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Kinetic modelling of NSAID action on COX-1: Focus on *in vitro/in vivo* aspects and drug combinations

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ABSTRACT

The detailed kinetic model of Prostaglandin H Synthase-1 (COX-1) was developed to *in silico* test and predict inhibition effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on target. The model takes into account key features of the complex catalytic mechanism of cyclooxygenase-1, converting arachidonic acid to prostaglandin PGH_2, and includes the description of the enzyme interaction with various types of NSAIDs (reversible/irreversible, non-selective and selective to COX-1/COX-2). Two different versions of the model were designed to simulate the inhibition of COX-1 by NSAIDs in two most popular experimental settings – *in vitro* studies with purified enzyme, and the experiments with platelets. The developed models were applied to calculate the dose-dependence of aspirin and celecoxib action on COX- 1 *in vitro* and *in vivo* conditions. The mechanism of the enhancement of aspirin efficiency in platelet as compared to its action on purified COX-1 was elucidated. The dose-dependence of celecoxib simulated with the use of the "*in vivo*" version of the model predicted potentially strong inhibitory effect of celecoxib on thromboxan production in platelets. Simulation of the combined effect of two NSAIDs, aspirin and celecoxib, on COX-1 allowed us to reveal the mechanism underlying the suppression of aspirin-mediated COX-1 inhibition by celecoxib. We discuss our modelling results in the context of the ongoing debates on the potential cardio-vascular risks associated with co-administration of various types of NSAIDs.

1. Introduction

The main target of nonsteroidal anti-inflammatory drugs (NSAIDs) is the enzyme Prostaglandin H Synthase, historically known also as cyclooxygenase (COX). This enzyme plays a key role in the synthesis of prostaglandins, and exists in two isoforms – COX-1 and COX-2. COX-1 is constitutively expressed in the majority of human tissues, whereas COX-2 expression is mainly associated with inflammation. Both isoforms catalyze the synthesis of PGH_2 from arachidonic acid through

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complex catalytic mechanism including cyclooxygenase and peroxidase activities (Kulmacz et al., 2003). The majority of antipyretic and analgesic effects of NSAIDs originate from the inhibition of the production of PGH_2, final product of COX, which serves as a precursor for synthesis of prostanoids, such as prostacycline (PGI_2), PGE_2, thromboxane (TxA_2) and others. Prostanoids involved in multiple physiological processes, among them gastric cytoprotection, maintenance of renal and vascular homeostasis, normal platelet function, blood clotting and inflammatory signaling. That is why the inhibition of the initial stage of prostanoids synthesis by NSAIDs may cause, along with therapeutic effects, some serious adverse reactions, in particular, gastrointestinal ulceration (Grosser et al., 2006). A new generation of NSAIDs – inhibitors selective to COX-2 isoform (rofecoxib, celecoxib, etc.) – has been developed specifically with the view to avoid gastrointestinal side effects. It was thought that selective inhibition of COX-2 would result in anti-inflammatory action without disrupting COX-1 – related production of gastroprotective prostaglandins (Grosser et al., 2006; Warner et al., 1999; Chan et al., 1999). However, the complexity of COX-1/COX-2 interplay and subtle balance between different multifunctional prostanoids have been underestimated. Therefore, administration of COX-2 selective NSAIDs resulted in the manifestation of the wide range of new adverse reactions, most importantly, an increased risk of heart attacks (Grosser et al., 2006). The understanding and predicting of both therapeutic and side effects of NSAIDs as well as the development of new effective anti-inflammatory drugs clear of adverse reactions requires thorough investigation of NSAID effects at all levels of system organisation including molecular, cellular, and organism ones.

The experimental research devoted to the characterisation of NSAIDs inhibition effects at molecular and cellular levels is commonly performed with the use of different assays, among them purified COX-1 and COX-2 (Gierse et al., 1999; Ouellet et al., 2001; Callan et al., 1996; Mitchell et al., 1994), intact cells (platelet, endothelia cells and others) (Kargman et al., 1996; Mitchell et al., 1994; Burch et al., 1978; Rome and Lands, 1975), and human whole blood assays (WBA) (Warner et al., 1999; Mitchell et al., 2006). The key properties of NSAIDs, their basic types, and mechanism of action were mainly characterised in *in vitro* experiments with the use of cell-free preparation of COX-1,2 (Gierse et al., 1999; Ouellet et al., 2001; Callan et al., 1996). The attempts to extrapolate these results to *in vivo* conditions have faced problems (Kargman et al., 1996). Several studies showed that for unknown reasons for the majority of NSAIDs the IC_{50} and selectivity measured in intact cells differ from the values obtained in the experiments with purified COX (Kargman et al., 1996). For example, IC_{50} and selectivity values obtained for the same NSAID in different experimental settings may differ from each other by up to two orders of magnitude (Kargman et al., 1996; Brooks et al., 1999). Note that the problem of extrapolation of *in vitro* measured NSAID characteristics to *in vivo* conditions is essential, i.e. if such work is to be used in the design of clinical trials, especially when optimising the drug dose and scheduling of drug administration. As long as the mechanism underlying the discrepancy between NSAID characteristics measured in different experimental settings remains unknown, it is unclear how to predict NSAID effects in humans using *in vitro* and *in vivo* data. Several experimental studies proposed that the key factor causing the observed variation in NSAID effects is the difference between *in vitro* experimental conditions and intracellular micro-environment in various cells (Mitchell et al., 2006).

In this work we demonstrate how the kinetic modelling approach can be applied to understand and predict the effect of various types of NSAIDs on the primary target – cyclooxygenase. Our modelling approach is based on the detailed mechanistic understanding of how drug target works, and how NSAIDs can interfere with COX catalytic activity. Our models allow to extrapolate how changes in micro-environmental conditions of drug target can influence the resulting drug effect.

The central component of our approach is the detailed kinetic model of COX-1 catalysis, accounting for inhibitory action of NSAIDs. For the first time, such a model was developed and applied to describe inhibition effects of different types of NSAIDs, among them irreversible and reversible inhibitors, time-dependent and time-independent, selective to COX-1 or COX-2. The developed model reproduces complex dynamics of COX-1 catalysis and takes into account the key features of COX functioning established experimentally.

We demonstrate the abilities of our approach by application of the developed models to study the inhibitory effects of two popular NSAIDs, aspirin and celecoxib, in different experimental settings. These drugs represent two polar classes of NSAIDs: aspirin is an irreversible, time-dependent traditional NSAID with preferential selectivity to COX-1 (Mitchell et al., 1994; Rome and Lands, 1975), whereas celecoxib belongs to a new generation of COX-2 selective inhibitors with fast reversible binding to COX-1,2 (Gierse et al., 1999). *In silico* we studied how aspirin and celecoxib act on COX-1 both as single drugs and in combination.

We adjusted our models to describe NSAID action on COX-1 in two types of experimental settings – *in vitro* studies with purified enzyme, and *in vivo* experiments with living cells (platelets). The results presented in this paper allow to get insight into the mechanism of several experimental phenomena observed for aspirin and celecoxib, which have not been previously explained. We managed to explain the origin of the discrepancy between inhibitory action of NSAIDs *in vitro* and *in vivo* (Kargman et al., 1996; Brooks et al., 1999). Modelling of the combined action of aspirin and celecoxib on target revealed a non-trivial mechanism of the suppression of aspirin inhibitory effect by celecoxib. In the light of on-going discussions on the potential risks and benefits of co-administration of two or more NSAIDs (Capone et al., 2005; Curtis and Krumholz, 2004; Ouellet et al., 2001), our approach provides the basis for the model-based assessment and prediction of safety and efficacy of NSAID combinations.

2. Kinetic model of COX-1 catalytic cycle with inhibitory action of NSAIDs

Prostaglandin H Synthase-1 (PGHS-1 or COX-1) is a bifunctional enzyme which has two distinct catalytic sites with corresponding catalytic activities: a cyclooxygenase site (cox), in which the substrate, arachidonic acid, is converted to the intermediate product, PGG_2, and a peroxidase site (pox) in