Interaction of Clonixin with EPC Liposomes Used as Membrane Models

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Received 15 December 2004; revised 15 February 2005; accepted 16 February 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20351

ABSTRACT: In this work, an overall analysis of clonixin interaction with liposomes was achieved using different techniques, which allowed the evaluation of the change in different membrane's characteristics as well as the possible location of the drug in the membrane. Clonixin acidity constants were obtained and the values are 5.5 ± 0.08 and 2.2 ± 0.04. Clonixin partition coefficient (K_p) between liposomes and water was also determined using derivative spectrophotometry, fluorescence quenching, and zeta-potential (ζ-potential). These three techniques yielded similar results. ζ-potential measurements were performed and an increase of the membrane negative charge with an increase of drug concentration was observed. Drug location within the bilayer was performed by fluorescence quenching using a set of n-(9-anthroyloxy) fatty acid probes ($n = 2$, 6, 9, and 12). The fluorescence intensity of all probes was quenched by the drug. This effect is more noticeable for the outer located probe, indicating that the drug is positioning in the external part of the membrane. These same probes were used for steady-state anisotropy measurements to determine the perturbation in membrane structure induced by clonixin. Clonixin increased membrane fluidity in a concentration dependent manner, with the highest perturbation occurring nearby the 2-AS probe, closely located to the bilayer surface. J Pharm Sci 94:1277–1287, 2005

Keywords: clonixin; liposomes; drug interaction; light scattering; UV/Vis spectroscopy; fluorescence spectroscopy; partition coefficient; drug location; membrane fluidity

INTRODUCTION

Cyclooxygenase (COX), a membrane related enzyme, is the pharmacological target of non-steroidal anti-inflammatory drugs (NSAIDs), which are therefore commonly used in inflammatory diseases treatment. Although low doses of NSAIDs inhibit prostaglandins biosynthesis, high concentrations interfere with processes not dependent on these mediators. Membrane related phenomena, such as neutrophil function inhibition, oxidative phosphorylation inhibition in mitochondria, signal transduction disruption, and the consequent interference with intracellular calcium mobilization and protein kinase C activity alteration, have all been reported by Klein et al.,[1] as well as a membrane fluidity alteration, which has been mentioned by several authors.[2–5] Furthermore, NSAIDs have been shown to inhibit the cellular proliferation rate, to alter the cell cycle regulation, and to induce apoptosis in cancer cell lines, in a mechanism independent from prostaglandin pathways.[1]

There is consensual evidence that the lipid affinity of the NSAIDs is of major significance

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Journal of Pharmaceutical Sciences, Vol. 94, 1277–1287 (2005)

for their toxic and therapeutic actions. Indeed, depending on their hydrolipophilic character, NSAIDs can be distributed between the membrane and the aqueous phases. This distribution determines their concentration in each phase and thereby controls the extents of their penetration into the membrane and/or their interactions with phospholipids or other membrane components, such as COX enzymes, which are embedded in the lipid bilayers.[6] Thus, for the study of NSAIDs' action mechanisms and their side-effects, it is of great importance to investigate the interactions between these drugs and biomembranes. For this purpose, this work was performed using liposomes of egg yolk phosphatidylcholine (EPC). Liposomes are generally accepted to be a suitable model for the study of membrane structure and properties, because they are surrounded by a lipid bilayer structurally similar to the lipidic matrix of the cell membranes.[6,7] Additionally, because of being constituted by natural lipids, EPC liposomes can mimic the chemical and structural anisotropic environment of cell membranes. EPC liposomes also appear to mimic the interfacial character as well as the ionic, H-bond, dipole–dipole, and hydrophobic interactions, which may define partitioning in real biomembranes.[6,7]

Traditionally, the octanol–water partition coefficient (K_p) has been used to measure compounds' hydrophobicity, which is correlated to drug activity. The octanol–water system is a good membrane model when polar group interactions between the solute and the phospholipid bilayer are minimal or absent. However, since octanol can only model non-polar interactions,[8] better systems are needed for molecules which can establish electrostatic interactions with polar groups in the membrane. According to this, the study of clonixin's partition in a liposome/buffer system has been performed. There is a more satisfactory correlation between this parameter and its pharmacological properties since clonixin has proved to be able to establish electrostatic interactions with polar groups in the biomembranes. The drug's K_p was evaluated by derivative spectrophotometry, a technique that can be used when a solute's spectral characteristic changes between one media to another. Derivative spectrophotometry eliminates the intense background signals that arise from light scattered by lipid vesicles, and it also improves the resolution of overlapping signals reported by several authors.[9–12] Moreover, the liposome/water K_p was also determined by other experimental techniques: zeta-potential (ζ-potential) and fluorescence quenching. Using the ζ-potential technique, it was possible to evaluate the interaction of clonixin with liposomes by measuring the membrane potential arising from the drug partitioning. In fact, biological membranes are charged, due to ionized components (lipids, glycolipids, glycoproteins), and the resulting surface potential plays a critical role in regulatory processes, membrane–membrane interactions, and in their binding capacity to solutes in solution.[13,14] Additionally, to electrostatic effects, which can affect the conformation and activity of membrane and membrane-bound enzymes,[15,16] several cell processes are also related to electrostatic or polarization effects on the cell membrane. In this context, the characterization of the electrostatic membrane properties induced by clonixin binding is a fundamental parameter, and it also allows the quantification of clonixin molecules in these membranes. Consequently, K_p values can be calculated.

Fluorescence quenching was also used to measure clonixin's coefficient partition. The fluorescent n-(9-anthroyloxy)-stearic acids (n-AS, $n = 2$, 6, 9, and 12) are the set of probes most widely used for obtaining information on molecular aggregates, such as liposomes and natural membranes.[17–22] For these probes, there is evidence that the anthroyloxy fluorophores are located at a graded series of depths inside a membrane, depending on its substitution position (n) in the aliphatic chain.[17] Therefore, these probes, due to their exceptional environmental sensitivity, have been employed to monitor the microenvironment of membranes. These appropriate measurements allow information about the local membrane structure to be inferred. According to this, besides the determination of clonixin's K_p, fluorescence quenching provides a mean to evaluate the position and orientation of the drug in the membrane by a comparative analysis of all probe's quenching. Furthermore, the fluorescent probes are capable of sensing a "fluidity" gradient through the bilayer leaflet and, therefore, they were used to assess the clonixin effect in the lipid membrane fluidity. This was achieved using steady-state anisotropy measurements, since that anisotropy depends upon the rotational motion of the fluorophore and it is sensitive to hindrance forces imposed by the microenvironment, property that has been widely used to estimate membrane fluidity.[18] Membrane fluidity assessment gives useful physiologic information as biomembranes need to be in a fluid state in order to maintain complete biological function. Indeed, any alteration in membrane fluidity tends