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Interaction of naproxen amphiphilic derivatives with biomembrane models evaluated by differential scanning calorimetry and Langmuir–Blodgett studies

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ABSTRACT

Anti-inflammatory drugs represent a potential new strategy for the treatment of Alzheimer's disease (AD). The ability to cross the blood–brain barrier and to reach brain tissues is a critical point for these drugs and is strictly related to their lipophilicity.

Naproxen (NAP) is a non-steroidal anti-inflammatory drug (NSAIDs) under active investigation for AD. To improve its lipophilic character, NAP was conjugated through a diethylamine spacer (EDA) to lipoamino acids (LAA), α-amino acids containing a long alkyl side chain, to obtain the NAP–EDA–LAA10 and NAP–EDA–LAA14 prodrugs.

The interaction of NAP and prodrugs with dimyristoylphosphatidylcholine phospholipids, forming either multilamellar vesicles or monolayers (at the air/water interface) and used as biomembrane models, was studied by differential scanning calorimetry and Langmuir–Blodgett techniques.

Experimental data showed that NAP conjugation with LAA residues was able to enhance the drug interaction with such biomembrane models.

1. Introduction

Alzheimer's disease (AD), one of the most frequent causes of dementia in elderly, is characterized by a slow, progressive decline in cognitive function and behavior. Inflammatory mediators have been detected in and around amyloid plaques in the brain of AD patients and, as a consequence, the inflammatory hypothesis and the possibility to treat this disease with anti-inflammatory drugs have been taken into account. Several studies have demonstrated that the use of non-steroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk of developing AD [1,2]. It has been shown that some NSAIDs decrease the production of β-amyloid protein (1–42) (Aβ42), the main component of senile plaques [3]. For this reason, these drugs are considered potential therapeutic agents for AD, including not selective inhibitors of cyclo-oxygenase (COX), such as indomethacin, ibuprofen, flurbiprofen, and naproxen, as well as COX-2 selective agents like rofecoxib [4].

Naproxen [(S)-(+)-6-methoxy-α-methyl-2-naphthalene acetic acid] (NAP; Fig. 1) possesses anti-inflammatory activity, moderate antipyretic, painkiller, and anti-platelet activities [5]. It plays an equipotent action on both COX isoforms, but its anti-inflammatory action also depends on the stabilization of lysosomal membranes and on the inhibition of chemotactic response of neutrophils [6,7].

Central nervous system is always difficult to reach by drugs administered systemically, especially at the concentrations occurring to obtain a pharmacological effect. The blood–brain barrier (BBB) strongly obstacles drugs to go inside the brain tissues [8]. However, the lipophilic part of blood vessel endothelia in BBB offers a large surface area for the passive transport of drugs. There is a general good correlation between the BBB penetration *in vivo* and lipophilicity of drugs [9].

To increase the lipophilic nature and amphiphilicity of NAP and, then, to overcome more easily the effect of BBB, the conjugation of this drug with lipoamino acids (LAA) has been used. LAA are α-amino acids with an alkyl side chain of varying nature and length [10]. They possess some structural features of lipids as well as of peptides; in this manner, they maintain a polar character but possess also a certain degree of lipophilicity [10]. LAA conjugation to different drugs has shown to impart to the drug more membrane affinity, allowing to enhance the interaction with cell membranes and/or crossing biological barriers [11,12]. We conjugated NAP with different LAAs through the spacer ethylenediamine (EDA) and used differential scanning calorimetry (DSC), Langmuir–Blodgett (LB) technique, and biomembrane models represented by multilamellar vesicles (MLV) and monolayers made of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) to investigate whether the LAA moiety can affect the interaction of the drug with the biomembranes. To this aim, two NAP–EDA–LAA prodrugs, showing a different alkyl side chain in the LAA moiety, were tested. MLV exhibit, under heating, a characteristic transition from the gel (L_β) phase

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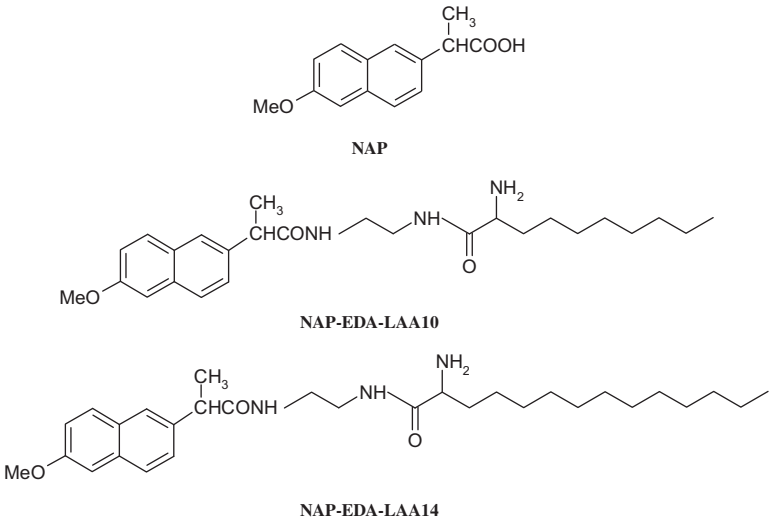

Fig. 1. Structures of naproxen and its LAA prodrugs.

to the liquid crystal (L_α) phase [13–16] that can be revealed by DSC by measuring the associated thermodynamic parameters (transition temperature, T_m, and enthalpy changes, ΔH). Amphiphilic compounds, interacting with the phospholipid bilayers, may cause modification of the lipid chain packing, resulting in a variation of the transition thermodynamic parameters [11,16–24]. This behavior, which can be analyzed by the Van't Hoff model of the freezing point depression of solutions, has been verified for several classes of chemical compounds, such as anesthetics [24], and applied on theoretical basis by some researchers [24–26]; the deviations from the model due to the complex structure as well as to the size of the compounds have been also taken into account [15,27].

Langmuir–Blodgett (LB) is commonly applied to study the interaction between drugs and phospholipid monolayers that represent a good model for biomembrane. In monolayers, two thermodynamic variables, temperature and pressure, can be easily controlled [28–30]. Analysis of LB results may provide important information on the disposition and organization of biological compounds in lipid membranes.

The film-balance method permits to obtain phase diagrams of phospholipids which, generally, are in the form of surface pressure/mean molecular area (π/Å^2) isotherm curves.

The results can give indications on the ability of NAP and its derivatives to dissolve in the biomembrane models and be uptaken by the membrane. In addition, information on the use of lipophilic system as carrier for the prodrugs can be obtained.

2. Materials and methods

2.1. Materials

Naproxen [(S)-(+)-6-methoxy-a-methyl-2-naphthalene acetic acid], 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDAC), and 1-hydroxybenzotriazole (HOBt) (Aldrich), triethylamine (TEA), N-Boc-ethylendiamine [*tert*-butil *N*-(2-amino-ethyl)carbamate], dry dimethylformamide (DMF), dry dichloromethane (DCM) (Fluka) and all other reactants and solvents were analytical grade or higher and were purchased from Sigma–Aldrich Chimica S.r.l. (Milan, Italy). The N-Boc-protected LAA were synthesized as described in the literature [31]. Synthetic DMPC was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland) and was pure as assessed by two-dimensional thin-layer chromatography [32].

IR spectra were registered in Nujol using a Perkin–Elmer 1600 FT-IR spectrophotometer. ^{1}H NMR spectra were obtained in CD_3OD on a Unity Inova Varian instrument operating at 200 MHz; chemical shifts are reported in ppm using TMS as internal standard. Mass analysis was performed with a triple quadrupole spectrometer (IPE sciex API 3000) operating in SIM mode with a positive ions electrospray. Analytical TLC was performed on silica gel aluminum plates (Merck $F_{254+356}$); spots were detected by UV light or treatment with ninhydrin.

2.2. Synthesis of NAP–EDA intermediate (Scheme 1)

NAP (5 mmoles, 1150 mg) was added with HOBt (5 mmoles, 675.6 mg), TEA (8 mmoles, 1115 ml), and EDAC (8 mmoles, 1533 mg) in 10 ml dry DCM. After stirring for 2 h at 0 °C, a solution of Boc-EDA (5 mmoles, 791 μl) in 5 ml of dry DMF was added, and the reaction was stirred at room temperature for 24 h. At the end, solvents were removed off under high vacuum, and the residue was redissolved in 30 ml of DCM and washed with subsequent 30 ml-aliquots of water, 5% sodium bicarbonate water solution, 5% acetic acid, and finally brine. The organic phase was dried for 6 h with anhydrous sodium sulfate and, after evaporation, the residue was purified by semi-prep TLC (0.5 mm 230–400 mesh silica gel plates; Macherey–Nagel GmbH & Co. KG, Düren, Germany), using a 9:1 by volume mixture of DCM and methanol. The Boc protecting group was removed by treatment with 20% TFA in dry DCM (30 min at room temperature). The final compound was purified by successive dissolution in DCM and evaporation under high vacuum, and finally stored at −20 °C until use.

N-(2-aminoethyl)-2-(6-methoxynaphthalen-2-yl)propanamide: MW: 272.34 ($C_{16}H_{20}N_2O_2$); IR (cm^{-1}): 3375, 1678, 1636, 1603, 1541, 1489, 1214, 1165, 1122, 1029, 846; ^{1}H NMR (ppm): 8.09 (br, 1H, NH), 7.88 (br m, 2H, aromatic), 7.41 (d, 2H, aromatic), 7.21–7.17 (t, 2H, aromatic), 3.80 (s, 3H, OCH_3), 3.50 (s, 1H, CH–CH_3), 3.46 (m, 2H, NH–CH_2), 2.68 (m, 2H, CH_2–NH_2), 1.49 (s, 3H, CH–CH_3); EI-MS: 273 $[M+H]^+$ (94%), 200 (100%).

2.3. Synthesis of NAP–EDA–LAA conjugates (Scheme 1)

The chosen N-Boc-LAA (0.29 mmoles) was dissolved in 2 ml of dry DCM and 5 ml of dry DMF and added with EDAC (0.35 mmoles,