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Research paper

Novel lipid-mimetic prodrugs delivering active compounds to adipose tissue

Andrea Mattarei [a, **, 1], Andrea Rossa [a, b, c], Veronica Bombardelli [a, b, c], Michele Azzolini [b, c], Martina La Spina [c], Cristina Paradisi [a], Mario Zoratti [b, c], Lucia Biasutto [b, c, *]

[a] University of Padova, Department of Chemical Sciences, Via F. Marzolo 1, 35131 Padova, Italy
[b] CNR Neuroscience Institute, Viale G. Colombo 3, 35121 Padova, Italy
[c] University of Padova, Department of Biomedical Sciences, Viale G. Colombo 3, 35121 Padova, Italy

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ABSTRACT

Obesity and associated pathologies are a dramatically growing problem. New therapies to prevent and/or cure them are strongly needed. Adipose tissue is a logical target for pharmacological intervention, since it is now recognized to exert an important endocrine function, secreting a variety of adipokines affecting, for example, adiposity and insulin resistance. This proof of principle work focuses on the development of novel lipid-mimetic prodrugs reaching fat deposits by the same lymphatic absorption route followed by dietary triglycerides.

Pterostilbene, a natural phenolic compound with potential anti-obesity effects, was used as model "cargo", attached via a carbamate group to an ω-aminodecanoate chain linked to either position 1 or position 2 of the glycerol moiety of synthetic triglycerides. The prodrugs underwent position-selective hydrolysis when challenged with pancreatic lipases *in vitro*. Pterostilbene-containing triglycerides as well as pterostilbene and its metabolites were present in the adipose tissue of mice fed an obesogenic diet containing one or the other of the derivatives.

For the first time this approach is used to deliver an obesity antagonist to the adipose tissue. The results demonstrate the feasibility of delivering active compounds to adipose tissue by reversibly incorporating them into triglyceride-mimetic structures. Upon release in the target site these compounds are expected to exert their pharmacological activity precisely where needed.

1. Introduction

Obesity has been firmly correlated with an increased hazard for a range of serious ailments [1,2]. The strongest link [3] may be that between the overweight/obese status and the metabolic syndrome, defined as "a compilation of risk factors that predispose individuals to the development of type 2 diabetes (T2D) and cardiovascular disease (CVD)" [4]. White adipose tissue (WAT) secretes adipokines (leptin, adiponectin and several others) which have major (patho) physiological effects and mediate interactions between WAT and other organs, including the immune system, liver, muscle and the central nervous system [5]. For example, adiponectin levels decrease in adiposity, contributing to obesity-associated cancerogenesis, CVD and T2D.

Upward violation of adipose tissue homeostasis leads to the immigration of macrophages and to an increase of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, as well as of iNOS and ROS, which can act in paracrine/endocrine fashion leading to a chronic inflamed state linked to a host of pathologies [6]. Systemic oxidative stress [7] contributes heavily itself to the onset and progression of obesity-associated health problems [8]. Thus, developing effective tools against adiposity on one hand and its consequences on the other are strong priorities of modern pharmacology. The most successful treatment for obesity is an appropriate reduction of food intake, accompanied by exercise [9]. Exercise seems also to ameliorate the inflammatory status, independently from weight loss [10], and to induce "browning" of white

* Corresponding author. CNR Neuroscience Institute c/o Dept. of Biomedical Sciences, Viale G. Colombo 3, 35121 Padova, Italy.
** Corresponding author.
E-mail addresses: andrea.mattarei@unipd.it (A. Mattarei), lucia.biasutto@cnr.it (L. Biasutto).
[1] Present address: Department of Pharmaceutical & Pharmacological Sciences, University of Padova, Via F. Marzolo 5, 35131 Padova, Italy.

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fat [11,12]. Brown fat (BAT) [11,13,14] is a mitochondria-rich type of adipose tissue, abundant in early life, which provides - normally when prompted by the sympathetic nervous system - non-shivering thermogenesis, dissipating food-derived energy to produce heat in mitochondria "uncoupled" by the expression of Uncoupling Protein 1 (UCP1). It can thus counteract obesity. An important role in the complex regulation of BAT is attributed to AMPK, expressed both in the controlling CNS structures and in adipose tissue: reduction of AMPK activity in the hypothalamus or upregulation of it in WAT and BAT increase "browning" and energy dissipation [15]. AMPK signaling, activated by exercise, has to do with insulin secretion, glucose transport, mitochondrial biogenesis, fatty acid oxidation, inflammation. Development of anti-obesity drugs has registered some failures, due to important side effects, and has so far not had a significant impact on population-wide obesity [16]. A few drugs are currently available which have shown some modest efficacy and side effects ranging from constipation to tumorigenesis (in experimental animals) [17]. They are costly, some are classified as controlled substances because of the possibility of abuse, all are off-limits in pregnancy and lactation. The possibility of BAT recruitment by pharmacological intervention is receiving much attention [13,18]. Organ-specific upregulation of AMPK activity may provide a strategy (see above). Indeed metformin, an AMPK activator and a major anti-diabetic drug, also induces weight loss [19].

Natural compounds may arguably be considered the best currently available weapons in the anti-obesity, anti-metabolic disorder arsenal [20–22]. For example berberine, genistein, flavonoids, catechins and resveratrol indirectly activate AMPK [23]. Resveratrol [24–26] is just the most talked-about member of the family of bioactive stilbene polyphenols, whose mechanisms of action are thought to overlap to a considerable degree [27]. Upregulation of UCP1 by resveratrol may account for the energy dissipation that must take place for a "slimming" effect [28]. Its efficacy is limited however by its prompt and extensive modification by Phase II metabolism enzymes, for which it represents a ready-made target [29]. Pterostilbene, i.e. 3,5-di-O-methylresveratrol [30–32], having only one free hydroxyl, is less prone to metabolic conjugation, has a relatively high bioavailability [33,34] and a potentially higher efficacy than resveratrol [35,36]. Of relevance, the prodrug approach to increasing absorption and reducing metabolic conjugation and elimination offers more promise with pterostilbene than with resveratrol, due to the need to protect only one hydroxyl instead of three [37].

The ability of pterostilbene to antagonize the metabolic syndrome has been reported by several studies [38–40]. Like resveratrol, it can activate the Keap/Nrf2/ARE anti-oxidant pathway, upregulate SIRT1, repress NF-kB activity, activate AMPK. It has been reported to upregulate adiponectin in an *in vitro* 3T3-L1 adipocyte cell model [41] and to downregulate instead the secretion of pro-inflammatory cytokines upon interaction of these cells with RAW 264.7 macrophages [42].

It is clear that a strategy for the selective pharmacological targeting of adipose tissue would represent a powerful tool in the fight against obesity and obesity-related problems. As discussed, the issue is not just that of reducing fat deposits, but also that of relieving chronic inflammation where needed.

Based on these premises, this work is focused on the development of a tool to selectively convey a representative active phenolic compound (APC), pterostilbene, to adipose tissue by imitating intestinal lipid absorption [43]. In the intestinal lumen dietary triglycerides are degraded by pancreatic lipases (PL), which hydrolyze the ester bonds selectively at position 1 in the glycerol backbone. Free fatty acids and monoglycerides pass then into enterocytes, where triglycerides are resynthesized and packed into chylomicrons. These are preferentially taken up into the lacteals of the lymphatic system rather than into the portal vein - thus avoiding first-pass metabolism - and eventually move to the thoracic duct and into the blood. Plasma triglyceride levels are regulated by the lipoprotein lipase (LPL) system [44], which hydrolyzes triglycerides to fatty acids for concomitant use or storage by the underlying tissue.

As a proof of principle study, we have thus undertaken the synthesis of lipid-mimetic prodrugs of pterostilbene to exploit the efficient and selective distribution of triglycerides, through the lymphatic system, to adipose tissue (Fig. 1).

In our approach the APC is incorporated into a long-chain triglyceride. Two classes of isomeric derivatives can be synthesized and tested, characterized by connection of the APC either to position 1 (Type-1) or 2 (Type-2) of the glycerol backbone (Fig. 2).

A similar strategy has been adopted in a few previous studies [45–48]. To our knowledge, however, in no case the construct was meant to target the adipose tissue.

The prodrugs we synthesized underwent position-selective hydrolysis when challenged with Pancreatic Lipase *in vitro*. Pterostilbene-containing triglycerides as well as pterostilbene and its metabolites were present in the adipose tissue of mice fed an obesogenic diet containing one or the other of the derivatives. These results provide evidence that this approach can succeed in delivering a cargo to the adipose tissue after oral administration.

2. Results and discussion

2.1. Synthesis of lipid-mimetic prodrugs of pterostilbene

Pterostilbene was reversibly linked to the triglyceride structure through a carbamate bond. The choice was based on previous results, which showed that this group is resistant to hydrolysis by digestive enzymes in the stomach and intestine, but releases the active compound at convenient rates after passing into the blood and tissues [49,50]. The length of the linker chain (*n* in Fig. 2) has been chosen arbitrarily as nine methylene units but can be modulated to optimize the performance of the derivatives. Details of the syntheses are provided in Materials and Methods.

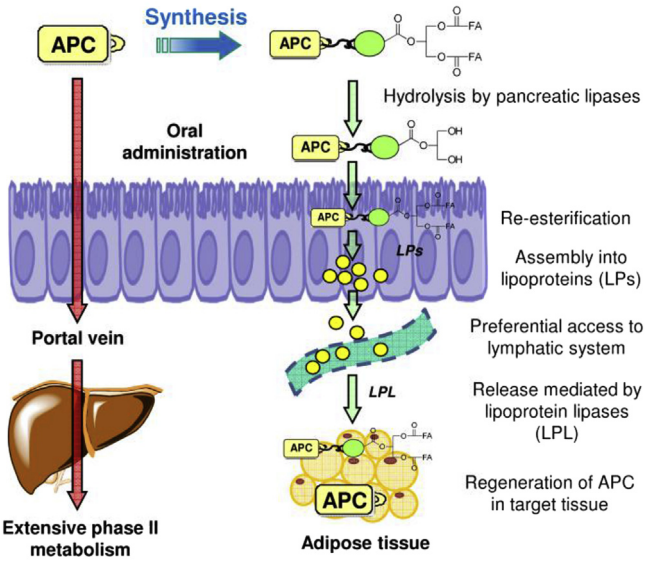

Fig. 1. Absorption mechanisms expected for an active phenolic compound (APC) and for its triglyceride-mimetic prodrug. Only the Type-2 derivative is depicted.