Journal of Controlled Release 286 (2018) 10–19

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Lipophilic activated ester prodrug approach for drug delivery to the intestinal lymphatic system

Jong Bong Lee[a], Atheer Zgair[a,b], Jed Malec[a,c], Tae Hwan Kim[d], Min Gi Kim[e], Joseph Ali[a], Chaolong Qin[a], Wanshan Feng[a], Manting Chiang[f], Xizhe Gao[f], Gregory Voronin[g], Aimie E. Garces[a], Chun Long Lau[a], Ting-Hoi Chan[a], Amy Hume[a], Tecashanell M. McIntosh[a], Fadi Soukarieh[a], Mohammed Al-Hayali[a], Elena Cipolla[a,h], Hilary M. Collins[a], David M. Heery[a], Beom Soo Shin[e], Sun Dong Yoo[e], Leonid Kagan[f], Michael J. Stocks[a], Tracey D. Bradshaw[a], Peter M. Fischer[a], Pavel Gershkovich[a,*]

[a] School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK
[b] College of Pharmacy, University of Anbar, Anbar 31001, Iraq
[c] DMPK, Evotec, Milton Park, Abingdon, Oxfordshire OX14 4RZ, UK
[d] College of Pharmacy, Catholic University of Daegu, Gyeongsan 38430, Republic of Korea
[e] School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea
[f] Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA
[g] Comparative Medicine Resources, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA
[h] School of Pharmacy, Universita di Roma Tor Vergata, Rome 00173, Italy

ARTICLE INFO

Keywords:
Lymphatic transport
Prodrugs
Bexarotene
Retinoic acid
Chylomicron
Activated esters

ABSTRACT

The intestinal lymphatic system plays an important role in the pathophysiology of multiple diseases including lymphomas, cancer metastasis, autoimmune diseases, and human immunodeficiency virus (HIV) infection. It is thus an important compartment for delivery of drugs in order to treat diseases associated with the lymphatic system. Lipophilic prodrug approaches have been used in the past to take advantage of the intestinal lymphatic transport processes to deliver drugs to the intestinal lymphatics. Most of the approaches previously adopted were based on very bulky prodrug moieties such as those mimicking triglycerides (TG). We now report a study in which a lipophilic prodrug approach was used to efficiently deliver bexarotene (BEX) and retinoic acid (RA) to the intestinal lymphatic system using activated ester prodrugs. A range of carboxylic ester prodrugs of BEX were designed and synthesised and all of the esters showed improved association with chylomicrons, which indicated an improved potential for delivery to the intestinal lymphatic system. The conversion rate of the prodrugs to BEX was the main determinant in delivery of BEX to the intestinal lymphatics, and activated ester prodrugs were prepared to enhance the conversion rate. As a result, an 4-(hydroxymethyl)-1,3-dioxol-2-one ester prodrug of BEX was able to increase the exposure of the mesenteric lymph nodes (MLNs) to BEX 17-fold compared to when BEX itself was administered. The activated ester prodrug approach was also applied to another drug, RA, where the exposure of the MLNs was increased 2.4-fold through the application of a similar cyclic activated prodrug. Synergism between BEX and RA was also demonstrated *in vitro* by cell growth inhibition assays using lymphoma cell lines. In conclusion, the activated ester prodrug approach results in efficient delivery of drugs to the intestinal lymphatic system, which could benefit patients affected by a large number of pathological conditions.

1. Introduction

The intestinal lymphatic system is an important organ of the immune system as it accommodates more than half of the body's lymphocytes [1, 2]. In addition, the intestinal lymphatic system plays an important role in the pathophysiology of multiple diseases including lymphomas, metastasis of some solid tumours, and human immunodeficiency virus (HIV) infection. Therefore, efficient delivery of drugs to the intestinal lymphatic system has potential to improve treatment of diseases such as autoimmune disorders, lymphatic system-

* Corresponding author at: School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK.
E-mail address: pavel.gershkovich@nottingham.ac.uk (P. Gershkovich).

https://doi.org/10.1016/j.jconrel.2018.07.022
Received 22 February 2018; Received in revised form 7 July 2018; Accepted 13 July 2018

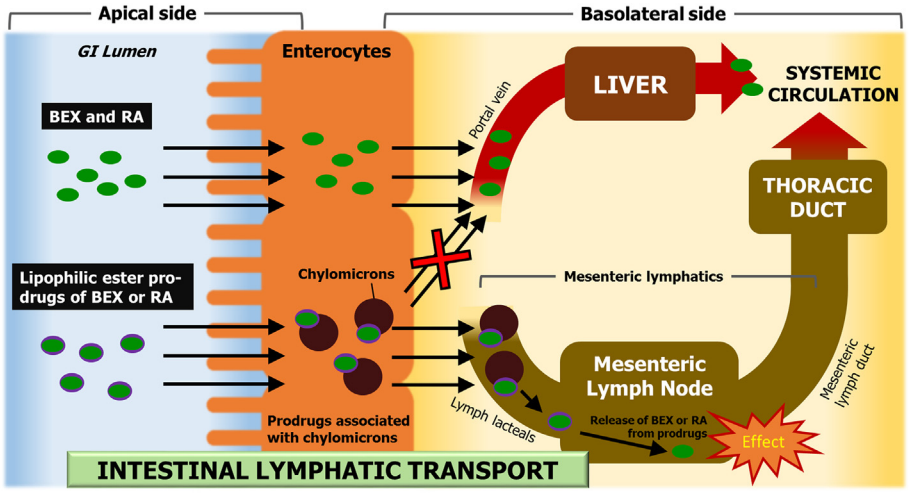

Fig. 1. Schematic diagram of the intestinal lymphatic transport pathway. Highly lipophilic drugs and prodrugs with appropriate physicochemical properties are able to associate with the chylomicrons (CM) in the enterocyte. The drug-CM complex is too large to penetrate the blood capillaries and therefore is passed on to the intestinal lymphatic system before reaching the systemic circulation. GI, gastrointestinal; BEX, bexarotene; RA, retinoic acid.

associated cancers, HIV infections and cancer metastasis [3–6]. However, only a very limited proportion of a drug can usually be distributed from the systemic circulation into the lymphatic system [4, 7]. As a result, in order to achieve sufficient concentrations of the drugs in the affected lymph nodes, the required levels in systemic circulation would be very high and associated with significant adverse side effects. Therefore, there is an unmet need for specific delivery of therapeutic agents to the intestinal lymphatics for treatment of patients affected by diseases associated with the intestinal lymphatic system.

Although most drugs absorbed from the gastrointestinal (GI) system are passed to the portal vein, lipophilic compounds may also gain access to systemic circulation through the intestinal lymphatics [8, 9] (Fig. 1). This distribution to the lymphatic system is determined mainly by the association of drugs with large lipoproteins, *i.e.* chylomicrons (CM), in the enterocytes. This is because drug molecules need to be associated with CM in order to utilise them as a carrier to the lymphatic system. In fact, a linear correlation between drug association with CM and lymphatic absorption is well established [10].

Intestinal lymphatic transport has been studied previously as an absorption pathway, with a primary focus on the fact that this absorption pathway can bypass the liver at the first pass following enteric drug administration, hence decreasing first-pass metabolism and thereby increasing the systemic bioavailability of drugs [11–14]. However, an issue that has been overlooked in the past is that the lymphatic system has functional importance, and drugs that are transported by this route can reach very high concentrations in lymph fluid and lymph nodes, and can therefore exert their pharmacological activity within the lymphatic system itself [12]. It is known that if a drug has the necessary physicochemical properties, an appropriate lipid-based formulation can facilitate the transport of the drug *via* the intestinal lymphatic system following oral administration [5, 15]. On the other hand, lipophilic prodrug approaches have been employed in the past to take advantage of the intestinal lymphatic transport of drug molecules that otherwise would not have the necessary physicochemical properties required for association with CM. However, previously suggested approaches were mainly based on glyceride mimetic or very bulky prodrug moieties [8, 14, 16–18]. It was believed that an alkyl ester prodrug approach would not be suitable for this purpose because of the instability of alkyl esters during the absorption phase [8].

We now report a study where a lipophilic prodrug approach was used to deliver bexarotene (BEX) and retinoic acid (RA) efficiently to the intestinal lymphatic system by a novel activated ester prodrug approach. Drugs with a wide range of indications and mechanisms of action could potentially benefit from delivery to the intestinal lymphatic system by the proposed approach. Here we focused on delivery of BEX and RA to potentially improve the treatment outcomes of non-Hodgkin's lymphoma (NHL) in patients with substantial mesenteric lymph node (MLN) involvement in the disease. Lymphoma is the most common cause of mesenteric lymphadenopathy [19]. In as many as 30–50% of NHL patients, especially those with diffuse large B-cell lymphoma (DLBCL), disease significantly affects MLNs [6]. This lymphadenopathy is persistent even in the disease remission stage, rendering patients susceptible to relapse [19]. In this study, 25 prodrugs of BEX were synthesised and assessed. The activated ester prodrug was shown as the most promising approach for efficient delivery to the intestinal lymphatic system. This successful approach was further applied to RA. In addition, our results show that the two drugs exert synergy in treatment of DLBCL *in vitro* at concentrations which are realistically achievable in the lymph nodes, when the suggested delivery approach is applied *in vivo*.

2. Materials and methods

2.1. Materials

BEX was purchased from LC Laboratories (Woburn, MA, USA). RA (all-*trans*), Intralipid®, esterase from porcine liver, sodium taurocholate (NaTc), NaCl, NaF and lecithin were obtained from Sigma (Gillingham, UK). Rat plasma was purchased from Sera Laboratories (West Sussex, UK). Thiazolyl blue tetrazolium bromide (MTT), sesame oil, polyethylene glycol 400 (PEG400) and all solvents (HPLC grade or higher) were purchased from Fisher Scientific (Loughborough, UK).

2.2. Chemical synthesis

2.2.1. General synthetic scheme for esterification

Ester prodrugs of BEX and RA were synthesised by adding 2.87 mmol of the desired alcohol to 0.287 mmol of BEX or RA. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (0.431 mmol) and 4-dimethylaminopyridine (0.057 mmol) were added, and dichloromethane (15 mL) was used as the solvent. The mixture was stirred magnetically overnight at room temperature. Purification was performed by flash chromatography using a 200–400 mesh silica gel-packed glass columns and hexane–ethyl acetate 50:2 (*v*/v) as the mobile phase. Specific details for compounds that were obtained by different synthetic schemes are described in Supplementary Material 1.

2.2.2. Characterisation of synthetic prodrugs

^{1}H NMR and ^{13}C NMR spectra were obtained using a Bruker 400 Ultrashield instrument at 400 and 100 MHz, respectively. Bruker TOPSPIN 2.1 software was used to analyse the spectra. Chemical shifts are reported as parts per million (ppm) relative to tetramethylsilane