Biochimica et Biophysica Acta 1808 (2011) 2995–3006

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Thermal, dynamic and structural properties of drug AT_1 antagonist olmesartan in lipid bilayers

Dimitrios Ntountaniotis [a,b], Gregor Mali [c,e], Simona Golic Grdadolnik [d,e], Halabalaki Maria [f], Alexios-Leandros Skaltsounis [f], Constantinos Potamitis [b], Eleni Siapi [b], Petros Chatzigeorgiou [g], Michael Rappolt [h,*], Thomas Mavromoustakos [i,**]

[a] Department of Chemistry, University of Patras, Patras 26500, Greece
[b] National Hellenic Research Foundation, Institute of Organic and Pharmaceutical Chemistry, 48 Vas. Constantinou, 11635, Athens, Greece
[c] Laboratory for Inorganic Chemistry and Technology, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia
[d] Laboratory of Biomolecular Structure, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia
[e] EN-FIST Centre of Excellence, Dunajska 156, SI-1000 Ljubljana, Slovenia
[f] University of Athens, Department of Pharmacy, Pharmacognosy Section, Zographou 15771, Athens, Greece
[g] University of Athens, Department of Chemistry, Laboratory of Physical Chemistry, Zographou 15771, Athens, Greece
[h] Austrian Academy of Sciences, Institute of Biophysics and Nanosystems Research (IBN), c/o Sincrotrone Trieste, Strada Statale 14, km 163.5, 34149 Basovizza, Italy
[i] University of Athens, Department of Chemistry, Laboratory of Organic Chemistry, Zographou 15771, Athens, Greece

ARTICLE INFO

Article history:
Received 26 May 2011
Received in revised form 14 July 2011
Accepted 1 August 2011
Available online 6 August 2011

Keywords:
Olmesartan
Dipalmitoyl-phosphatidylcholine bilayer
Small-angle and wide angle X-ray scattering
Raman spectroscopy
Differential scanning calorimetry
Solid state NMR spectroscopy

ABSTRACT

It is proposed that AT_1 antagonists (ARBs) exert their biological action by inserting into the lipid membrane and then diffuse to the active site of AT_1 receptor. Thus, lipid bilayers are expected to be actively involved and play a critical role in drug action. For this reason, the thermal, dynamic and structural effects of olmesartan alone and together with cholesterol were studied using differential scanning calorimetry (DSC), ^{13}C magic-angle spinning (MAS) nuclear magnetic resonance (NMR), cross-polarization (CP) MAS NMR, and Raman spectroscopy as well as small- and wide angle X-ray scattering (SAXS and WAXS) on dipalmitoyl-phosphatidylcholine (DPPC) multilamellar vesicles. ^{13}C CP/MAS spectra provided direct evidence for the incorporation of olmesartan and cholesterol in lipid bilayers. Raman and X-ray data revealed how both molecules modify the bilayer's properties. Olmesartan locates itself at the head-group region and upper segment of the lipid bilayers as ^{13}C CP/MAS spectra show that its presence causes significant chemical shift changes mainly in the A ring of the steroidal part of cholesterol. The influence of olmesartan on DPPC/cholesterol bilayers is less pronounced. Although, olmesartan and cholesterol are residing at the same region of the lipid bilayers, due to their different sizes, display distinct impacts on the bilayer's properties. Cholesterol broadens significantly the main transition, abolishes the pre-transition, and decreases the membrane fluidity above the main transition. Olmesartan is the only so far studied ARB that increases the *gauche*:*trans* ratio in the liquid crystalline phase. These significant differences of olmesartan may in part explain its distinct pharmacological profile.

1. Introduction

Hypertension is a chronic medical condition, which affects approximately one billion people worldwide [1,2]. The renin–angiotensin aldosterone system (RAAS) modulates blood pressure and it is the major system associated with hypertension. Many classes of antihypertensive medication are developed to act on RAAS. Angiotensin II receptor blockers (ARBs) have been designed to inhibit the binding of angiotensin II (AII) onto the G-protein coupled AT_1 receptor, and consequently decrease blood pressure [3–6]. Apart from complications such as stroke, ischemic heart disease, vascular remodeling and diabetic nephropathy, AII is associated also with inflammation, oxidative stress and cell growth [1,7–9]. Olmesartan medoxomil (Fig. 1A) belongs to the antihypertensive class of ARBs. This drug is an ester prodrug of the active metabolite (Fig. 1B), which is deesterified in the gastrointestinal tract [10,11]. The IUPAC name of this active metabolite olmesartan is 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl] imidazole-4-carboxylic acid.

Olmesartan medoxomil is commonly prescribed with a thiazide diuretic (in general, hydrochlorothiazide (HCT)) and/or a calcium channel blocker to ameliorate its effect [12–14]. The Food and Drug Administration (FDA) approved olmesartan medoxomil on April

* Corresponding author. Tel.: +39 040 375 8708; fax: +39 040 375 8029.
**Corresponding author. Tel.: +30 2107274475; fax: +30 2107274761.
E-mail addresses: michael.rappolt@elettra.trieste.it (M. Rappolt), tmavrom@chem.uoa.gr (T. Mavromoustakos).

0005-2736/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2011.08.001

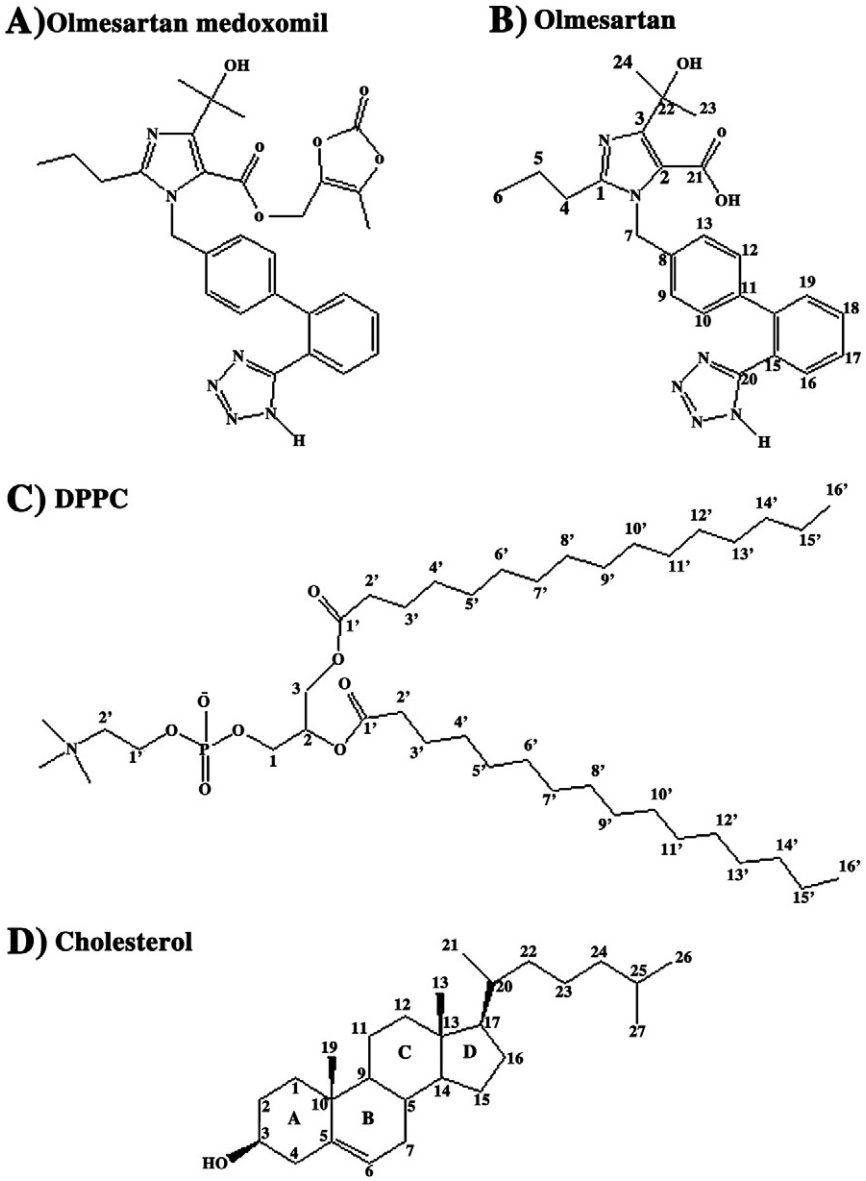

Fig. 1. The chemical structures of (A) prodrug olmesartan medoxomil; (B) olmesartan; (C) DPPC; and D) cholesterol.

2002, which was the seventh drug in the class of ARBs [15]. It has been marketed as an antihypertensive drug in United States, Japan and European countries [16]. The most recent approval by FDA was announced on July 2010 for Tribenzor (olmesartan medoxomil, amlodipine, hydrochlorothiazide), a new three-in-one combination product for the treatment of hypertension, which contains an ARB, a calcium channel blocker and a diuretic [17]. Although olmesartan belongs to ARB class, it is proposed that its pharmacological profile is distinct from the others. Generally, not only pharmacological similarities but also differences are observed among different ARBs [11,18].

In this study, liquid and solid state nuclear magnetic resonance (NMR), differential scanning calorimetry (DSC), Raman spectroscopy as well as small- and wide angle X-ray scattering (SAXS, WAXS) were applied to investigate the thermal, dynamic and structural properties of olmesartan in dipalmitoyl-phosphatidylcholine (DPPC) bilayers in the absence and presence of cholesterol.

In our previous studies we have put forward a two-step model in which the AT_1 prototype antagonist losartan first inserts into the bilayer core and diffuses towards the active site (first step), and then anchors to the active site (second step) [19]. As a continuation of our studies we are now examining and comparing the effects of AT_1 antagonist olmesartan with losartan and other AT_1 antagonists in liposomal formulations to reveal their role in distribution and eventually try to explain their drug efficacies.

In this work, multilamellar vesicles (MLVs) of dipalmitoyl-phosphatidylcholine (DPPC) (Fig. 1C) formed in excess of water were used to model the plasma membranes of vasculature. Note, that saturated phosphatidylcholines are the most abundant lipid species in the plasma membrane of vasculature (40–65%) [20].

In the past DPPC MLVs have been extensively used in NMR and DSC experiments to study the interactions of lipid-soluble drugs with biological membranes as the main phase transitions occur at convenient temperatures and are close to physiological ones [21–26]. Cholesterol (Fig. 1D) is a major component of the cell plasma membrane and its role is essential to establish proper membrane permeability and fluidity as well as to interfere with drug action [27–29].

The aim of this research work is to study olmesartan–lipid interactions in a temperature range from 20 °C up to 50 °C covering all mesomorphic phases. DSC experiments using samples with different molar ratios (olmesartan:phospholipid) were prepared.