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A calorimetric evaluation of the interaction of amphiphilic prodrugs of idebenone with a biomembrane model

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Abstract

Lipoamino acids (LAA) are useful promoieties to modify physicochemical properties of drugs, namely lipophilicity and amphiphilicity. The resulting membrane-like character of drug–LAA conjugates can increase the absorption profile of drugs through cell membranes and biological barriers. To show the role of amphiphilicity with respect to lipophilicity in the interaction of drugs with biomembranes, in the present study we evaluated the mode of such an interaction of lipophilic conjugates of LAA with the antioxidant drug idebenone (IDE). DSC analysis and transfer kinetic studies were carried out using dimyristoylphosphatidylcholine (DMPC) multilamellar liposomes (MLVs) as a model. For comparison, two esters of IDE with alkanoic acids were synthesized and included in the analysis. The experimental results indicate that based on their different structure, IDE–LAA conjugates interacted at different levels with respect to pure IDE with DMPC bilayers. In particular, a progressive penetration inside the vesicles was observed upon incubation of IDE–LAA compounds with empty liposomes. The enhanced amphiphilicity of the drug due to the LAA moieties caused more complex interactions with DMPC bilayers, compared to those registered with the native drug or IDE alkanoate esters.

Keywords: Idebenone; Lipoamino acids; Lipophilicity; Amphiphilicity; DSC; Biomembrane model

1. Introduction

In previous studies, some ester prodrugs of idebenone (IDE) with short-chain 2-alkylamino acids were described. These prodrugs were characterized by their biological activity and stability profile [1,2].

IDE [2-(10-hydroxydecyl)-5,6-dimethoxy-3-methylbenzo-1,4-quinone] is a synthetic analogue of coenzyme Q_{10} (Fig. 1). IDE is a potent antioxidant agent and, thanks to its ability to inhibit lipid peroxidation, it protects cell and mitochondrial membranes from oxidative damage [3]. This drug has clinical applications in many central nervous system degenerative diseases associated with oxidative stress, such as Parkinson's and Alzheimer's diseases, as well as cerebral ischemia and brain aging [4,5], and especially in the therapy of Friedreich's ataxia [6].

The above-mentioned studies belong to a wider project aimed at evaluating the advantages of conjugating drugs to lipoamino acids (LAA). Conjugation of LAA to drug molecules can modify many physicochemical properties of the latter, namely lipophilicity and amphiphilicity. The resulting *membrane-like character* of these conjugates can ultimately affect the absorption of drugs through cell membranes and biological barriers, as well as increase their stability in the bloodstream [7,8].

LAA have been conjugated to many drugs, including antitumor and anti-inflammatory agents [9–12]. Shorter-chain LAA

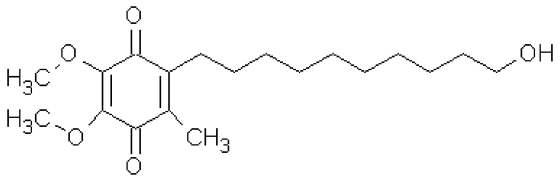

Fig. 1. Chemical structure of idebenone.

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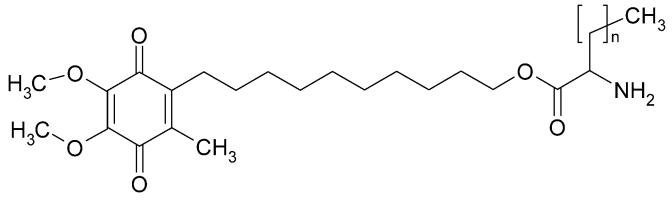
Table 1
Chemical structure and properties of IDE–LAA esters



Compound	n	MW	$c\log P$[a]	$c\log P$[b]	$c\log D_{7.4}$[c]	Water solubility[b] ($\log S$)[d]
IDE–LAA4	1	423.55	4.52	4.08	4.13	−3.79
IDE–LAA5	2	437.58	5.06	4.54	4.53	−4.06
IDE–LAA6	3	451.61	5.59	5.00	4.95	−4.33
IDE–LAA7	4	465.64	6.12	5.47	5.17	−4.60
IDE	–	*338.45*	*3.49*	*4.21*	–	*−3.16*

[a] ACD log *P* 5.15 software.
[b] Osiris Property Explorer.
[c] Pallas 3.1.1.2, CompuDrug Chemistry Ltd.
[d] Unit stripped logarithm (base 10) of the solubility measured in mol/l.

have more recently been used by us instead of the originally proposed long-chain ones [7]. Short- and medium-chain 2-alkylamino acids (C_4 to C_8) can, in fact, still exert the lipophilicity modifier properties of LAA without dramatically reducing the solubility of conjugates in biological and experimental media.

Studies on IDE were aimed at exploring the effects caused by LAA conjugation upon an already lipophilic drug (IDE has a log *P* around 3.5–4, Table 1). Most studies on LAA have, in fact, been focused on peptides and hydrophilic drugs [13–15]. However, by their nature LAA are able to enhance not only the lipophilic character of drugs, but also their amphiphilicity, a critical property associated with crossing through and interaction with biological membranes [16,17]. For instance, in a recent study we demonstrated that conjugation of a model drug with LAA allows the drug to interact in a complex mode and at different levels with a biomembrane model, consisting of pure phospholipid liposomes [18]. Compared to LAA conjugates, the corresponding drug derivatives with simple fatty (alkanoic) acids gave poorer interactions with such a model.

DSC analysis of the degree of interaction of xenobiotics, such as drugs, with such an anisotropic biphasic system has already been shown to correlate with their biological behavior [19–21]. The partitioning into and binding of a drug to cell membranes/barriers, as well as to models such as liposomes, follow complex mechanisms and are related to so-called "anisotropic lipophilicity" [17]. The latter results from the hydrophobicity of the drug, but also from its ability to make polar and ionic bonds with the membranes. In IDE–LAA conjugates the presence of a free ionizable amine group allows polar and ionic interactions to enhance the partition of these compounds within DMPC bilayers, causing deep changes in the thermotropic parameters of the pure phospholipid.

Therefore, in the present study we evaluated the mode of interaction of IDE lipophilic prodrugs, described in Table 1, with a biomembrane model. DSC analysis and transfer kinetic studies were performed using pure dimyristoylphosphatidylcholine (DMPC) multilamellar liposomes (MLVs) as a model. Two esters of IDE with alkanoic acids were synthesized and included in the study for comparison.

2. Materials and methods

2.1. Materials

IDE was kindly provided by Wyeth Lederle SpA (Catania, Italy). 1,2-Dimyristoyl-*sn*-glicero-phosphocholine (DMPC) (purity ⩾99.0% by TLC) is a Fluka product (No. 41803; Sigma-Aldrich Chimica Srl, Milan, Italy). IDE–LAA esters (Table 1) were synthesized as previously described [1]. Purity was checked by TLC on silica gel aluminum sheets ($F_{254+356}$, Merck); spots were detected either by UV light, ninhydrin, or acid–base reactant treatment. Reactants and solvents were all commercial products of at least analytical grade. HPLC-grade water was used throughout the study.

IR spectra were registered in nujol with an FT-IR Perkin–Elmer 1600 spectrophotometer. ^{1}H NMR spectra were obtained in DMSO-d_6 with a 200-MHz Brüker instrument; chemical shifts are reported in ppm with TMS as the internal standard. Mass spectra were recorded on a Perkin–Elmer Sciex API 3000 triple quadrupole mass spectrometer, checked using a Sample Control 1.4 software package in selected ion monitoring mode (SIM). Data were analyzed using Multiview and MacQuan 1.6 software packages (Perkin–Elmer Sciex, Toronto, Canada).

2.2. Synthesis of IDE esters with alkanoic acids (IDE–FA)

Butyric (C_4) or caproic acids (C_6) (0.15 mmol) were dissolved in 5 ml of dry dichloromethane and 1-hydroxybenzotriazole (0.3 mmol, 46 mg), triethylamine (0.375 mmol, 52 µl), and 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDAC) hydrochloride (0.45 mmol, 64 mg) were added to the solution. The mixture was stirred for 2 h in an iced-water bath and then a solution of IDE (0.15 mmol, 25.5 mg) in 5 ml of dry dichloromethane was added. The reaction mixture was magnetically stirred at room temperature for an additional 24 h. At the end of this period, the solvent was removed under vacuum and the residue dissolved in 30 ml of dichloromethane and extracted with a 5% aqueous sodium bicarbonate solution (30 ml) and then brine (2 × 30 ml). The organic phase was desiccated with anhydrous sodium sulphate and then filtered and evaporated to dryness in vacuo. The structure and properties of the obtained esters are reported in Table 2.

IDE–FA4: 10-(4,5-dimethoxy-2-methyl-3,6-dioxocyclohexa-1,4-dien-1-yl)decyl butyrate. IR (nujol; cm^{-1}): 1736 (ester C=O). MS (m/z): 409 [M + 1]. ^{1}H-NMR (ppm, δ): 4.09 (m, 2H, O–CH_2), 3.79 (s, 6H, CH_3), 2.50 (t, 2H, ∅–CH_2), 1.99 (t, 3H, CH_3), 1.66 (m, 2H, CH_3–*CH_2*), 1.33–1.25 (broad m, 16H, CH_2).

IDE–FA6: 10-(4,5-dimethoxy-2-methyl-3,6-dioxocyclohexa-1,4-diene-1-yl)decyl hexanoate. IR (nujol; cm^{-1}): 1709 (ester C=O). MS (m/z): 437 [M + 1]. ^{1}H NMR (ppm, δ): 4.12 (m, 2H, O–CH_2), 3.65 (s, 6H, CH_3), 2.51 (t, 2H, ∅–CH_2), 1.88 (t,