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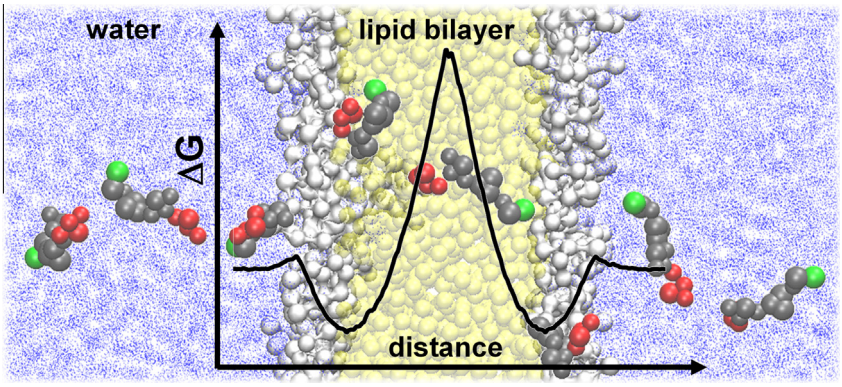
Structural perturbation of a dipalmitoylphosphatidylcholine (DPPC) bilayer by warfarin and its bolaamphiphilic analogue: A molecular dynamics study

Manuela Aseye Ayele Ayee [a,b], Charles William Roth [a], Belinda Sena Akpa [a,c,*]

[a] Department of Chemical Engineering, University of Illinois at Chicago, 810 S. Clinton St., Chicago, IL 60607, USA
[b] Department of Medicine, University of Illinois at Chicago, 909 S. Wolcott Ave., Chicago, IL 60612, USA
[c] Department of Molecular Biomedical Sciences, North Carolina State University, 1060 William Moore Dr., Raleigh, NC 27607, USA

GRAPHICAL ABSTRACT



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ABSTRACT

Compounds with nominally similar biological activity may exhibit differential toxicity due to differences in their interactions with cell membranes. Many pharmaceutical compounds are amphiphilic and can be taken up by phospholipid bilayers, interacting strongly with the lipid–aqueous interface whether or not subsequent permeation through the bilayer is possible. Bolaamphiphilic compounds, which possess two hydrophilic ends and a hydrophobic linker, can likewise undergo spontaneous uptake by bilayers. While membrane-spanning bolaamphiphiles can stabilize membranes, small molecules with this characteristic have the potential to create membrane defects via disruption of bilayer structure and dynamics. When compared to the amphiphilic therapeutic anticoagulant, warfarin, the bolaamphiphilic analogue, brodifacoum, exhibits heightened toxicity that goes beyond superior inhibition of the pharmacological target enzyme. We explore, herein, the consequences of anticoagulant accumulation in a dipalmitoylphosphatidylcholine (DPPC) bilayer. Coarse-grained molecular dynamics simulations reveal that permeation of phospholipid bilayers by brodifacoum causes a disruption of membrane barrier function that is driven by the bolaamphiphilic nature and size of this molecule. We find that brodifacoum partitioning into

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; VKOR, vitamin K epoxide reductase; NPT, isobaric, isothermal ensemble, constant number of particles, pressure, and temperature; PMF, potential of mean force; WAR, warfarin; BDF, brodifacoum.

* Corresponding author at: Campus Box 8401, 1060 William Moore Dr., Raleigh, NC 27607, USA.
E-mail addresses: mayee2@uic.edu (M.A.A. Ayee), croth6@uic.edu (C.W. Roth), bsakpa@ncsu.edu (B.S. Akpa).

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Brodifacoum
Defect
Hydroxycoumarin

bilayers causes membrane thinning and permeabilization and promotes lipid flip-flop – phenomena that are suspected to play a role in triggering cell death. These phenomena are either absent or less pronounced in the case of the less toxic, amphiphilic compound, warfarin.

1. Introduction

Bolaform amphiphiles are compounds that possess two hydrophilic end groups joined by a hydrophobic linker. Like traditional amphiphiles, bolaamphiphiles are surface-active agents. In aqueous solutions, they form nanostructures such as micelles, ribbons, and fibers [1–3]. Bola species occur naturally in the monolayer membranes of thermophilic microorganisms, conferring the superior stability required for existence in extreme environments [4]. Synthetic bolaform species have found application as bilayer membrane stabilizers, synthetic ion channels [5–8], and as additives in drug delivery formulations [1,9]. They are also of considerable interest in monolayer functionalization of solid surfaces [10–12].

While much of the appeal of bolaform additives to bilayer membranes lies in their ability to fortify membrane structure by spanning the full thickness of the bilayer, hydrophobic mismatch and other molecular features can make these two-headed molecules function as disruptors of the lamellar structure formed by phospholipid amphiphiles. By tuning the groups making up the hydrophobic linker, bolaamphiphiles can be made more or less membrane stabilizing [13,14]. Rigid aromatic rings confer more stability than unbranched saturated aliphatic chains [2,14]. Rates of phospholipid flip-flop (transit of phospholipid molecules from one bilayer leaflet to another) have been observed to increase in the presence of membrane-spanning bolaamphiphiles. Flip-flop rates decrease as the stiffness of the aliphatic linker is increased [4,14]. Highly flexible linkers allow bolaform compounds to adopt U-shaped or hairpin conformations in lipid bilayers rather than spanning their thickness [13,14]. In addition to linker rigidity, linker length is critical. As membrane stabilization is a typical objective, few studies have probed the properties of bolaamphiphiles that are shorter than the thickness of typical phospholipid membranes. Short bolaamphiphiles have been reported to have a destabilizing tendency that can drive lateral phase segregation [13]. Still fewer studies consider bolaform species that have asymmetric head groups.

In this paper, we consider the interaction of a bolaform anticoagulant compound with a dipalmitoylphosphatidylcholine (DPPC) bilayer. The compound is an anionic, asymmetric molecule with length similar to naturally-occurring phospholipids. Using coarse-grained molecular dynamics simulations, we identify a potential for membrane disruption by defect formation that is a specific consequence of the additive's bolaamphiphilic structure. By contrasting this with the behavior of an amphiphilic analogue, we characterize a potential biophysical mechanism for the heightened cytotoxicity of the bola-species.

2. Warfarin and its bolaamphiphilic counterpart, brodifacoum

Both warfarin and brodifacoum are compounds that inhibit blood coagulation by interfering with an essential metabolic cycle, the reduction of vitamin K. This anticoagulant [15–26] action is due to a shared hydroxycoumarin moiety that mimics the structure of vitamin K and enables these compounds to function as inhibitors of the enzyme vitamin K epoxide reductase (VKOR) [27–29]. VKOR is an integral membrane protein that resides inside cells of organs such as the liver; consequently, hydroxycoumarin anticoagulants must cross plasma membranes to reach their site of action. Whether compounds traverse membranes via passive or active mechanisms depends on their size and physicochemical characteristics. Passive transport (diffusion across the membrane down a concentration gradient) is facilitated by low molecular weight, high lipid solubility, and absence of charge [30–33]. However, even charged amphiphiles of modest size can bind efficiently to lipid interfaces, with slower subsequent transport across the membrane's hydrophobic core mediated by conformational and orientational changes [34,35]. Indeed, liposome partitioning of ionized drugs demonstrates higher bilayer affinity than would be suggested by octanol–water partitioning [36,37]. As bulk phase partitioning is an inadequate predictor of membrane association and experimental approaches are limited with respect to probing the molecular phenomena that govern drug association with and permeation through membranes, computer simulations have become an invaluable tool in elucidating these processes. Several recent publications and reviews demonstrate the utility of molecular simulation methods and transfer free energy calculations for predicting and clarifying biophysical interactions between drug-like molecules and phospholipid membranes [38–43]. These approaches have been used to predict drug bioavailability and membrane permeability, with outcomes that correlate well with experimental measures. Interrogation of aggregation and pore-forming behavior provides insight as to mechanisms of toxicity. Furthermore, the molecular level examination of events occurring on femto- to microsecond time-scales elucidates conformational, orientational, and clustering phenomena that underlie structural and dynamical perturbations imposed on bilayers during drug permeation.

Warfarin and brodifacoum are both lipophilic and small enough to plausibly cross membranes by passive permeation. Transmembrane diffusion of coumarin compounds and their derivatives has been the subject of a number of computational and experimental investigations [44–48]. Findings suggest that coumarin species do permeate bilayers, with molecular orientation, intramolecular hydrogen bonding and isomerization evolving to accommodate the different molecular environments comprising the bilayer structure [36,44–46]. During their transit, we posit that these hydroxycoumarin compounds may disrupt membrane barrier function, creating a potential for cytotoxicity. Warfarin is, in fact, known to cause acute cell damage [49–52]. However, its use as a clot-preventing therapeutic ensures conservative dosing and limited tissue exposure. In contrast, brodifacoum is a so-called 'agent of opportunity', a commercially available chemical that has the potential to be weaponized [53]. The compound is found in consumer-accessible rodenticides. It is odorless and tasteless, and malicious or accidental release could lead to large exposures. A further concern is that brodifacoum can remain sequestered in organs for up to a year [15–17,20,24,54]. Hence, the potential for cell damage by brodifacoum is likely higher simply due to degree and duration of exposure. We further posit that the structure of brodifacoum lends itself to greater disruption of bilayer structure. Both warfarin and brodifacoum contain the hydroxycoumarin active group for anticoagulation, which constitutes a polar region in the molecular structure (Fig. 1). However, where warfarin possesses a hydrophobic substituent in the form of a phenyl ring, brodifacoum is characterized by an extended hydrophobic region capped by a bromophenyl group whose dipole imbues the