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Review

Interaction of nonsteroidal anti-inflammatory drugs with membranes: *In vitro* assessment and relevance for their biological actions

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ABSTRACT

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs in the world due to their anti-inflammatory, analgesic and antipyretic properties. Nevertheless, the consumption of these drugs is still associated with the occurrence of a wide spectrum of adverse effects. Regarding the major role of membranes in cellular events, the hypothesis that the biological actions of NSAIDs may be related to their effect at the membrane level has triggered the *in vitro* assessment of NSAIDs-membrane interactions. The use of membrane mimetic models, cell cultures, a wide range of experimental techniques and molecular dynamics simulations has been providing significant information about drugs partition and location within membranes and also about their effect on diverse membrane properties. These studies have indeed been providing evidences that the effect of NSAIDs at membrane level may be an additional mechanism of action and toxicity of NSAIDs. In fact, the pharmacokinetic properties of NSAIDs are closely related to the ability of these drugs to interact and overcome biological membranes. Moreover, the therapeutic actions of NSAIDs may also result from the indirect inhibition of cyclooxygenase due to the disturbing effect of NSAIDs on membrane properties. Furthermore, increasing evidences suggest that the disordering effects of these drugs on membranes may be in the basis of the NSAIDs-induced toxicity in diverse organ systems. Overall, the study of NSAIDs-membrane interactions has proved to be not only important for the better understanding of their pharmacological actions, but also for the rational development of new approaches to overcome NSAIDs adverse effects.

Contents

Abbreviations: ATP, adenosine triphosphate; COX, cyclooxygenase; Coxibs, COX-2 selective inhibitors; CV, cardiovascular; DPPC, dipalmitoylphosphatidylcholine; GI, gastrointestinal; GUVs, giant unilamellar vesicles; LB, Langmuir-Blodgett; LOX, lipoxygenase; LUVs, large unilamellar vesicles; MD, molecular dynamics; MLVs, multilamellar lipid vesicles; mPGES-1, microsomal prostaglandin E synthase-1; NO-NSAIDs, nitric oxide-releasing nonsteroidal anti-inflammatory drugs; NSAIDs, nonsteroidal anti-inflammatory drugs; PC-NSAIDs, phosphatidylcholine-associated nonsteroidal anti-inflammatory drugs; PG, prostaglandin; SLB, supported lipid bilayers; SUVs, small unilamellar vesicles; Tx, thromboxane.

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1. Introduction

The prescription of products with anti-inflammatory, analgesic and antipyretic properties dates back at least 2500 years ago, when Hippocrates recommended the use of an extract of willow barb to relieve the pain of childbirth and to reduce fever. The identification of salicin as the active ingredient of this plant led to the massive production of salicylic acid in 1860 and later onto the development of a more palatable form of this drug, the acetylsalicylic acid, which is greatly used worldwide until today [1,2]. This was just the beginning of the long story of nonsteroidal anti-inflammatory drugs (NSAIDs). Briefly, Vane discovered the mechanism of action of these drugs in 1971: the inhibition of prostaglandins biosynthesis by cyclooxygenase pathway [3]. About 20 years later, a new isoform of cyclooxygenase (COX) was discovered, called COX-2, whose expression is induced by inflammatory mediators in various tissues [4]. In the meantime, a significant quantity of chemically diverse NSAIDs was commercialized but the gastrointestinal (GI) toxicity was soon identified as a major drawback of the chronic use of these drugs. In this context, the discovery of COX-2 led to the prompt development of COX-2 selective inhibitors (coxibs) with the rational basis that the selective inhibition of COX-2 would maintain the therapeutic actions of these drugs, while avoiding their GI toxicity [1]. Despite the initial expectations of academic community and pharmaceutical companies, these selective inhibitors were rapidly associated with the occurrence of cardiovascular (CV) adverse effects, which led to the withdrawal of some coxibs from the market and raised questions about the CV safety of non-selective NSAIDs [1,5]. The increasing concern about NSAIDs toxicity has led to the reduction of the worldwide consumption of these pharmaceuticals [5], thereby inducing the further study of the pharmacological actions of NSAIDs toward the development of more effective and safer drugs to treat inflammatory conditions.

In the 1980's, Lichtenberger et al. have proposed for the first time the role of surface-active phospholipids as a cytoprotective mechanism in the surface of gastric mucosa [6,7]. Shortly, the deleterious effect of aspirin on the surface hydrophobicity of gastric mucosa was described by the same laboratory and attributed to its interaction with the hydrophobic or non-wettable lining of phospholipids [8,9]. This was the beginning of the assessment of NSAIDs-phospholipids interaction, which has continued in the 1990's still due to the contribution of Lichtenberger laboratory [10,11]. Since 2000, the *in vitro* evaluation of NSAIDs-membrane interaction has spread in the scientific community with significant contributions from different laboratories around the world. In this regard, this review aims to explore the *in vitro* assessment of NSAIDs-membrane interactions in the last decade and to discuss the relevance of this interaction for the pharmacokinetics and the therapeutic and toxic effects of these anti-inflammatory drugs, after briefly reviewing some fundamental aspects concerning NSAIDs and biological membranes.

1.1. Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous class of compounds with anti-inflammatory, antipyretic and analgesic properties, which arise from the inhibition of the prostanoids biosynthesis through the cyclooxygenase pathway [5]. In fact, these pharmaceuticals are commonly used worldwide for the treatment of inflammatory conditions, such as musculoskeletal diseases (rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, acute gouty arthritis), and painful conditions of low to moderate intensity, including dental pain, menstrual pain, postoperative pain and migraine [12].

NSAIDs are classified according to different characteristics, including chemical properties and COX selectivity. These drugs are grouped in several chemical classes (Fig. 1). However, in general, they are weak organic acids with hydrophobic properties, which facilitate their penetration in inflamed tissues where pH is lower and their approach to COX [13], since it is a monotopic membrane protein and the cyclooxygenase active site is located in the end of a hydrophobic channel [14,15]. In addition, these pharmaceuticals are also classified as non-selective NSAIDs and COX-2 selective NSAIDs (Fig. 1), based on the ratio of COX-1/COX-2 inhibitory potencies.

The chemical diversity of NSAIDs justifies their distinct pharmacokinetics but they share some general properties. After oral ingestion, most NSAIDs are rapidly absorbed and are extensively bound to plasma proteins, especially albumin. NSAIDs are metabolized in the liver, involving oxidation and hydroxylation catalyzed by cytochrome P450 enzymes and/or glucuronidation or other conjugation catalyzed by the hepatic transferases. Renal excretion is the main route of elimination of NSAIDs. Plasma half-life times are extremely variable in this class of drugs, for instant ibuprofen, indomethacin and diclofenac undergo rapid elimination, while oxaprozin and piroxicam are slowly excreted [13,16].

The mechanisms by which NSAIDs exert their therapeutic effects are briefly summarized in Table 1. Besides its anti-inflammatory, analgesic and antipyretic properties, aspirin is also used worldwide due to its antiplatelet effect, which results from the irreversible inhibition of the TXA_2 production in platelets, avoiding the vasoconstriction and platelet aggregation induced by this prostanoid [17,18]. However, NSAIDs are associated with a wide range of adverse effects, which are also partially due to their mechanism of action. In fact, the most common adverse effect of NSAIDs is GI toxicity, but it is nowadays believed that coxibs and non-selective NSAIDs are associated with an increased risk of CV and renal adverse effects. The main mechanisms of toxicity of NSAIDs are displayed in Table 1.

It has been a long journey so far since the launch of aspirin in the market in 1899 by Bayer. However, scientific community and pharmaceutical companies seek to reveal all the pharmacological actions of NSAIDS and to develop the golden NSAID, an effective and safe drug to treat inflammation, pain and fever. In this regard, different drugs have been developed to overcome the toxicity of NSAIDs, such as mPGES-1 inhibitors, NO-NSAIDs, PC-NSAIDs and dual COX/LOX inhibitors [1,19].

1.2. Biological membranes

Most cellular functions occur in or around cell membranes [24,25], which highlights the importance of biological membranes in the human body. Actually, biological membranes define cells and organelles, acting as a selective barrier, allowing the segregation of specific chemical reactions and thus increasing the biochemical efficiency of cells. Besides protecting cells from the external environment, biological membranes are involved in a wide range of important functions, mediated by both lipids and