaluate the absorptive function of the intestine (Craig & Atkinson Jr, 1988). Furthermore, the inhibition induced by PL-81 can be promptly reversed by discontinuing its administration (Hayashi et al., 1990; Tso et al., 1980). The noted reversible inhibition of PL-81 aligns consistently with findings observed both in the *in-vivo* rat model and the *in-vitro* CaCo2 cellular model (Han et al., 2016).

Our group published a recent study that documented the use of PL-81 in an *in-vitro* setting to inhibit the intestinal lymphatic uptake of drugs. The research explored this phenomenon in an *in-vitro* model utilizing artificial chylomicrons. The study findings indicated that concentrations of PL-81 at ≥1% effectively inhibited the uptake. The results showed that PL-81 exerted its effect through a mechanism involving the surrounding/coating of artificial chylomicrons, preventing the drug from being packaged into the Intralipid® chylomicrons (Yousef et al., 2023).

2.2. Puromycin model

The antibiotic, puromycin, has also been investigated as a chylomicron-blocking agent. In 1963, the potential of puromycin as a protein inhibitor was investigated. Puromycin was administered intraperitoneally at a rate of 75 mg/kg per hour for 5 h. The findings indicated that puromycin could induce clinical and biochemical protein alterations in rabbits, rats, and mice (Young et al., 1963).

However, the use of puromycin as a lymph blocker can be traced back to Sabesin and Isselbacher in 1965. In that study, female Sprague-Dawley rats weighing 180 to 200 g experienced a 24-h fasting period before receiving intraperitoneal injections of puromycin. Dissolved in a buffered salt solution, puromycin was delivered *via* a regimen of hourly injections, initially at a dose of 2.5 mg over 4 h, followed by five subsequent injections, each containing 1 mg. Following the administration of the fourth injection by one hour, the animals were intubated with 1.5 mL of corn oil, and euthanasia occurred 2, 4, and 6 h later. Under these conditions, rats accumulated triglyceride within the intestinal cells and failed to develop normal post-prandial hyperlipemia due to impaired chylomicron formation (Sabesin & Isselbacher, 1965).

In 1969, Redgrave and Zilversmit investigated the impact of puromycin on fat absorption using various approaches. Female Holtzman and Sprague-Dawley rats weighing 200–225 g were infused with a lipid mixture equivalent to 63.7 μmoles of triolein to examine the effect of puromycin on lymph flow and fat absorption. The lipid mixture was infused through lymph fistula and duodenal cannula preparations, while puromycin (2 mg/mL dissolved in the same buffered salt solution as used in the previous study) was administered intraperitoneally. During puromycin administration, lymph flow remained constant, and the hourly output of triglycerides in puromycin-treated animals was lower than in control animals, although the concentrations in both groups were comparable. For the assessment of oral fat absorption, female Sprague-Dawley rats (180–200 g) were intraperitoneally injected with puromycin. Initial 6-h infusions (2.5 mg each hour) were followed by 1 mg each hour until the end of the experiment. After the fifth injection, rats received 1.5 mL of corn oil containing [^{14}C]-linoleic acid *via* a stomach tube. Puromycin at this dosage caused liver triglyceride accumulation, moderately suppressing protein synthesis. Gastric emptying delay in the puromycin group indicated no inhibition of chylomicron formation or transport at this dose, challenging the conclusion of Sabesin and Isselbacher (Redgrave & Zilversmit, 1969).

In the same year, another study investigated the metabolic comparison of short and long-chain fatty acids, along with triglycerides, in

Table 1
Studies of *in-vivo* xenobiotics lymphatic transport using Pluronic-81 (PL-81) as a chylomicron blocking agent.

Species	PL- 81 Dose and timing	Xenobiotics Tested by the Model	Reference
Male Wistar rats (300–325 g)	Intraduodenal infusion of Pluronic L-81 (1 mg/h) given 4 h before the drug	Vitamin D3	Dahan & Hoffman, 2005
Male Wistar rats (200–250 g)	5 mL/kg Pluronic L-81was added to diet containing ß-carotene	ß-carotene	Schweigert et al., 2002

rats. The study distinguished between control and puromycin-treated groups. In Fisher-strain female rats weighing 150–175 g, puromycin was administered hourly at a rate of 2.75 mg/h for four doses. Subsequently, a continuous puromycin infusion was maintained at a rate of 1.1 mg/h throughout the study. The study observed the hindrance of long-chain fatty acid absorption into the thoracic duct lymph, particularly in the form of chylomicron triglycerides, due to puromycin supporting the findings of Sabesin and Isselbacher (Kayden & Medick, 1969).

Linked to the previous studies, an examination of the impact of puromycin on the apo-lipoproteins of chylomicrons revealed intriguing findings. This agent, known for prematurely ending protein translation, led to the secretion of incompletely synthesized apo B polypeptides as lipoprotein particles by HepG2 cells incubated with puromycin (Spring et al., 1992). Additionally, a separate study involving similar cells treated with puromycin suggested that the length of apo B might play a role in determining the size of lipoproteins during biosynthesis, potentially influencing the chylomicrons produced during puromycin treatment (Schumaker et al., 1994).

The impact of puromycin on cholesterol absorption in male and female Wistar strain rats (200–300 g) with indwelling catheters in the left thoracic lymphatic duct revealed important findings (Vahouny et al., 1977). Following the protocol outlined earlier (Spring et al., 1992), the administration of puromycin led to a decrease in cholesterol absorption, accompanied by inhibition of simultaneously administered fatty acid absorption. In male rats subjected to the same treatment, a reduction in cholesterol absorption was observed, yet it had no impact on the absorption of fatty acids. Despite the reduced lipid absorption observed in puromycin-treated animals, there was no accumulation of cholesterol or fatty acids in the intestinal mucosa, regardless of gender. The study proposed that the altered lymph production in fed animals treated with the protein synthesis inhibitor puromycin was attributed to delayed gastric emptying rather than the lymph blockage effect of the substance (Vahouny et al.,1994) which is a conclusion similar to that of Redgrave and Zilversmit (Redgrave & Zilversmit, 1969).

However, Miura et al. in 1979 reported another study supporting Sabesin and Isselbacher in which they observed a disruption in lipid transport due to impaired chylomicron formation resulting from the effects of puromycin. In the male Wistar rats (350-400 g) used in that study, puromycin demonstrated a block in lipoprotein formation in intestinal cells and a reduction in the lymphatic absorption of [1-^{14}C]-linoleic acid (Miura et al., 1979).

Literature reports provide evidence of the ability of puromycin ability to block intestinal chylomicrons, as illustrated in various studies (Kayden & Medick, 1969; Miura et al., 1979; Redgrave & Zilversmit, 1969; Sabesin & Isselbacher, 1965). Additionally, some investigations suggest that the delay in lymph uptake, previously attributed to the chylomicron-blocking effect of puromycin, may be linked to delayed gastric emptying (Redgrave & Zilversmit, 1969; Vahouny et al., 1977). The Miura et al. study (Miura et al., 1979) shed light on a potential explanation for these discrepancies. Their research indicated that the impact of puromycin on the lymphatic transport of fatty acids is influenced by the type of fatty acids being transported. Puromycin seemed to exert more effect on the lymphatic uptake of highly unsaturated fatty acids. This specificity was critical according to that study, as more unsaturated long-chain fatty acids are prime candidates for chylomicron packaging. The effect of puromycin appeared to be targeted specifically at chylomicrons, as opposed to other lipoproteins like very low-density lipoproteins (VLDL), which transport less unsaturated fatty acids. Still, further studies are essential to investigate the detailed effects of puromycin and the mechanism by which it blocks chylomicrons. Currently, there are no reports on whether puromycin inhibits chylomicron exocytosis from enterocytes or targets other phases of chylomicron formation, such as intracellular movement or the re-esterification of fatty acids. Besides, conducting studies on the inhibition of intestinal lymphatic uptake of xenobiotics would contribute valuable data for this model. It would also enable an exploration of its potential advantages and disadvantages when compared to other chylomicron-blocking agents.

2.3. Colchicine model

Colchicine is derived from the *Colchicum autumnale* plant and has a history of being used for joint pain since at least 1500 BCE (Dasgeb et al., 2018). Despite its use for thousands of years, clinical and experimental interest in the mechanism of action and the toxicological data of colchicine can be traced back to 1950s.

In 1954, Sternberg and Ferguson conducted a toxicology study on colchicine using Wistar rats and cats. Rats, evenly divided by sex and weighing 90 to 150 g, received either a single intravenous dose or intraperitoneal doses administered five times weekly. The intraperitoneal doses started with non-toxic amounts and doubled weekly until significant mortality occurred. For the single intravenous dose in rats, 4 mg/kg was administered to 31 rats, with sacrifice occurring seventeen hours after administration. In ten rats, repeated injections started at 0.1 mg/kg/day and increased to 1.6 mg/kg/day in the fifth week, with sacrifice during the fourth and fifth weeks. Cats received a single intravenous dose varying between 0.5 or 5 mg/kg per cat, and they were sacrificed after eight to twenty-four hours. Repeated doses in four cats started at 0.025 mg/kg/day and increased to 0.2 mg/kg/day in the fourth week, with sacrifice during the third and fourth weeks (Sternberg et al., 1954).

Upon further investigation, it was found that the LD_{50} of colchicine in Sprague-Dawley female rats weighing approximately 200 g was 1.6 mg/kg when administered through intravenous injections at dosages ranging from 0.25 to 2.0 mg/kg (Rosenbloom & Ferguson Jr, 1968). This closely aligned with the previously reported value by Sternberg and Ferguson (Sternberg et al., 1954). Death did not occur until around 8 h after administering the drug dose. Rats displayed symptoms including lethargy, diarrhea, loss of appetite, and others, at doses equal to or >0.5 mg/kg. Consequently, it was decided to utilize the 0.5 mg/kg dose of colchicine for further metabolic investigations (Rosenbloom & Ferguson Jr, 1968).

In 2007, the oral toxicity of colchicine was assessed through a single gavage administration (10, 20, or 30 mg/kg body weight) in young, mature male, and female Sprague-Dawley rats. Colchicine toxicity resulted in progressively more severe, dose-related clinical signs in both male and female rats. The observed signs encompassed mortality, decreased body weight, and reduced feed intake in the days immediately following dosing, followed by recovery in surviving animals. Gender-related discrepancies in the toxic reaction to colchicine were observed between male and female rats, with female rats displaying twice the susceptibility to the lethal effects of colchicine compared to their male counterparts. The calculated oral LD_{50} for saline-pretreated female rats was 26 mg/kg body weight, while for saline-pretreated male rats, it was 51 mg/kg (Wiesenfeld et al., 2007).

The elucidation of the effects of colchicine gained prominence in the 1970s. In 1974, Stein and colleagues investigated the regulation of secretory processes in the liver by conducting an intraperitoneal colchicine injection study, using female and male Albino rats weighing around 200 g from the Hebrew University strain. Two colchicine doses, 0.5 mg/kg and 5.0 mg/kg, were administered. The study specifically investigated triglyceride secretion into the serum, monitoring the process for 90 min following the injection of Triton WR 1339 and [^{14}C]-palmitic acid. Triton WR 1339, also known as tyloxapol, is a substance known to inhibit the catabolism of lipoproteins and was utilized to showcase the action of colchicine in non-Triton-treated rats. Triton WR 1339 interferes with the hydrolysis of chylomicrons and VLDL, causing a linear increase in serum levels of triglycerides and inhibiting their removal from circulation. The release of triglyceride was reduced to approximately 20–30% of control values. The impact of colchicine on serum triglyceride levels was observed to be independent of Triton WR

1339 presence, demonstrating similarity in both males and females, as well as in fed and fasted rats. Since the intestine contributes only about 10% of serum triglyceride in fasted rats, the researchers concluded that colchicine primarily affected the liver. The dose-dependent effect of colchicine was reversible 6–7 h after injection of 0.05 mg/100 g body weight after 270 min. Non-secreted triglyceride accumulated in liver cells within secretory vesicles. The study also observed a noticeable reduction in microtubules and a slight increase in microfilaments. It was suggested that microtubules play a role in regulating the release of lipoproteins into the bloodstream by preserving the structural arrangement of the cell membrane, which is crucial for fusion with secretory vesicles (Stein et al., 1974).

More than a year later, a study delving into the influence of colchicine on intestinal chylomicrons employed female Sprague-Dawley rats weighing between 230 and 250 g. Intraperitoneal injections of colchicine (0.5 mg/100 g of body weight) were administered one hour before the consumption of a margarine emulsion (1 g in 2 mL of saline). During the following hours, this group did not display the elevation in plasma triacylglycerol levels observed in the control group. The impact of colchicine became more pronounced when the experiment followed the prior administration of Triton WR-1339. Colchicine-treated rats also displayed a five-fold increase in triacylglycerol in the proximal jejunum. These results supported the colchicine disrupts the intracellular phase of fat absorption, proposing the involvement of the microtubular-microfilamentous system in releasing chylomicrons from intestinal cells into circulation. The same study investigated the potential general toxic effects of colchicine on the intestine using a D-xylose absorption test. The results indicated that the 3-h urine excretion of xylose in controls (6.9 ± 0.5 mg/3 h) and treated rats (7.9 ± 1.6 mg/3 h) did not exhibit statistically significant differences. The comparable xylose absorption capacity of controls and colchicine-treated rats suggested that the colchicine dose in this experiment did not induce a general toxic effect on the intestinal mucosa (Arreaza et al., 1976). In the same year, Glickman *et al* investigated the impact of colchicine on the lymphatic transport of oleic acid, suggesting again a potential involvement of microtubules in the mechanism. The study, conducted in Male Holtzman rats (250 to 300 g) with indwelling mesenteric lymph cannulas, revealed that animals treated with colchicine (0.5 mg per 100 g) exhibited delay and reduction in the lymphatic absorption of [^{14}C]-oleic acid. The findings pointed towards a potential role of microtubules in intestinal lipid transport particularly in chylomicron secretion (Glickman et al., 1976).

Additional studies in subsequent years supported the earlier findings. In one study involving male Wistar rats weighing 300–350 g, a decrease in lymphatic transport was observed after administering colchicine *via* intraperitoneal injection (5 mg/kg). This pre-administration preceded the administration of a 5 mL mixed micellar solution containing [1-^{14}C]-linoleic acid. The administered lipid showed a slower transit to the lymphatics, mainly in the form of free fatty acids. Colchicine was reported to act by inhibiting microtubule function through its binding with tubulin, leading to the predominant presence of triglycerides as chylomicrons, whose release was hindered from intestinal epithelial cells in colchicine-treated animals. Thus, this resulted in an increased abundance of free fatty acids rather than triglycerides as lipids were transported from epithelial cells into the lymphatics (Miura et al., 1982).

In a different investigation using an intraduodenal pulse injection of [^{14}C]-oleic acid, the administration of colchicine at a dose of 0.5 mg/100 g in female Albino rats (200–250 g) was found to have no impact on the uptake of fatty acids by the intestinal mucosa. However, it had contrasting effects on fatty acid esterification, promoting their integration into triglycerides rather than phospholipids. Additionally, colchicine led to the buildup of endogenous diglycerides, triglycerides, and cholesterol esters within the intestinal epithelium. Ultra-structural and morphometric analyses showed a reduction in visible microtubules and a displacement of the smooth and rough endoplasmic reticulum and Golgi apparatus. Also, microvilli were observed at the lateral plasma membrane of enterocytes following colchicine treatment (Pavelka & Gangl, 1983).

The colchicine model was utilized to investigate the impact of chylomicrons on the lymphatic absorption of various drugs (Table 2). For instance, male Wistar rats weighing 230–270 g received an intravenous dose of 5 mg/kg colchicine to study vitamin D3 absorption. In this experiment, rats were administered a soybean oil emulsion containing vitamin D3, prepared using either milk fat globule membrane (MFGM) or Tween 80 as emulsifiers. In colchicine-untreated rats, the cumulative percentage of vitamin D3 absorbed in lymph at 12 h post-dose was 19.2% and 13.8% for MFGM and Tween 80 emulsions, respectively. This absorption decreased to 2.05% and 2.23% in each case when rats were treated with colchicine (Liu et al., 1995).

In a succeeding study, Dahan and Hoffman extended the findings of previous researchers by administering colchicine to rats through intraperitoneal injection (5 mg/kg). One hour post-injection, male Wistar rats (300–325 g) received oral gavage of vitamin D3. Colchicine substantially reduced the intestinal absorption of vitamin D3, resulting in a relative bioavailability of 12.5% compared to the control group. Markedly, the vitamin D3 elimination rate constant increased from 0.04 ± 0.005 in the control to 0.08 ± 0.0009 after colchicine treatment. The elimination half-life of vitamin D3 from the plasma in both control and colchicine-treated animals was 15.5 (± 0.9) h and 8.6 (± 1.7) h, respectively (Dahan & Hoffman, 2005). Eighteen hours after the colchicine dose, rats displayed lethargy and began to die, as had been seen in earlier studies (Rosenbloom & Ferguson Jr, 1968; Sternberg et al., 1954). A lower colchicine dose (2.5 mg/kg) did not create adequate inhibition of lipid transport and proved to be as fatal as the higher one. Results from the d-xylose loading test indicated impaired d-xylose concentrations in colchicine-treated animals compared to other groups, implying a degree of toxicity to the absorptive function of the intestine after colchicine treatment (Dahan & Hoffman, 2005).

Following this, Iwanaga *et al* examined the intestinal absorption of Solvent Green (SG) 3, a model poorly water-soluble compound, using colchicine. They orally administered SG to male Wistar rats (300–350 g) with inhibited chylomicron synthesis, achieved by pre-treatment with intravenous colchicine at 5 mg/kg one hour before the administration of SG and its lipid-based formulations. These formulations included a soybean oil emulsion and a self-microemulsifying drug delivery system

Table 2
List of studies investigating colchicine as a blocker of lymphatic uptake *via* chylomicrons for various xenobiotics.

Species	Colchicine Dose	Colchicine Pre-treatment Timing hours	Xenobiotics Tested with the Model	Reference
Male Wistar rats (230–270 g)	IV injection (5 mg/kg)	1	Vitamin D3	Liu et al., 1995
Male Wistar rats (300–325 g)	IP injection (5 mg/kg)	1	Vitamin D3	Dahan & Hoffman, 2005
Male Wistar rats (300–350 g)	IV injection (5 mg/kg)	1	Solvent Green 3	Iwanaga et al., 2006
Female Sprague-Dawley rats (200–220 g)	IP injection (5 mg/kg)	1	Docetaxel	Valicherla et al., 2016
Female Sprague-Dawley rats (220–250 g)	IP injection (5 mg/kg)	_	Paclitaxel	Meher et al., 2020

*Abbreviations: IV = Intravenous, IP = Intraperitoneal.

(SMEDDS). Colchicine effectively impeded the intestinal absorption of SG across all tested lipid-based formulations, indicating that SG was absorbed from the intestine through a lymphatic route (Iwanaga et al., 2006).

Moreover, in 2016, Valicherla *et al* developed self-emulsified drug delivery systems loaded with Docetaxel (D-SEDDS) to enhance both the oral bioavailability and antitumor properties of the drug. To assess the intestinal transport of D-SEDDS following oral administration, they utilized the colchicine model in female Sprague Dawley rats (200–220 g). After intraperitoneal colchicine administration (5 mg/kg), the C_{max} of D-SEDDS experienced an 8.69-fold decrease, dropping from 125.5 ± 2.5 to 14.44 ± 4.72 ng/mL. Furthermore, the $AUC_{0-\infty}$ exhibited a reduction of over 10-fold, decreasing from 260.23 ± 51.8 to 11.29 ± 1.03 h·ng/mL (Valicherla et al., 2016).

Similarly, Meher *et al* recently used the same approach by formulating a silica-based solid self-emulsifying drug delivery system (Si-PTX-S-SEDDS) to encapsulate another anticancer agent, paclitaxel (PTX). The goal was to enhance the oral bioavailability of PTX and address challenges associated with conventional delivery systems. Using the colchicine model in female Sprague Dawley rats (220–250 g), the study aimed to elucidate the transport mechanism of the developed formulation. The results revealed a 4–6-fold decrease in oral bioavailability in colchicine-treated animals, highlighting the involvement of chylomicrons in the oral absorption of the Si-PTX-S-SEEDS formulation in rats (Meher et al., 2020).

Research on colchicine as a lymph-blocking agent has centered on its toxicity, role as a microtubule inhibitor, and relevance in investigating the lymphatic absorption of formulations designed for this route. Additionally, studies have delved into the pharmacokinetic profile of colchicine within a practical dose range (1–10 mg/kg) in rats (Chen et al., 2008). The chylomicron-blocking effect of colchicine is primarily linked to its ability to disrupt the secretion of chylomicrons from enterocytes, thereby impeding their uptake into intestinal lymphatics. However, the exact extent and duration of inhibition by colchicine remains unclear despite these efforts. In studies examining the lymphatic uptake of xenobiotics, the reported dose of colchicine was 5 mg/kg, a dose deemed effective in blocking lymphatic uptake when administered intravenously and intraperitoneally, but also potentially associated with toxicity. Although the analytical feasibility for lower oral and intravenous doses of colchicine in the rat model (0.1 mg/kg) has been documented (Al Nebaihi et al., 2021), the effectiveness of low doses (0.1 to 0.5 mg/kg) in blocking lymphatic uptake was not found to be effective (Al Nebaihi et al., 2024). Even higher intraperitoneal dose of 2.5 mg/kg has been reported to be ineffective in blocking chylomicrons (Dahan & Hoffman, 2005). Further exploration of lower doses using different routes of administration would support the reported data and assess the potential use of lower effective doses, if feasible.

2.4. Vinca alkaloids model

Vinca alkaloids (VA), first extracted in the 1950s by Canadian researchers Robert Noble and Charles Thomas Beer from the Madagascar Periwinkle plant, *Catharanthus roseus*, have a rich history of use in Ayurveda and traditional Chinese medicine. Yet, the connection between VA and cancer was established following animal studies that revealed fatalities attributed to septicemia and leukopenia (Martino et al., 2018).

Initial investigations into VA centered on their hypolipidemic effects. Swiss/H-Riop outbred male mice (30 to 35 g) were subjects in experiments designed to explore the serum lipid-lowering properties of VA, both in normal mice and those with ascites tumor-induced hyperlipidemia (Ehrlich, NK/Ly). Administered through intraperitoneal injections ranging from 0.2 to 5 mg/kg, VA demonstrated a rapid and reversible reduction in serum lipid levels following a single, non-toxic dose. The dose-dependent increase in the serum lipid-lowering effect showed the same changes in serum lipid and lipoprotein composition across all doses. Vinca alkaloids were observed to decrease levels of neutral lipids (triglycerides) and very low-density lipoproteins (VLDL) in the serum. Simultaneously, higher doses resulted in an elevation of total lipid and triglyceride content in the liver. These findings suggested that VA might hinder the release of lipoproteins in the liver by either obstructing active sites of membrane systems (cytoplasmic, Golgi) responsible for secretion or altering the physicochemical properties of the membranes, such as membrane fluidity (Kremmer et al., 1979).

An additional study in rhesus monkeys with vincristine and vinblastine revealed a decrease in plasma low-density lipoprotein cholesterol concentrations, accompanied by an elevation in plasma triacylglycerol concentrations. Within 7–10 days post-injection, plasma lipid levels reverted to normal concentrations. Electron micrograph examinations of hepatocytes in monkeys subjected to the stated VA showcased an accumulation of glycogen particles and a proliferation of smooth endoplasmic reticulum. This coincided with an increase in the number of vesicles containing lipoproteins (Sethi et al., 1983).

Later, one study looked into the effect of intestinal lymphatic blocking in the previously quoted study by Stein *et al* using colchicine for the same purpose. In that study, female and male Albino rats of the Hebrew University strain weighing 200 g were administered intraperitoneal injections of vinblastine sulfate at doses of 0.1 or 1.0 mg/100 g body weight (equivalent to 1 or 10 mg/kg). Subsequently, they were subjected to intravenous injections of [l-^{14}C]-palmitic acid and Triton WR 1339 at different time intervals. The inhibition of triglyceride secretion into the serum was observed to a reduced extent, with reductions of 19.5% and 57.2% in female rats. The inhibition required a higher dose of vinblastine compared to colchicine. Additionally, the effect of vinblastine lasted for a shorter duration in comparison to that of colchicine, and the inhibition was nearly entirely reversed within 270 min (Stein et al., 1974).

Similar to puromycin, VA were not found to be used in studying the intestinal lymphatic uptake of drugs or formulations. Data regarding more detailed mechanisms of action and related aspects is still lacking as well. However, an intriguing literature finding suggests and involves a group of VA with lipid-lowering effects beyond the well-known drugs vincristine and vinblastine (Kremmer et al., 1979). Most of these compounds are commercially available and they can be further explored for their potential chylomicron-blocking effect. Table 3 presents some of these VA, their LD50, and the doses examined for their hypolipidemic effects.

2.5. Cycloheximide model

Acetoxycycloheximide (ACH), derived from *Streptomyces albulus* cultures, has been recognized for its inhibitory effects on transplanted tumors and demonstrated toxicity to yeast, mammalian cells in tissue

Table 3
Some of the vinca alkaloids (VA) that have shown hypolipidemic effects (Kremmer et al., 1979).

Vinca Alkaloid	LD 50 (mg/kg)	Doses Studied for the Lipid-Lowering Effect (mg/kg)
Vincristine	4.2	0.2 and 1
Dimethylaminoacetyl-Vincristine	> 200	10
Vinblastine	7.6	1
Penta-hydroxy-Vinblastine	> 200	10
Dimethylaminoacetyl-Vinblastine	> 200	5
Leurosine	29	3
Vinleurosine	90	6
N-Formyl-Leurosine	29	3
Vindoline base	>200	200
Vindoline HCl-salt	~ 280	50 and 200
Desacetyl-Vindoline	>200	10
Catharantine	>200	20
Velbanamine	>200	3 and 10

culture, and intact animals since the 1960s. Concurrently, research has explored the impact of this antibiotic on the inhibition of [^{14}C]-amino acid incorporation into tissue proteins. The blockade of protein synthesis by ACH was found to induce a syndrome marked by various symptoms and eventual death. Toxicity was observed across various animals, including female Albino rabbits at a dose of 0.5 mg/kg, female mongrel dogs at doses ranging from 2 to 4 mg/kg, and mice (Carworth *CF*-1 strain) and rats (Wistar strain) at 5 mg/kg. Actidione-cycloheximide or cycloheximide, though described as less toxic to intact mammals but closely related to ACH, exhibited over 90% inhibition of [^{14}C]-amino acid incorporation into rabbit liver proteins when administered intraperitoneally at a dose of 50 mg/kg. The study concluded that cycloheximide can induce clinical and biochemical changes in rabbits, rats, and mice similar to those produced by ACH (Young et al., 1963).

Similar to puromycin, the utilization of cycloheximide as a chylomicron lymph blocker has roots in the work of Sabesin *et al* in 1965. In the previously mentioned study, female Sprague-Dawley rats (180–200 g) were subjected to ACH treatment. The administration of ACH (0.2 mg per kg), followed by 3 h and 1.5 mL corn oil, resulted in lipid accumulation within the intestinal mucosa, accompanied by low concentrations of plasma triglycerides. Interestingly, the administered corn oil failed to induce the typical post-prandial hyperlipemia, suggesting interference in lipid transport due to impaired chylomicron formation (Sabesin & Isselbacher, 1965).

A few years later, an in-depth exploration of the effect of cycloheximide on protein synthesis unfolded, using a dose of 1.5 mg/kg in male white Wistar rats (160–175 g). This dosage exerted a reduction in hepatic protein synthesis throughout 7 h. Despite the pronounced decline in protein synthesis, the liver manifested only minimal ultra-structural alterations. These alterations encompassed a partial disorganization of the customary parallel arrays of rough endoplasmic reticulum, with closer proximity of its lamellae to various cytoplasmic organelles, particularly mitochondria. Additionally, the Golgi cisternae exhibited a near absence of contents, and the vacuoles, which typically contained multiple electron-dense particles in controls, were lacking in the cycloheximide-treated animals (Verbin et al., 1969).

A couple of years later, the same author contributed to a toxicological assessment of cycloheximide conducted in both male and female white Wistar rats weighing 160–175 g. Cycloheximide, prepared at a concentration of 1 mg/mL, was intraperitoneally administered as a single dose ranging from 1.5 to 4.5 mg/kg of body weight. In preliminary dose-response studies, it was found that, among the tested doses, only 1.5 mg/kg of the antibiotic was non-lethal to animals of both sexes. Doses in the range of 3.0–4.5 mg/kg approached or exceeded the LD_{100}, particularly in female rats. Specifically, 3.3 mg/kg was the only dosage within this range where at least one animal of each sex survived the entire 72-h test period. In addition, both male and female rats exhibited symptoms such as diarrhea, lethargy, and semi-comatose states at the time of sacrifice (Verbin et al., 1971).

Then, the research shifted its focus to investigating the impact of inhibiting protein synthesis on lipid absorption, employing ACH in rats with mesenteric lymph fistulas. Male Sprague-Dawley rats (200–250 g) equipped with indwelling mesenteric lymph cannulas received an intraduodenal infusion of a micellar solution of oleic acid labeled with [^{14}C]-oleic acid. Protein synthesis inhibition was achieved through an intraperitoneal dose of 0.25 mg/kg ACH administered one hour before lipid infusion. A temporary increase in chylomicron size occurred during maximal triglyceride absorption in control animals, while ACH-treated animals exhibited a sustained increase in chylomicron size, lasting up to 4 h after lipid infusion. The mean recovery of labeled lymph triglycerides was 65% in control animals, compared to 43% in the ACH-treated group. These findings underscored the role of protein synthesis in the formation and transport of chylomicrons from the mucosal cell into the lymph (Glickman et al., 1972). Following studies by the authors demonstrated that ACH-induced impairment of intestinal protein synthesis was associated with a deficiency in a key chylomicron apoprotein, offering a potential explanation for the observed decline in lipid absorption under conditions of impaired protein synthesis (Glickman & Kirsch, 1973). Further investigations provided direct evidence of a substantial decrease in the mucosal content of two major chylomicron apo-proteins, namely apo B and apo A-I, during inhibition of protein synthesis by ACH (Glickman et al., 1978).

In the early studies applying the cycloheximide model to chylomicron blockade, male Wistar rats (200–250 g) with lymphatic fistulas were subjected to protein synthesis inhibitors: cycloheximide (administered intraperitoneally at a dose of 1.4 to 1.6 mg/kg) or acetoxycycloheximide (0.4 mg/kg). Following this, an intraduodenal infusion of a lipid emulsion, consisting of an equimolar blend of monopalmitin, palmitic acid, and [^{14}C]-oleic acid, was conducted. In normal rats, 75% of the introduced radioactivity was recovered in the intestinal lymph 6 h post-infusion, contrasting with 4.5% in rats treated with cycloheximide. Moreover, in normal rats, 65% of the radioactivity was found in the intestinal lymph 4 h after infusion, whereas in rats treated with acetoxycycloheximide, only 43% of the radioactivity passed into the lymph. The findings of the study affirmed the inhibitory impact of both cycloheximide and acetoxycycloheximide on the lymphatic absorption of oleic acid (Bernard & Carlier, 1981).

Research using the cycloheximide model to study the lymphatic absorption of drugs began with a study evaluating the intestinal absorption of vitamin D and 25-hydroxy vitamin D [25(OH)D_3]. In that study, male Holtzman rats (250–300 g) were treated with 3 mg/kg intraperitoneal cycloheximide to inhibit chylomicron synthesis. Findings indicated that 53% of vitamin D was found in the chylomicron fraction, in contrast to only 13% of [25(OH)D_3]. Inhibiting chylomicron synthesis with cycloheximide resulted in a 46% reduction in vitamin D absorption but only a 30% decrease in [25(OH)D_3] absorption. Several lipophilic and hydrophilic components were included for comparative analysis. The intestinal absorption of the hydrophobic oleic acid and retinol decreased by approximately 70%, surpassing the reductions observed for vitamin D and [25(OH)D_3]. Interestingly, the absorption of the water-soluble substance phenylalanine remained unaffected by cycloheximide in this experimental system (Sitrin et al., 1982).

In succeeding investigations by the same previous group on 1,25-dihydroxy vitamin D_3[1,25$(OH)_2D_3$], a similar approach using cycloheximide to impede chylomicron synthesis did not result in reduced absorption of [1,25$(OH)_2D_3$]. Almost all [1,25$(OH)_2D_3$] was directly released from the intestine into the portal blood, evading the need for chylomicron packaging for transport into the intestinal lymph. Inhibiting protein synthesis did not decrease the intestinal absorption of 1,25-dihydroxyvitamin D; 80.2 $\pm$ 7.6% of the absorbed 1,25-dihydroxyvitamin D was observed in cycloheximide-treated rats, compared to 88.6 $\pm$ 0.4% in the control animals (Sitrin et al., 1985).

In 2005, Dahan and Hoffman extended their exploration beyond the previously detailed PL-81 and colchicine models to include the cycloheximide model for investigating vitamin D absorption. The administration of cycloheximide (3 mg/kg) through intraperitoneal injection before oral gavage of Vitamin D3 in male Wistar rats (300–325 g) exhibited an association with the mesenteric lymph duct cannulation model. This observation implied that the integration of this lipophilic molecule into chylomicrons played a vital role in its lymphatic absorption. Similar to the PL-81 model and in contrast to the colchicine model at 4-h time point, the oral d-xylose loading test indicated that, at this dosage, cycloheximide did not affect intestinal absorptive functions, and no adverse effects were recorded (Dahan & Hoffman, 2005).

>50 distinct experimental studies employing cycloheximide as a lymphatic blocker since 1982 have been identified (Table 4). Originally demonstrated as a non-invasive chylomicron flow block approach in male Holtzman rats, this method has been extended to include various rat strains, such as Wistar, Sprague-Dawley, Swiss Albino and Carlie Foster rats. Moreover, ICR mice have also been subjects in this model as have both male and female Swiss albino mice. Cycloheximide pretreatment conducted an hour before administering the drug under

Table 4
List of literature reports in which cycloheximide was used as a chylomicron blocker.

Species	Cycloheximide Dose	Cycloheximide Treatment Timing	Xenobiotics Tested with the Model	Reference
Male Holtzman rats (250–300 g)	IP injection (3 mg/kg)	3 h before administering the drug	Oleic Acid Retinol Vitamin D3 25-Hydroxy Vitamin D3	Sitrin et al., 1982
Male Holtzman rats (250–300 g)	IP injection (3 mg/kg)	3 h before administering the drug	1,2- Dihydroxy Vitamin D3	Sitrin et al., 1985
Male Wistar rats (300–325 g)	IP injection (3 mg/kg)	1 h before administering the drug	Vitamin D3	Dahan & Hoffman, 2005
Male Wistar rats (275–300 g)	IP injection (3 mg/kg)	1 h before administering the drug	Vitamin D3	Dahan & Hoffman, 2006
Male Sprague-Dawley rats (270–310 g)	IP injection (3 mg/kg)	1 h before administering the drug	Halofantrine	Lind et al., 2008
Male Sprague-Dawley rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Candesartan Cilextil	Gao et al., 2011
Male Sprague-Dawley rats (190–230 g)	IP injection (3 mg/kg)	1 h before administering the drug	Sirolimus	Sun et al., 2011
Male Sprague-Dawley rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Nimodipine	Fu et al., 2013
Male Sprague-Dawley rats (225–275 g)	IP injection (3 mg/kg)	1 h before administering the drug	Puerarin	Tang et al., 2013
Male Sprague-Dawley rats (300–325 g)	IP injection (3 mg/kg)	1 or 1.5 h before administering the drug	Docetaxel	Attili-Qadri et al., 2013
Male and Female Charle Foster strain Albino rats (230–270 g)	IP injection (3 mg/kg)	1 h before administering the drug	Praziquantel	Mishra et al., 2014
Male Wistar rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Acetylpuerarin	Sun et al., 2014
Male Wistar rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Terbinafine	Baheti et al., 2016
Male Wistar rats (200–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Carvedilol	Arzani et al., 2015
Wister Albino rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Diacerin	El-Laithy et al., 2015
Male Sprague-Dawley rats (230–270 g)	IP injection (3 mg/kg)	1 h before administering the drug	Efavirenz	Makwana et al., 2015
Male Wistar rats (180–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Darunavir	Bhalekar et al., 2016
Male Wistar rats (200–300 g)	IP injection (3 mg/kg)	1 h before administering the drug	Lopinavir	Garg et al., 2016
Female Sprague-Dawley rats (200–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Docetaxel	Valicherla et al., 2016
Male Sprague-Dawley rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Piroxicam	Xing et al., 2016
Male Sprague-Dawley rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Huperzine A	Li, Hu, et al., 2017, Li, Zhuang, et al., 2017
Male Sprague-Dawley rats (250–300 g)	IP injection (3 mg/kg)	1.5 h before administering the drug	Doxorubicin-Quercetin	Alrushaid et al., 2017
Male Wistar rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Lopinavir	Ravi & Vats, 2017
Male Wistar rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Topotecan	Wang et al., 2017
Male Sprague-Dawley rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Berberine	Elsheikh et al., 2018
Male Wistar rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Pueraria Flavones	Qiao et al., 2018
Male Sprague-Dawley rats (180–260 g)	IP injection (3 mg/kg)	1 h before administering the drug	Canagliflozin	Singh et al., 2018
Male Sprague-Dawley rats (180-220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Bacalein	Wu et al., 2018
Male Sprague-Dawley rats (250–300 g)	IP injection (3 mg/kg)	1 h before administering the drug	Bacalein	Xu et al., 2019
Rat Male Sprague-Dawley (230–270 g)	IP injection (3 mg/kg)	1 h before administering the drug	Baicalein	Liao et al., 2019
Wistar rats (~250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Exenatide	Lin et al., 2019
Female Sprague-Dawley rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Asenapine Maleate	Patel, Mundada, & Sawant, 2019, Patel, Shah, et al., 2019
Female Sprague-Dawley rats (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Lurasidone	Patel & Sawant, 2019
Female Sprague Dawley rats (190 g–230 g)	IP injection (3 mg/kg)	1 h before administering the drug	Raloxifene Hydrochloride	Li et al., 2020
Female Sprague-Dawley rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Paclitaxel	Meher et al., 2020

(*continued on next page*)

Table 4 (*continued*)

Species	Cycloheximide Dose	Cycloheximide Treatment Timing	Xenobiotics Tested with the Model	Reference
Male Sprague-Dawley rats (270–330 g)	IP injection (3 mg/kg)	1 h before administering the drug	Nislodipine	Mundada et al., 2020
Male Wistar rats (213.5–237.5 g)	IP injection (3 mg/kg)	1 h before administering the drug	Ibrutinib	Rangaraj et al., 2020
Female Sprague-Dawley rats (200–240 g)	IP injection (1 mg/kg)	0.5 h before administering the drug	Raloxifene	Ye et al., 2020
Male Wistar rats (250–280 g)	IP injection (3 mg/kg)	1 h before administering the drug	Quetiapine	Agarwal et al., 2021
Male ICR mice	IP injection (6 mg/kg)	1 h before administering the drug	(Cy5)-Labeled Insulin	Bao et al., 2021
Male Sprague-Dawley rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Andrographolide	Elsheikh et al., 2021
Male and Female Swiss Albino Mice (30–40 g)	IP injection (3 mg/kg)	1 h before administering the drug	Nintedanib Esylate	Patel & Patel, 2021
Male Sprague-Dawley rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Baicalin	Rizk et al., 2021
Male Wistar rats (300–450 g)	IP injection (3 mg/kg)	1 h before administering the drug	Abiraterone and Cinacalcet	Ryšánek et al., 2021
Male Wistar rats (230–270 g)	IP injection (3 mg/kg)	1 h before administering the drug	Mebendazole	Shrivastava et al., 2021
Male Wister Albino rats (250–280 g)	IP injection (3 mg/kg)	1 h before administering the drug	Resveratrol	Gausuzzaman et al., 2022
Male Wister Albino rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Revaprazan	Goo et al., 2022
Male Wistar rats (215–235 g)	IP injection (3 mg/kg)	1 h before administering the drug	Atazanavir	Gurumukhi & Bari, 2022
Male Wistar rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Ritonavir	Jitta et al., 2024
Rat Male Sprague-Dawley (180–220 g)	IP injection (3 mg/kg)	1 h before administering the drug	Madecassic Acid	Lin et al., 2023
Male Wistar Albino rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Gefitinib	Harisa et al., 2023
Male Wistar rats	IP injection (3 mg/kg)	1 h before administering the drug	Hydroxymethylnitrofurazone	De Souza et al., 2024
Wistar rats (each is 250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Etravirine and Darunavir Ethanolate	Muheem et al., 2024
Male Wistar rats (220–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Efavirenz	Fuentes et al., 2024
NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice (30–35 g)	IP injection (3 mg/kg)	0.5 h or 2 h before administering the drug	Niclosamide	Liu et al., 2024
Sprague-Dawley (SD) male rats (200 ± 20 g)	IP injection (3 mg/kg)	1 h before administering the drug	Dihydroartemisinin	Zheng et al., 2024
>Male Wistar rats (200–250 g)	IP injection (3 mg/kg)	1 h before administering the drug	Idarubicin Hydrochloride	Emzhik et al., 2024

*Abbreviations: IP = Intraperitoneal.

study, has been a common approach in various investigations. Attili *et al* and Alrusahid *et al* proposed enhanced blocking efficacy when the pre-treatment occurred at 1.5 h rather than at 1 h after administration (Alrushaid et al., 2017; Attili-Qadri et al., 2013). A common dosage used in the cycloheximide model has been 3 mg/kg, although alternative doses have also been explored (Al Nebaihi et al., 2023; Bao et al., 2021). While the intraperitoneal route is predominantly utilized, other administration routes have also been examined (Al Nebaihi et al., 2023). A detailed compilation of reported studies can be found in Table 4.

Various formulations have been developed to specifically target the intestinal lymphatics as a route of absorption. Table 5 provides examples of the different reported formulations. One recent study showcased the potential of specially formulated nanoparticles as a promising oral delivery system for insulin, employing cycloheximide as a chylomicron blocker. In this investigation, diabetic mice underwent pre-treatment with cycloheximide to explore the lymphatic transport of orally administered nanoparticles. The bioavailability of insulin- and cholic acid-loaded zein nanoparticles with dextran surfaces was significantly reduced by 43% and 49% in formulations A1 and A2, respectively, in the presence of cycloheximide. This suggested that approximately half of the nanoparticles were transported *via* the lymphatic pathway. Conversely, the bioavailability of a specific A2 nanoparticle formulation decreased by 22% in the presence of cycloheximide. The difference between A1 and A2 nanoparticles in intestinal lymphatic transport indicated that embedded cholic acid played a role in promoting the intestinal absorption of A1 nanoparticles through the lymphatic pathway, a feature lacking in A2 (Bao et al., 2021).

A comprehensive examination of the pharmacokinetic profile of cycloheximide and its associated effects and toxicity has been undertaken by Al Nebaihi *et al* to enhance the understanding of its impact. In adult Sprague-Dawley male rats, cycloheximide was administered at 0.5 mg/kg through oral, intraperitoneal, and intravenous routes. The findings showed that cycloheximide exhibited high clearance and volume of distribution, with an oral absolute bioavailability of 0.47 at the 0.5 mg/kg dose. The pharmacokinetics of cycloheximide was dose- and route-dependent, showing over a 3-fold relative bioavailability after intraperitoneal doses. Cycloheximide also demonstrated low plasma protein binding and minimal urinary excretion. Metabolism appeared to occur through oxidation and glucuronidation. Oral administration of 2.5 mg/kg cycloheximide led to reductions in plasma lipids (24–40%), accompanied by signs of inflammation and increased liver enzymes for a week following the dose. Markers of hepatic function and inflammation were elevated after the oral administration of 2.5 mg/kg. Additionally, cycloheximide was found to induce hepatotoxicity within 2 h after a single intraperitoneal dose of 5 mg/kg (Al Nebaihi et al., 2023).

Another study highlighted an important observation; when the

Table 5
Examples of formulations analyzed for their lymphatic uptake tendency through the inhibition models of colchicine and cycloheximide. All studies were done in a rat animal model.

Delivery System	Compound	Reference
SEDDS	Docetaxel	Valicherla et al., 2016
	Paclitaxel	Meher et al., 2020
	Resveratrol	Gausuzzaman et al., 2022
SMEDDS	Solvent Green 3	Iwanaga et al., 2006
	Sirolimus	Sun et al., 2011
	Terbinafine	Baheti et al., 2016
	Huperzine A	Li et al., 2017
	Canagliflozin	Singh et al., 2018
	Asenapine Maleate	Patel et al., 2019
	Bacalein	Liao et al., 2019
	Pueraria Flavones	Qiao et al., 2018
	Lurasidone	Patel & Sawant, 2019
	Niclosamide	Liu et al., 2024
SNEDDS	Revaprazan	Goo et al., 2022
	Madecassic Acid	Lin et al., 2023
SNESNS	Diacerein	El-Laithy et al., 2015
S-SNEOFs	Lopinavir	Garg et al., 2016
SLN	Praziquantel	Mishra et al., 2014
	Efavirenz	Makwana et al., 2015
	Darunavir	Bhalekar et al., 2016
	Lopinavir	Ravi & Vats, 2017
	Topotecan	Wang et al., 2017
NLC	Ibrutinib	Rangaraj et al., 2020
	Mebendazole	Shrivastava et al., 2021
	Hydroxymethylnitrofurazone	De Souza et al., 2024
	Atazanavir	Gurumukhi & Bari, 2022
	Gefitinib	Harisa et al., 2023
	Ritonavir	Jitta et al., 2024
	Etravirine and Darunavir Ethanolate	Muheem et al., 2024
ME	Puerarin	Tang et al., 2013
	Piroxicam	Xing et al., 2016
MD	Raloxifene Hydrochloride	Li et al., 2020
NCs	Docetaxel	Attili-Qadri et al., 2013
Nanoemulsion	Acetylpuerarin	Sun et al., 2014
	Baicalin	Wu et al., 2018; Xu et al., 2019
	Nisoldipine	Mundada et al., 2020
Niosomes	Carvedilol	Arzani et al., 2015
Nanocrystals	Nimodipine	Fu et al., 2013
Bioactive Chylomicrons	Berberine	Elsheikh et al., 2018
Nanoemulsomes	Andrographolide	Elsheikh et al., 2021
Bioemulsomes	Baicalin	Rizk et al., 2021
NMs	Efavirenz	Fuentes et al., 2024
Biomimetic Liposomes	Dihydroartemisinin	Zheng et al., 2024
Cerasomes	Idarubicin Hydrochloride	Emzhik et al., 2024

Abbreviations:
Self-Microemulsifying Drug Delivery System (SMEDDS), Nanocapsules (NCs), Microemulsion (ME), Solid-Lipid Nanoparticles (SLN), Self-Nanoemulsifying Self-Nanosuspension (SNESNS), Solid Self-Nanoemulsifying Oily Formulations (S-SNEOFs), Self-Emulsified Drug Delivery Systems (SEDDS), Nanostructured Lipid Carrier (NLC), Matrix Dispersion (MD), Nanomicelles (NMs).

lymphatic absorption pathway is obstructed, the primary route for drug absorption in the intestine is through the portal blood. Consequently, drugs relying on lymphatic transport may undergo hepatic first-pass metabolism when lymphatic uptake is hindered, leading to a reduction in both the rate and extent of drug absorption, reflected in decreased AUC, C_{max} and T_{max}. This phenomenon was evident with abiraterone, as pre-treatment with cycloheximide reduced the rate and extent of its absorption, resulting in a threefold decrease in C_{max} and a twofold decrease in AUC. Although the mean half-life was approximately 2 h after both oral and intravenous administrations, it appeared to be extended to 18 h in the cycloheximide pre-treated group. Yet, the specific pharmacokinetic mechanisms underlying this observation, including alterations in the volume of distribution, total body clearance, or both, remained unclear (Ryšánek et al., 2021). In a similar manner, pre-treatment with cycloheximide modified the pharmacokinetic profile of cinacalcet, suggesting a potential alteration in absorption in this model (Ryšánek et al., 2021).

Although being the most used chylomicron-blocking model compound, concerns have recently surfaced regarding the potential exaggeration of lymphatic uptake, particularly for drugs with low lipophilicity (log $P < 5$), when utilizing the cycloheximide model. This issue came to the forefront during a study evaluating abiraterone and cinacalcet using the cycloheximide model. The results may suggest a substantial overestimation of relative bioavailability (28-fold higher for abiraterone and 2.7-fold higher for cinacalcet) compared to the cannulation method, which directly measures drugs from the lymphatic vessel draining the intestinal region (mesenteric lymph duct). In light of these findings, the study concluded that the cycloheximide model may not be suitable for accurately assessing lymphatic transport and advocates for a critical re-evaluation of previously obtained data (Ryšánek et al., 2021). Further studies would be required to support this claim as there have been other reports supporting the good correlation between chylomicron blocking and cannulation methods (Dahan & Hoffman, 2005).

The cycloheximide model is the most extensively studied and applied one among the mentioned chylomicron-blocking models. Various xenobiotics, ranging from peptides like insulin to drugs addressing diverse conditions such as anticancer, antiviral, and hypertensive medications, have been investigated using this model. The advantage of cycloheximide lies in its capacity to block chylomicrons effectively at concentrations (3 mg/kg) without inducing appreciable toxicity, distinguishing it from the antitubular chylomicron blocker, colchicine.

3. Outlook and future perspectives

Upon reviewing the literature on chylomicron blockers, in addition to considering the various points discussed in different sections, three specific aspects have surfaced as areas that merit more detailed examination beyond what is covered in the individual sections. These key points are delineated below:

3.1. *Chylomicron blockade: an underappreciated drug interaction?*

Chylomicron blockers are being used and designed to reduce lymphatic drug absorption experimentally. However, the suitability of cycloheximide for human use is challenged by its toxicity, limiting its application primarily to *in vitro* and animal experiments (Wexler & Anderson, 2005). Moreover, puromycin, a cytocidal antibiotic, is utilized principally in cell culture experiments when a selective agent is required to eliminate all cells without resistance genes, leaving only specific targets behind (Miyata et al., 2022). PL-81, employed in formulation and drug delivery, has also been identified as a potent anti-obesity drug in animal models (Au et al., 2009; Batrakova et al., 1998; Senthilkumar et al., 2022). Nevertheless, clinically, some of these blockers are available and have been used for decades for a number of malignancies. Colchicine, a remedy with a history spanning over a millennium for treating gout, is now being explored for potential applications in dermatological conditions, and cardiovascular diseases, as well as in immunology and oncology (Dasgeb et al., 2018; McEwan & Robinson, 2021; Schlesinger et al., 2020). Additionally, vinca alkaloids, such as vincristine, vinblastine, vinorelbine, vindesine, and vinflunine, are widely used chemotherapeutic agents. Vincristine and vinblastine, particularly studied in the context of chylomicron blockade, demonstrate clinical availability for its antitumor effects (Martino et al., 2018; Taher et al., 2017).

The hypothesis proposed is that *in-vivo* use of chylomicron blockers may interact with lipophilic xenobiotics, partially absorbed through the chylomicron pathway, resulting in a reduced absorption of the latter. The combination of clinical use of drugs (colchicine, vinca alkaloids) or

an excipient (PL-81) inhibiting chylomicron formation or secretion, along with a substrate intended for absorption through the chylomicron pathway, could lead to a reduced T_{max} and a decreased area under the concentration-time curve and consequently less drug available to exert effects. Reports on drug interactions, particularly with colchicine and vinca alkaloids, attribute these interactions to competition and effects related to transporters and enzymes that both inhibitors and concurrently administered drugs interact with (Griffin & D'Arcy, 1997; Hansten et al., 2023; Moudi et al., 2013; Terkeltaub, 2009; Zhou & Rahmani, 1992).

However, to the best of our knowledge, there is no documented evidence that PL-81, colchicine, or vinca alkaloids in clinical use have led to a demonstrable reduction in drug concentrations as a result of the chylomicron blockage effect. Several factors may contribute to this lack of support, such as the percentage of PL-81 in formulations potentially not reaching the threshold for inducing chylomicron blockage and subsequent reduction in drug concentration. Additionally, the developed formulations may not have been tested *in-vivo* or even *in-vitro* for the chylomicron blocking effect (Krupka et al., 2010; Oh et al., 2004; Senthilkumar et al., 2022).

Also, formulations of vinca alkaloids still in development may not have be used at effective doses to impart the blockage effect. Furthermore, many formulations are designed to enter the M cells pathway rather than the chylomicron pathway, as will explained in the next section. Therefore, drugs taken with such formulations may not be affected, as they follow a different pathway to the general circulation (Lee et al., 2015). Another factor that may obscure the evidence of chylomicron blockage in clinical use is that these agents might not be suitable for oral administration. For instance, vinca alkaloids, known to have adverse effects and fatalities when administered *via* routes other than intravenous, prompted the FDA to add a warning to the labeling of vinca alkaloids such as vincristine and vinblastine, advising intravenous administration only (Aschenbrenner, 2021).

Despite its utilization in various ailments, including gout (Slobodnick et al., 2015), colchicine also lacks clinical documentation as a chylomicron blocker in humans. Taking it as an example, where a reported dose of 0.5 mg/kg has demonstrated a chylomicron blocking effect in a rat model (Stein et al., 1974), the Human Equivalent Dose (HED) can be calculated. The HED (mg/kg) calculation follows the method reported in the literature (FDA, 2005), considering the rat as the animal model and using rats with a weight of 200 g, falling within the standard weight range specified for the method (90–400 g) (Stein et al., 1974). The equation is as follows:

$$\text{HED (mg/kg)} = \text{rat dose (mg/kg)} \times 0.16 = 0.08 \text{ (mg/kg)}.$$

It is worth mentioning that the oral recommended dose of colchicine in humans is (0.015–0.03 mg/kg) (Finkelstein et al., 2010), and the toxic oral dose in humans is >0.1 mg/kg (Niel & Scherrmann, 2006). Also, it is important to note that the effectiveness of colchicine at the dose specified in the rat model has been questioned in other reports, as mentioned earlier in section (3.3). Likewise, the animal dose was administered intraperitoneally, whereas the recommended and toxic doses specified for humans are for oral tablets. Thus, an effective dose range for various routes of administration of colchicine and other blockers, complemented by additional toxicity studies would be necessary to broaden the applicability of the findings to humans.

3.2. Other pathways for intestinal lymphatic uptake

As previously demonstrated, in addition to drugs moving between the enterocytes (paracellularly) to get into the intestinal vasculature, they can gain entry into the intestinal lymphatics through two distinct routes: chylomicrons and M cells. While these pathways initially operate independently, they eventually converge into the mesenteric lymph for further transportation (Fu et al., 2013). Although the inhibitory models discussed earlier primarily target the chylomicron pathway, some drugs can be found in the mesenteric lymph as a result of their uptake through the M cells. Therefore, it is essential to clarify the role of the M cells pathway as well.

M cells play a critical part in transporting antigens and microorganisms to the underlying mucosal lymphoid tissues (Peyers' patches) for antigen presentation (Gebert et al., 1996; Kobayashi et al., 2019). They may also transport microparticles or particulates from the intestinal lumen, releasing them at the basolateral side for transport through the mesenteric lymph and, ultimately, the systemic circulation (Zhang et al., 2021). As M cells recognize pathogens by specific ligands on their surfaces, some particles including drugs and vaccines can be engineered to mimic specific ligands, enhancing their uptake through this pathway (Brayden et al., 2005; Patel, Mundada, & Sawant, 2019; Patel, Shah,

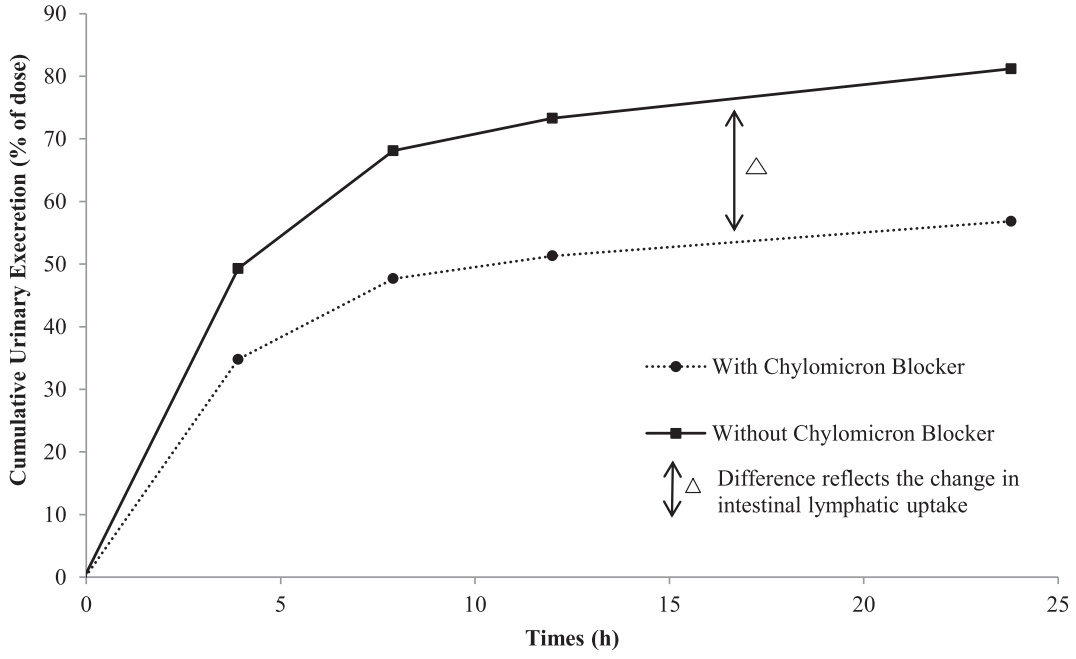

Fig. 6. Hypothetical graph of drug urinary excretion with and without chylomicron blocker for a drug that can be absorbed through intestinal lymphatics.

et al., 2019; Wu et al., 2024).

Factors influencing drug transport *via* the M cell pathway are still under investigation. Though, studies have explored the impact of particle size, hydrophobicity, surface charge, and shape on transport through this pathway. Particles measuring 600 and 2000 nm have been reported to undergo transport *via* the M cell pathway (He et al., 2018). Increasing hydrophobicity has been found to enhance uptake through M cells as studied with rifampicin nanoparticles coated with hydrophobic polymers (Bachhav et al., 2018). Studies on the effect of surface charges on the M cell pathway have different conclusions. In some studies, it has been noted that positively charged particles are more easily taken up by M cells than negatively charged ones, due to electrostatic affinity with intestinal mucus or cell membranes (Shi et al., 2016). These findings have been documented in a few reports including one with an oral DNA vaccine targeting Peyers' patches (Channarong et al., 2010). Other studies have shown a higher uptake through the M cells for the neutral and the positively charged particles compared to the negatively charged counterparts (Ensign et al., 2012; Schleh et al., 2011). Also, compared to spherical-shaped particles, rod- and disc-shaped particles achieved a higher extent of lymphatic transport through this pathway (Rivera-Gil et al., 2012; Li, Hu, et al., 2017, Li, Zhuang, et al., 2017).

3.3. Urinary excretion to quantify the lymphatic uptake

Pharmacokinetically, plots of cumulative urinary excretion over time are often used to analyze the excretion of a drug through urine. This method involves collecting urine samples over a period of time and plotting the cumulative amount of the drug excreted *versus* the time of collection. These plots help in understanding the rate and extent of drug excretion from the body (Taft, 2009). They are particularly useful in calculating urinary pharmacokinetic parameters such as the total amount excreted ($A_{e\infty}$) which can be used to determine the bioavailability of drugs (dos Serra et al., 2015; Thompson & Toothaker, 2004).

The rate at which the body eliminates drugs through urine correlates directly with the concentration of drugs in the bloodstream. This relationship enables the determination of bioavailability by comparing the quantity of unchanged drug excreted in urine following the administration of test and reference formulations (dos Serra et al., 2015). This method is based on the principle that the parameters obtained from the urinary excretion data reflect the absorption of the drug. However, it is important to note that this approach applies only to drugs that are excreted unchanged in the urine, and the relationship between serum concentration and renal clearance should be established before using urinary excretion data to assess bioavailability (Otoom et al., 2004; Cawello et al., 2012). Therefore, urinary excretion data can be a valuable tool in assessing the bioavailability of certain drugs, particularly when coupled with plasma level-time data (Cawello et al., 2012). To our knowledge, the use of urinary excretion of drugs has not yet been studied with chylomicron blockers but could be a useful non-invasive alternative approach to study drugs that are absorbed lymphatically and undergo renal excretion (Fig. 6).

The equation for bioavailability (F) when there are two different input pathways (p1 and p2) for a drug into the body, and the numerator is made up of two amounts, one from each pathway, can be expressed as:

$$F = \frac{(\textit{Amount of the drug excreted into the urine via } p1 + \textit{Amount of the drug excreted into the urine via } p2)}{\text{Dose of the drug administered.}}$$

This equation considers the total amount of the drug excreted into the urine *via* each pathway and relates it to the dose of the drug administered. It allows for the estimation of drug bioavailability when there are multiple input pathways contributing.

4. Conclusions

Numerous xenobiotics have been employed to obstruct the formation and/or secretion of chylomicrons, with the aim of hindering the uptake of associated drugs through the intestinal lymphatic pathway. Since this pathway constitutes a distinct route into the general circulation besides the portal pathway, here we reported a comprehensive pharmacokinetic equation for bioavailability that accounts for this pathway. The same principle when applicable, urinary data can be utilized as a non-invasive alternative to provide insights into the portion of the drug taken up through intestinal lymphatics.

Research in this field dates back to the 1940s, yet essential data for various chylomicron blockers are still lacking. Reported compounds in the literature include PL-81, puromycin, vinca alkaloids, colchicine, and cycloheximide. Among these, PL-81 has stood out as a compound with a reversible effect and minimal toxicity and can be used *in-vitro*, and *in-vivo* in animals. Additionally, it is unique as it does not function as a protein synthesis inhibitor; instead, it exerts its action by destabilizing the surface of forming chylomicrons. It also disrupts the transport of triglycerides within enterocytes and alters the conformation of chylomicron apo-lipoproteins. PL-81 was tested *in-vitro* using a cellular Caco-2 model and a non-cellular model with artificial chylomicrons. However, no reports were found on its clinical blockage of the chylomicron pathway, attributed probably to the low concentrations used in the formulations, which are likely well below the EC_{50} to induce this effect.

Puromycin and vinca alkaloids (vincristine and vinblastine) have been sporadically reported in the literature, focusing on their toxicity and effects on lipid absorption through chylomicrons. However, documentation on their application in drug intestinal lymphatic uptake is lacking. Moreover, beyond vincristine and vinblastine, other vinca alkaloids have been reported to affect the triglyceride profile and lower their concentrations. Consequently, these alternatives present promising prospects for further investigation as potential agents for blocking chylomicron pathways. Despite the clinical use of vinca alkaloids in many applications, especially as anticancers, their likelihood of being associated with chylomicron blockage in clinical practice is low.

Colchicine and cycloheximide have been the most commonly used chylomicron blockers in the pharmaceutical development of lymphotropic drugs and formulations. The latter is preferred due to its lower toxicity compared to colchicine in animal models, even at concentrations inducing chylomicron blockage. By relying on a reported effective colchicine dose in rats to block chylomicrons and employing allometric scaling, it was found that recommended doses used in humans might have a similar effect. However, further investigation into the effective and toxic doses of colchicine is required to understand its clinical impact on blocking the chylomicron pathway in humans.

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CRediT authorship contribution statement

Malaz Yousef: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Nadia Bou-Chacra:** Writing – review & editing, Validation, Supervision. **Raimar Löbenberg:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Neal M. Davies:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work the authors utilized Grammerly and ChatGP T3.5 in order to improve the writing quality. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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