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The oral absorption of phospholipid prodrugs: *In vivo* and *in vitro* mechanistic investigation of trafficking of a lecithin-valproic acid conjugate following oral administration

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Abstract

The purpose of this study was to evaluate the oral absorption characteristics of a phospholipid–drug conjugate, comprising direct conjugation between the lecithin and the drug moiety through the *sn*-2 position. We investigated the mechanisms involved with the trafficking of this conjugate following oral administration in the gastrointestinal (GI) lumen, within the enterocyte and further. A phospholipid-valproic acid conjugate (DP-VPA) was utilized as a model molecule. The oral absorption of this conjugate in rats was investigated following administration in long (LCT) vs. medium (MCT) chain triglyceride formulations, and in the postprandial vs. fasted state. Oral administration within the LCT solution caused more than a 3-fold increase in DP-VPA bioavailability in comparison to the MCT solution. Moreover, a significant food effect was evident for DP-VPA. Hence, we evaluated the lymphatic transport of DP-VPA in mesenteric lymph duct cannulated freely moving rats. Sixty percent of the absorbed DP-VPA was associated with lymphatic transport. Similar DP-VPA absorption was obtained in secretory type II PLA_2 knockout mice (C57BL/6) and in control mice (BALB/c). Moreover, nil DP-VPA degradation in serum and very low (4.8%) degradation by bee venom PLA_2 *in vitro* were obtained. In conclusion, direct conjugation between the drug and the phospholipid produces a complex having unique absorption properties that include: (1) a stable complex that does not undergo degradation in the GI tract; (2) permeation through the gut wall and entering intact to the enterocyte; and (3) association with chylomicron in the enterocyte and reaching the systemic circulation via the lymphatic route. These unique properties may be of interest in drug delivery.

Keywords: Phospholipid–drug conjugate; Oral prodrug; Drug delivery; Valproic acid; Lymphatic transport; Food effect; Pharmacokinetics; Phospholipase A_2

1. Introduction

The prodrug approach has been proven to be an effective way of overcoming various ADME barriers that restrict the application of many chemical entities as orally administered drugs [1,2]. Lipidic prodrugs are chemical entities comprising a drug covalently bound to the lipid moiety. Three main different lipid carriers have been introduced: fatty acids, triglycerides and phospholipids. While the use of triglycerides and fatty acids as lipidic carriers has been extensively investigated and applied for various drugs [3–5], the use of phospholipids as the lipidic vector in the prodrug approach has been investigated to a lesser degree [6]. Few works examined this approach when the drug is bound to the phosphate group [7–12]; whereas a phospholipid bearing the drug instead of a fatty acid is a novel approach of drug delivery system [13].

We have recently investigated the oral absorption of phospholipid–drug conjugates with the drug (indomethacin) attached to

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the *sn*-2 position of the phospholipid through a carbonic linker [14–17]. We have shown that their absorption characteristics are highly dependent on the length of the linker between the phospholipid and the drug moiety. Oral administration of conjugate with a 5-carbon length linker enabled recognition of the conjugate by phospholipase A_2 (PLA_2) enzymes in the gut lumen, and consequent liberation and absorption of the free drug to the systemic circulation. On the other hand, oral administration of a conjugate with a shorter linker (2-carbon) was not degraded, and no absorption, neither of the whole conjugate nor of the free drug, was obtained. The *in vitro* degradation of these phospholipid–drug conjugates by PLA_2 was in correlation with the *in vivo* results [14]. The high dependency of the performance of these lecithin–drug conjugates on the length of the linker between the phospholipid and the drug moiety has aroused an interesting question regarding the possibility of direct conjugation between the phospholipid and the drug, with no linker at all.

Phosphatidylcholine is an insoluble swelling amphiphile which is not absorbed intact, and undergoes hydrolysis by PLA_2 in the *sn*-2 position [18]. The hydrolytic product, lysophosphatidylcholine, is absorbed from the gastrointestinal (GI) lumen into the enterocyte, followed by resynthesis to lecithin and entrance to the mesenteric lymphatic system in association with the lipoprotein chylomicron [18,19]. A complex comprising direct conjugation between the phospholipid and the drug moiety may behave in different ways: since such a conjugate resembles lysophosphatidylcholine in terms of structure, size and configuration, it may be absorbed intact into the enterocyte. Alternatively, since unlike lysophosphatidylcholine, the *sn*-2 position is occupied by a drug moiety, this conjugate may undergo degradation by PLA_2 in the GI tract (similarly to the 5-carbon linker conjugate), or neither degradation nor absorption of this conjugate may occur (similarly to the 2-carbon linker conjugate).

DP-VPA is a phosphatidylcholine esteric conjugate of valproic acid, comprising the drug attached directly to the *sn*-2 position of the lecithin, with no linker at all between the phospholipid and the drug moiety (Fig. 1). DP-VPA has a broad antiepileptic potency that was evident in several experimental models of epilepsy [20–22]. In addition, reduced circulating plasma levels of free valproic acid and a concurrent reduction in side effects was observed following DP-VPA administration [23,24]. However, its absorption following oral administration has not yet been fully elucidated. A recent proof-of-concept study on 2 greyhounds has indicated that significant proportion of a DP-VPA oral dose gain access to the systemic circulation via the intestinal lymphatics [25], however, a more thorough investigation may be of benefit in order to illustrate the oral absorption characteristics of this conjugate.

The purpose of the present study was to evaluate the oral absorption characteristics of a phospholipid–drug prodrug, comprising direct conjugation between the lecithin and a drug moiety through the *sn*-2 position of the phospholipid. DP-VPA was utilized in this research as a model complex with no linker between the lecithin and the drug. In particular, we investigated the mechanisms involved in the trafficking of this conjugate following oral administration, both in the lumen of the gastrointestinal tract (i.e. pre-enterocyte processes) and mechanisms occurring following the uptake into the enterocyte and further, including the effect of food, various formulations, transport routes and the stability of the conjugate to secretory type II PLA_2 *in-vitro* and *in-vivo* (in knock-out mice). This research clarifies the advantages and limitations of the oral absorption of such phospholipid–drug conjugates, and also elucidates the inclusive role of the linker length between the lecithin and the drug moiety.

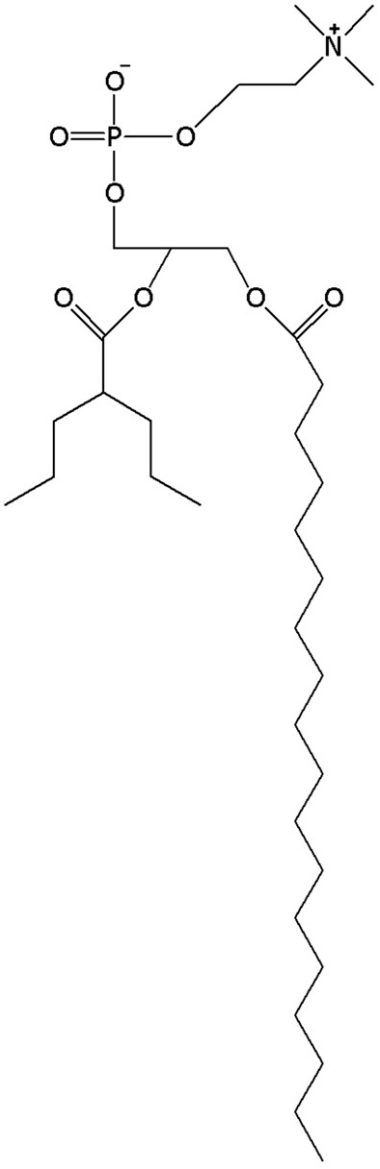

Fig. 1. Chemical structure of DP-VPA. The drug moiety (valproic acid) is directly conjugated to the *sn*-2 position of the phospholipid, with no linker at all.

2. Materials and methods

2.1. Materials

DP-VPA was supplied by D-Pharm Ltd. (Rehovot, Israel). Peanut oil (long chain triglycerides, LCT), ammonium acetate, Tris–HCl, NaCl, $CaCl_2$ and propylene glycol were purchased from Sigma Chemical Co. (St. Louis, MO). Captex 355, triglycerides of