British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12801

Celecoxib interferes to a limited extent with aspirin-mediated inhibition of platelets aggregation

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Keywords
celecoxib, COX-1 enzyme, drug–drug interaction, low dose aspirin anti-aggregation effect, pharmacokinetic–pharmacodynamic modelling

Received
14 July 2015

Accepted
9 October 2015

Accepted Article Published Online
11 October 2015

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Celecoxib and other COX-2 selective inhibitors interact with COX-1 and can interfere with the antiplatelet effects of aspirin.

WHAT THIS STUDY ADDS

- We investigated the interaction between celecoxib and aspirin and its consequences for platelet aggregation using *ex vivo* experiments and pharmacokinetic–pharmacodynamic modelling analysis.
- Celecoxib can attenuate to a limited extent the *in vivo* antiplatelet effects of low dose aspirin, but the consequences of this interaction for the risk of cardiovascular and cerebrovascular events in patients treated concomitantly with low dose aspirin and celecoxib remains unknown.

AIMS

The aim of the study was to analyze the interaction between celecoxib and low dose aspirin for COX-1 binding and its consequences on the aspirin-mediated antiplatelet effects.

METHODS

We investigated *ex vivo* the interaction between celecoxib and aspirin for COX-1 binding and measured the resulting antiplatelet effects. We applied mechanism-based pharmacokinetic–pharmacodynamic (PKPD) modelling to analyze these data and to predict *in vivo* platelet aggregation for different doses and administration schedules of aspirin and celecoxib.

RESULTS

The predictions of the PK-PD model were consistent with results from previous studies that investigated interaction between aspirin and celecoxib. The modelling results indicate that celecoxib can attenuate to a limited extent the *in vivo* antiplatelet effects of low dose aspirin. The extent of this interaction can be substantial (up to 15% increase in platelet aggregation by 200 mg day^{-1} celecoxib when combined with low dose aspirin) during the first days of aspirin administration in patients who are already treated with celecoxib, and it cannot be prevented by separate administration of the interacting drugs.

CONCLUSIONS

At the recommended therapeutic doses, celecoxib can attenuate to a limited extent the *in vivo* antiplatelet effects of low dose aspirin. Patients receiving a combination of low dose aspirin and the recommended doses of celecoxib were not identified to have increased risk of cardiovascular and cerebrovascular events due to competition between these drugs for COX-1 binding. Interaction between low dose aspirin and other COX-2 inhibitors and its clinical consequences requires further investigation.

Introduction

The constitutive and the inducible cyclo-oxygenase enzymes (COX-1 and COX-2, respectively) are prostaglandin-endoperoxide synthases, membrane-associated heme-containing homodimers (composed of two 70 kDa subunits), responsible for conversion of arachidonic acid into precursors for numerous important biological mediators, including thromboxanes (Tx), prostaglandins and prostacyclin. COX-1 and COX-2 enzymes are important pharmacological targets that are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs).

Most of the NSAIDs inhibit both COX-1 and COX-2 isoenzymes (e.g. ibuprofen, diclofenac and others), induce a variety of pharmacological effects and are predominantly used to manage diseases that are accompanied by fever and pain. However, pharmacological activities can differ widely between the individual NSAIDs. Specifically, low dose aspirin (75–100 mg day^{-1}) induces irreversible inhibition of platelet COX-1 leading to reduced production of thromboxane A_2 (and other eicosanoids) and a decrease in platelet aggregation and blood clotting. Due to these pharmacological effects, low dose aspirin is widely used in patients with cardiovascular risk factors to prevent vascular thrombotic events like heart attacks and strokes [1, 2]. COX-2-selective inhibitors, such as celecoxib, were designed to target predominantly the COX-2 isoenzyme, and to reduce inflammation and pain while minimizing COX-1-mediated effects, for example, gastrointestinal adverse drug reactions.

Because of the wide availability and frequency of use of NSAIDs that affect different isotypes of the COX enzyme, there is high potential for drug–drug interactions between the individual NSAID agents. Interactions between the specific NSAIDS have been analyzed in numerous studies using *in vitro* and *ex vivo* experimental systems, and in clinical experimental settings. Specifically, interaction between aspirin and ibuprofen has attracted the attention of several research groups and was thoroughly investigated. Outcomes of clinical studies [1, 3, 4] and of pharmacokinetic–pharmacodynamic modeling (PK-PD) analysis [5-8] indicated that ibuprofen can interfere with the antiplatelet effect of aspirin at the clinically-relevant dosages and render aspirin less effective when used for cardioprotection and stroke prevention. Therefore, concomitant administration of ibuprofen and aspirin should be avoided [2, 9].

Possible interactions of COX-2-selective inhibitors such as celecoxib with other NSAIDs gained importance due to the reports of an increased incidence of cardiovascular events in patients who are treated with COX-2 inhibitors. At the present time, there is some controversy regarding the interaction between celecoxib (a selective COX-2 inhibitor) and low dose aspirin (that induces anti-aggregation effects via irreversible inactivation of COX-1) (see Table 1). No significant differences in platelet aggregation response induced *ex vivo* by arachidonic acid or

Table 1

Summary of studies that analyzed the interaction between low dose aspirin and celecoxib and the resulting antiplatelet effects

Study	Subjects	Treatments and sample collection time	Analyzed parameters	Conclusions
Wilner *et al.* [10]	Healthy volunteers (aged 18–48 years, *n* = 8–9)	The patients received celecoxib 200 mg twice daily or matched placebo orally for 4 days. On day 5, all volunteers received 325 mg aspirin orally concomitantly with 20 mg celecoxib or with placebo orally, and samples were collected before and 2 and 8 h after this dose.	Serum TxB_2 levels, platelet aggregation response by aggregometry	Celecoxib did not affect the platelet aggregation response to arachidonic acid in healthy volunteers receiving aspirin
Renda *et al.* [3]	Osteoarthritis and stable ischaemic disease patients (aged 45–73 years, *n* = 29)	The patient were undergoing long term treatment with 100 mg aspirin once daily orally, and received 200 mg celecoxib or placebo twice daily orally for 7 days. Samples were collected before and 4 and 24 h after the last dose.	Platelet TxB_2 and LPS-stimulated PGE_2 production in whole blood, urinary 11-dehydro-TxB_2 excretion rate, platelet aggregation by turbidimetric analysis	Celecoxib did not interfere with the inhibition of platelet COX-1 activity and function by aspirin
Gladding *et al.* [4]	Healthy volunteers (*n* = 24)	Volunteers received three doses of 200 mg celecoxib or other NSAIDs or placebo orally at 12 h intervals. 2 h after the last dose, the patients received 300 mg aspirin orally, and 10 h later (24 h after the last NSAID dose) samples were collected.	PFA-100 closure time	Celecoxib did not demonstrate any significant antiplatelet effect or reduce the antiplatelet activity of aspirin
Rimon *et al.* [12]	Healthy beagle dogs (10–12 kg, *n* = 6)	The dogs received 1.16 mg kg^{-1} aspirin once daily orally and/or 1.43 mg kg^{-1} celecoxib twice daily orally for 3 days, and samples were collected.	Platelet aggregation response by aggregometry	Administration of celecoxib to dogs interferes with the ability of a low dose of aspirin to inhibit arachidonic acid-induced *ex vivo* platelet aggregation.

other platelet agonists were found in clinical studies performed in healthy volunteers [4, 10] and in patients with osteoarthritis and stable ischaemic disease [3] who received different doses of aspirin and celecoxib (see Table 1).

Contrary to the results of these studies, celecoxib (at a dose corresponding to 140 mg human dose) did attenuate the anti-aggregation effects of high dose aspirin (at a dose corresponding to 322 mg human dose) in a dog model of thrombosis [11]. Detailed recent biochemical and pharmacological investigation revealed that celecoxib and other coxibs are able to bind quite tightly to one subunit (i.e. the regulatory subunit) of the COX-1 enzyme [12]. This binding did not affect the normal catalytic activity of the enzyme, but it did interfere with the inhibition of COX-1 by aspirin in experiments in intact cells and proteosomal preparations, and in studies with purified COX-1 enzyme *in vitro* [12]. Attenuation of the aspirin effect on *ex vivo* platelet aggregation was observed in samples that were collected from dogs treated with low dose aspirin (at a dose corresponding to 81 mg daily human dose) and celecoxib (at a dose corresponding to 100 mg twice daily human dose).

This controversy regarding the interaction between celecoxib and aspirin may originate from several reasons, related to the differences in the drug doses and administration times that were applied in the individual studies, and differences in the efficiency of platelet aggregation in humans *vs*. experimental animals. In addition, results of *ex vivo* platelet aggregation responses in the presence of potent aggregation inducers (e.g. arachidonic acid), that were applied in all the above-mentioned studies [3, 4, 10, 12], cannot be easily compared since different platelet agonists and laboratory instruments were used (see Table 1 and [13]). Due to use of aggregation inducers at high concentrations, this test may overestimate the *in vivo* platelet aggregation and its contribution to blood clotting. Other tests can be used for analysis of *in vivo* platelet COX-1 (e.g. serum TxB_2 levels [14–16]) and of low dose aspirin pharmacological activity (e.g. *in vivo* blood clotting time), but their use in clinical settings is uncommon.

Due to the wide clinical use of aspirin and celecoxib and the clinical importance of an interaction between these drugs, we sought to investigate this interaction and its clinical consequences. The objective of this study was to analyze the interaction between celecoxib and aspirin for COX-1 binding and its consequences for the aspirin-mediated antiplatelet effects at therapeutic doses and different administration schedules of these drugs. To this end, we established an *ex vivo* assay of interaction between celecoxib and aspirin for COX-1 binding and measured the resulting antiplatelet effects. Subsequently, we applied mechanism-based PK-PD modelling to analyze these data and to predict *in vivo* platelet aggregation for different doses and administration schedules of aspirin and celecoxib.

Methods

Volunteers

Healthy male and female volunteers were recruited as plasma donors for the purpose of this study. The inclusion criteria were age of 18–50 years, lack of chronic diseases (including blood clotting and cardiovascular diseases, metabolic disorders, infectious diseases, etc.), and no use of medications. The study was approved by the Soroka Hospital Research Ethics Committee (study approval number 0068–15-SOR) and conducted according to the Declaration of Helsinki. All volunteers gave written informed consent prior to their recruitment.

Materials

Aspirin and celecoxib were generously provided by Teva Pharmaceutical Industries Ltd (Kfar Saba, Israel). Arachidonic acid solution (5 mg ml^{-1}) was purchased from Helena Laboratories (Beaumont, TX, USA).

Platelet aggregation assay

We applied analysis of platelet aggregation in the 96-well plates format which has undergone extensive validation and comparison with the ‘standard’ tests of platelet aggregation by other researchers [17–19] and our research group. Blood samples (30–50 ml) were collected by venipuncture into tri-sodium citrate (3.2%) tubes, and were centrifuged at 180 *g* for 15 min to obtain platelet rich plasma (PRP). Aspirin (0–10 μM) and celecoxib (0–2 μM, each drug individually or together) or control solution (saline) were added to the PRP aliquots, and these samples were incubated at room temperature for 1 h. Then, 200 μl from each of the samples were transferred to 96 wells plate, and 25 μl of 5 mg ml^{-1} arachidonic acid solution (or 25 μl of saline for the negative control samples) were added to the wells. The plate was vortexed for 30 s, and immediately after that u.v. absorbance at 595 nm was determined using a plate reader (Model 680, Bio-Rad Ltd.) every 15 s for 16 min, while the plates were kept at 37°C and were subjected to vigorous shaking in the plate reader between the reading cycles [19]. Changes in absorbance were converted to % aggregation by reference to the absorbance of the negative control (PRP with saline, without aspirin or celecoxib) and of the positive control samples (PRP with arachidonic acid solution, without aspirin or celecoxib). The calculation of the platelet aggregation response was based on the endpoint value, without taking into account the slope of the graph, as commonly applied in clinical practice [20], especially when arachidonic acid is used as an aggregation agonist (that induces a single wave response) [21].

The PK-PD model and modelling analysis

The applied model was based on the available pharmacokinetic and pharmacodynamic data of COX-1 enzyme and its interaction with aspirin and celecoxib. The turnover of COX-1 enzyme and its interaction with the drugs (aspirin and celecoxib, see Figure 1) were assumed to follow the following equations:

$$\frac{dEA}{dt} = k_{ASA} \cdot C_{ASA} \cdot E - k_{out} \cdot EA \quad (1)$$

$$\frac{dE}{dt} = k_{in} - (k_{out} + k_{ASA} \cdot C_{ASA} + k_{CEL} \cdot C_{CEL}) \cdot E + k_{off} \cdot EC \quad (2)$$

$$\frac{dEC}{dt} = k_{CEL} \cdot C_{CEL} \cdot E - (k_{out} + k_{off}) \cdot EC \quad (3)$$

where *EA*, *E*, and *EC* are the concentrations of aspirin-bound, unbound and celecoxib-bound COX-1 enzyme, respectively, C_{ASA} and C_{CEL} are the plasma concentrations of aspirin and celecoxib, respectively, that were known in *ex vivo* studies, or were calculated in *in vivo* simulations assuming one compartmental pharmacokinetic behaviour (see Table 2; equations not shown), k_{ASA} and k_{CEL} are the second order rate constants of aspirin and celecoxib binding to the COX-1 enzyme (the rate of reaction is proportional to the concentration of the individual reactants, C_{ASA} and E, and C_{CEL} and E, respectively), k_{off} is the COX-1–celecoxib complex dissociation rate constant, k_{in} is the zero order rate constant of COX-1 production and k_{out} is the first order rate constant of COX-1 elimination that equals k_{in} divided by the plasma COX-1 concentration in the absence of drug treatment ($[COX\text{-}1]_0$). Similar to previous studies [5, 8], it was assumed that the degradation kinetics of the COX-1 enzyme was not affected by its association with the studied drugs (i.e. k_{out} represents the degradation rate constant of the unbound, aspirin-bound, and celecoxib-bound COX-1 enzyme).

The platelet aggregation effect was assumed to be exerted by the unbound and celecoxib-bound COX-1 enzyme (*E* and *EC*, respectively, and not the aspirin-bound enzyme) and was calculated as % of the initial platelet aggregation effect (in absence of drugs) using the following equation:

$$\text{Effect} = \frac{E + EC}{[COX\text{-}1]_0} \cdot 100\% \quad (4)$$

where it is assumed that $[COX\text{-}1]_0$, the plasma COX-1 concentration in absence of drug treatment, is equal to the sum of concentrations of COX-1 enzyme in all of its forms (*E*, *EC*, and *EA*).

Estimation of the k_{ASA}, k_{CEL} and k_{off} values was performed based on simultaneous analysis of the *ex vivo* platelet aggregation data points from aspirin and celecoxib samples (each drug individually or together) and the positive and negative controls using Monolix 4.2.1 software (Lixoft, Orsay, France) with maximum likelihood estimation based on a SAEM (stochastic approximation version of EM) algorithm with a Markov Chain Monte Carlo (MCMC) procedure. Success of the individual model was judged by the fits (observed *vs.* predicted data), visual predictive checks, the percentage of the standard errors of the parameters and the values of the Akaike Information Criterion and the Bayesian Information Criterion. The fitted parameters were assumed to be log-normally distributed. A combined (additive and proportional components) model of variance was used to describe the residual variability of the parameters.

Model-based predictions

All predictions were executed utilizing MATLAB® 7.11 (The MathWorks, Inc., Natick, MA, USA). The system of equations was based on equations 1–4, turnover of platelet COX-1 enzyme and the pharmacokinetics of aspirin and celecoxib (see Table 2). These equations were solved using MATLAB®'s ode23s command, a variable order method for solving a system of stiff differential equations.

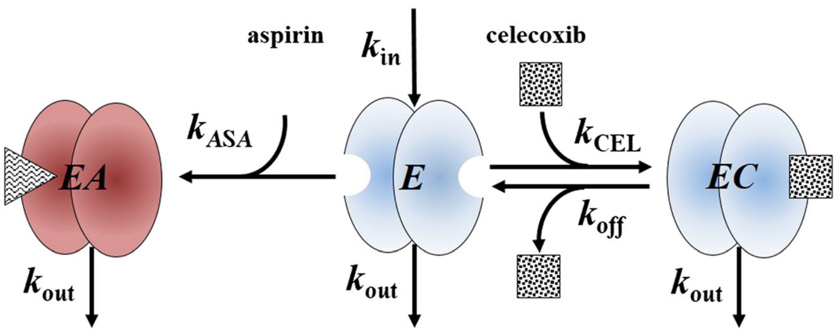

Figure 1

The model of aspirin and celecoxib interaction with two different subunits of COX-1 enzyme. Free and celecoxib-bound COX-1, but not the aspirin-bound COX-1, contribute to platelet aggregation

Table 2

The pharmacokinetic and pharmacodynamic parameters that were collected from the scientific literature or were estimated. The coefficients of variation for the estimated parameters are presented in brackets

Group	Parameter	Units	Value	Source
ASA PK	k_a ASA	h^{-1}	1.1	Hong *et al.* [5]
	k_{10} ASA	h^{-1}	2.1	
	V/F ASA	l	10.0	
CEL PK	k_a CEL	h^{-1}	1.084	Celecoxib prescribing information [33]
	k_{10} CEL	h^{-1}	0.0619	
	V/F CEL	l	429	
COX-1 turnover	$[COX\text{-}1]_0$	μM	$2.676 \cdot 10^{-4}$	Bautista *et al.* [34]
	k_{in}	μM h^{-1}	$1.208 \cdot 10^{-6}$	
ASA and CEL interaction with COX-1	k_{ASA}	1/(μM·h)	0.124 (14%)	Estimated
	k_{CEL}	1/(μM·h)	12.32 (31%)	
	k_{off}	h^{-1}	19.84 (52%)	

Sensitivity analysis

We used the developed PK-PD model to predict the effect of alterations of individual parameter values (increase or decrease by 10%) on the antiplatelet effect of low dose aspirin during co-treatment with celecoxib in *ex vivo* and *in vivo* experimental settings. *In vivo* sensitivity analysis was based on simulations for a patient who constantly receives 81 mg day^{-1} aspirin and 100 mg celecoxib twice daily (the first daily dose of celecoxib and the daily dose of aspirin are taken at the same time).

Comparison of the model-based predictions with the results of previous studies

The experimental settings (i.e. drugs doses, administration and sampling times) of the clinical and pre-clinical studies that investigated effect of celecoxib on aspirin antiplatelet effects (see Table 1) were used as inputs for the developed PK-PD model. The time course of predicted antiplatelet effects was compared with the results reported in the individual studies.

Model-based simulations of aspirin-celecoxib co-treatment scenarios

We predicted the time courses of plasma drug concentrations and of platelet aggregation for different administration schedules of aspirin and celecoxib using the developed PK-PD model. The administration schedules included 75–325 mg daily aspirin doses, alone or in combination with 200 mg daily celecoxib doses, at the same time or at different times during the same day. In all these simulations, area under the effect *vs.* time curve (AUEC) values were calculated (i.e. area below the 100% platelet aggregation *vs.* time line).

Results

Ex vivo interaction between aspirin and celecoxib and its description by the PK-PD model

The data obtained in *ex vivo* experiments (the observed values, Figure 2) indicate that celecoxib interfered with the antiplatelet effects of aspirin in a concentration-dependent fashion. For instance, platelet aggregation increased from 74.8 ± 4.6 to 93.0 ± 18.4 (2.5 μM aspirin alone or in presence of 2 μM celecoxib) and from 2.0 ± 2.0 to 56.5 ± 5.9 (10 μM aspirin alone or in presence of 2 μM celecoxib). The developed PK-PD model described appropriately the platelet aggregation data observed *ex vivo* for different concentrations of aspirin and celecoxib, except of a single data point (10 μM aspirin and 0 μM celecoxib, see Figure 2A) and allowed prediction of the extent of drug interactions for a wide range of concentrations of interacting drugs (see Figure 2B). The values of the estimated pharmacodynamic parameters (k_{ASA}, k_{CEL}, k_{off}) are shown in Table 2. Sensitivity analysis using the applied PK-PD model revealed that the magnitude of antiplatelet effects was more sensitive to the changes of k_{ASA}, as compared with k_{CEL} and k_{off} (7.3%, −2.4% and 2.2% change in platelet aggregation effect, respectively, following a 10% increase in the corresponding parameter value, see Figure 3A).

Comparison of the model-based predictions with the results of previous studies

Simulations using the developed PK-PD model predicted rapid increase and decline in aspirin plasma concentrations, and more gradual increase and decrease in plasma concentrations of celecoxib (see Figure 4). Indeed, due to the differences in the volume of distribution, elimination half-life values (see Table 2) and the doses,

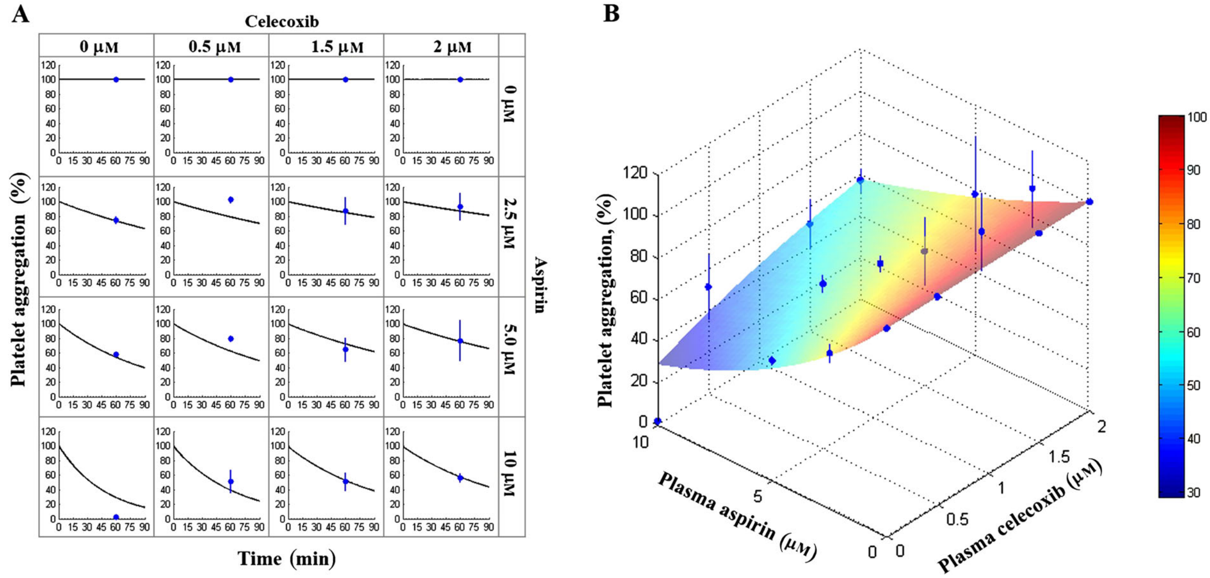

Figure 2

Aspirin-celecoxib observed *vs.* predicted data. Samples of platelet-rich plasma from healthy volunteers were incubated for 60 min with different concentrations of aspirin or/and celecoxib. Arachidonic acid was added and platelet aggregation was measured. The observed values are shown as blue dots and lines (average ± SD of four studies), and modelling-based predictions for studied aspirin and celecoxib concentrations (A, black lines) and for the wide range of aspirin and celecoxib concentrations (B, color-coded surface) are shown

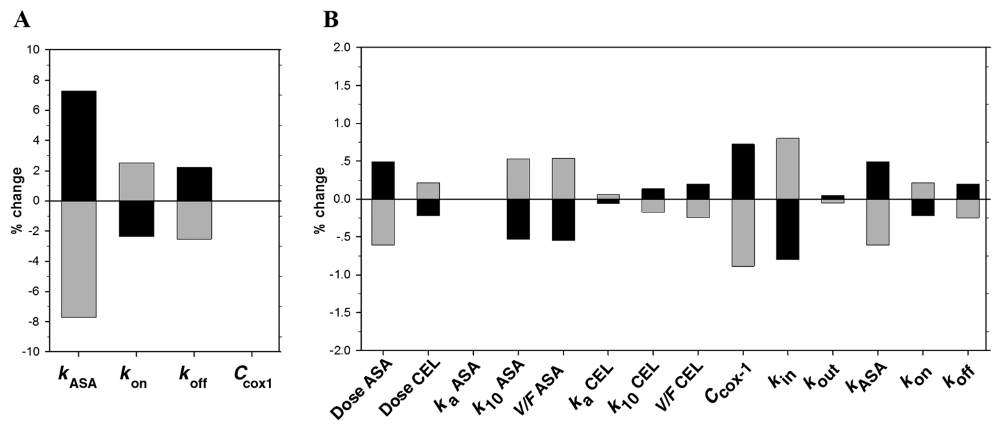

Figure 3

Sensitivity analysis: effect of alterations of individual parameter values (increase or decrease by 10%) on the anti-aggregation effect of low dose aspirin during co-administration with celecoxib. (A) *Ex vivo* sensitivity analysis and (B) *in vivo* sensitivity analysis, based on simulations for a patient who is constantly treated with 81 mg day^{-1} aspirin and 100 mg twice daily celecoxib (the first daily dose of celecoxib is given together with aspirin). (▬) 10% increase, (▭) 10% decrease

pharmacokinetic curves of aspirin were characterized by higher fluctuations (higher peak and lower trough values), as compared with celecoxib.

The developed PK-PD model predicted some increase in platelet aggregation *in vivo* due to administration of celecoxib (i.e., aspirin *vs.* aspirin with celecoxib administration) for experimental settings of studies by Wilner *et al.* (from 9.2% to 30.5% at 8 h, Figure 4A) and Gladding *et al.* (from 14.7% to 34.0% at 48 h, Figure 4C). On the other hand, a minor change in platelet aggregation due to administration of celecoxib was predicted for the experimental settings of studies by Renda *et al.* (from 10.0% to 13.1% at 48 h, Figure 4B) and Rimon *et al.* (from 10.0% to 11.6% at 24 h, Figure 4D).

Model-based simulations of aspirin-celecoxib co-treatment scenarios

Simultaneous administration of aspirin (100 mg day−1) and celecoxib (200 mg day^{-1}) is expected to produce aspirin plasma concentrations in the 0–11.5 μM range (without accumulation between the daily doses) and celecoxib plasma concentrations in the 0.38–1.35 μM range (with some accumulation between the daily doses; Figure 5A).

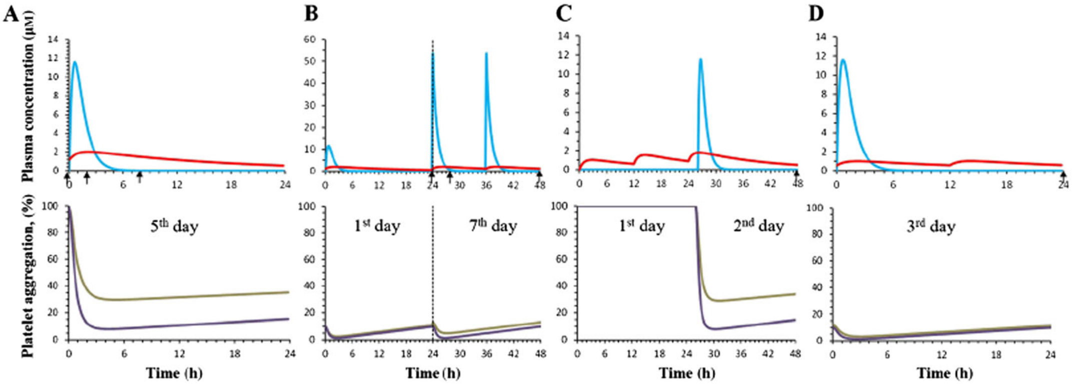

Figure 4

Modelling-based predictions based on protocols of previous studies of aspirin and celecoxib interaction (see Table 1). (A) Wilner *et al.* [10], (B) Renda *et al.* [3], (C) Gladding *et al.* [4], (D) Rimon *et al.* [12]. Arrows, sample collection times. Upper graphs: (—) ASA, (—) CEL; lower graphs: (—) ASA+CEL, (—) ASA.

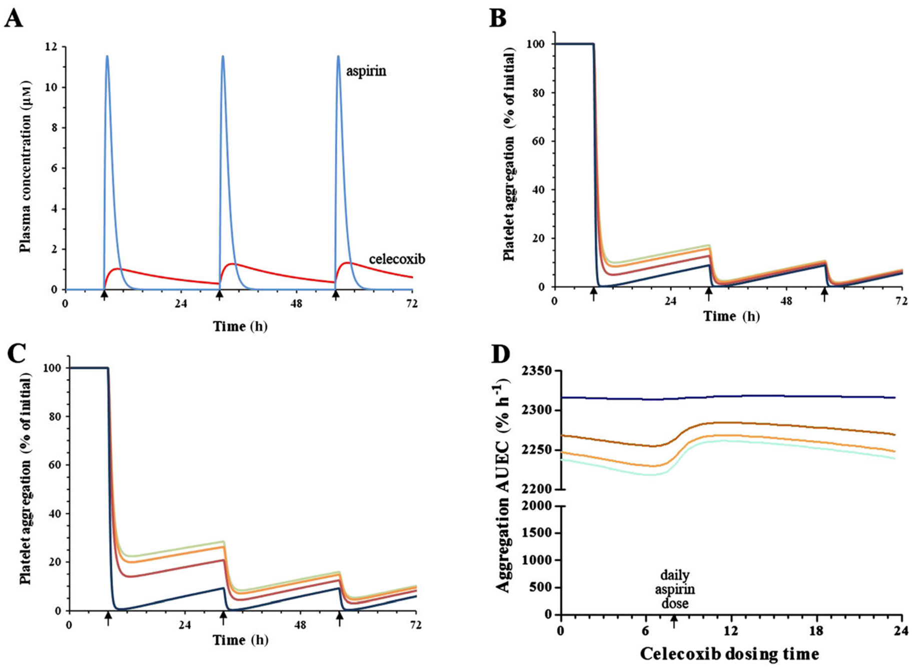

Figure 5

Modelling-based predictions of different clinical scenarios. (A) predicted plasma concentrations of aspirin (81 mg day^{-1}) and celecoxib (200 mg day^{-1}) in a typical patient, (B) predicted time course of platelet aggregation in a patient who starts to take different daily doses of aspirin, (C) predicted time course of platelet aggregation in a patient who starts to take different daily doses of aspirin in combination with 200 mg day^{-1} celecoxib and (D) predicted efficiency of platelet aggregation in a patient constantly treated with different daily doses of aspirin (at 08.00 h) in combination with 200 mg day celecoxib (simultaneously with aspirin or at other dosing times, see the arrows). (—) 75mg, (—) 81 mg, (—) 100 mg, (—) 325 mg

In absence of celecoxib treatment, 75–325 mg day^{-1} aspirin administration is expected to decrease the platelet aggregation in a dose-dependent fashion on the first day of treatment, but starting from the second day and after that, these aspirin doses are expected to induce antiplatelet effects of similar magnitude (Figure 5B). Addition of 200 mg day^{-1} celecoxib to the treatment schedule (administered simultaneously with the daily dose of aspirin) is expected to interfere substantially with the antiplatelet effects of aspirin on the first treatment day, but the extent of this interaction is expected to decline on subsequent treatment days (Figure 5C).

At steady-state conditions (75–325 mg day^{-1} aspirin and 200 mg day^{-1} celecoxib), the timing of aspirin *vs.* celecoxib daily dose had a minor effect on the predicted aspirin anti-aggregation effects (see Figure 5D). The highest dose of aspirin (325 mg day^{-1}) is expected to induce the maximal extent of antiplatelet effects that are not mitigated by celecoxib administration (simultaneously with aspirin or at other times during the day).

For lower aspirin doses (75–100 mg day^{-1}), timing of celecoxib administration of 200 mg day^{-1} celecoxib is expected to affect to a limited extent the magnitude of interaction between these drugs (e.g. 2220%·h for celecoxib administered 2 h before aspirin *vs.* 2262%·h for celecoxib administered 2 h after aspirin, the minimal and maximal antiplatelet effects, respectively; i.e. 1.9% difference in the AUEC values, see Figure 5D).

In vivo sensitivity analysis

The results of the sensitivity analysis indicate that at steady-state conditions (81 mg day^{-1} aspirin and 100 mg celecoxib twice daily) the alterations in several parameters can affect the extent of interaction between the studied drugs (Figure 5B). The pharmacokinetic factors with the highest impact were the dose of aspirin, its volume of distribution and elimination rate constant (Dose ASA, *V/F* ASA, k_{10} ASA). The change in the absorption rate constants of both drugs (k_a ASA, k_a CEL) and in the pharmacokinetic parameters of celecoxib had much lower impact on the antiplatelet effects. The pharmacodynamic factors with the highest impact were the COX-1 turnover parameters ($[\text{COX-1}]_0$, k_{in}) and the rate constant of COX-1 association with aspirin (k_{ASA}).

Discussion

Our study is based on the results of previous investigations that analyzed an interaction between aspirin and ibuprofen. In these studies, ibuprofen was found to interfere substantially with the antiplatelet effects of low dose aspirin [5, 8]. Subsequently, it was suggested that other NSAIDs that can bind the COX-1 enzyme ('COX-1 selective', 'COX-2 selective' and non-selective agents), can interfere with aspirin binding and acetylation of COX-1 and increase the risk of thromboembolic effects (heart attacks and strokes) [22]. The major parameters that govern the extent of this drug–drug interaction are the pharmacokinetic properties of the individual drugs, drug doses and their timing, and drug–COX-1 enzyme affinities.

To determine the interaction between celecoxib and aspirin due to competition for COX-1 binding, we applied the previously published mechanism-based PK-PD model [5, 8], to analyze *ex vivo* experimental data and to predict *in vivo* antiplatelet effects. Our analysis revealed that celecoxib can efficiently mitigate the antiplatelet effects of aspirin *ex vivo* (Figure 2). However, the magnitude of this interaction in *in vivo* settings was limited (Figure 4 and 5). The major factors that were found to affect *in vivo* platelet aggregation were the rate of turnover of COX-1 enzyme *vs.* the changes in the plasma concentrations of the investigated drugs (both these factors are absent in *in vitro* settings). The combined effects of the individual parameters on platelet aggregation *in vivo* are complex, and specialized tools (PK-PD models and appropriate software) are needed to reveal the interaction between aspirin and celecoxib at the clinically relevant doses and administration schedules of these drugs.

PK-PD modelling results indicate that that celecoxib can attenuate to a limited extent the *in vivo* antiplatelet effects of low dose aspirin. The extent of this interaction can be substantial during the first days of aspirin administration in patients who are already treated with celecoxib, and it cannot be prevented by separate administration of the interacting drugs. On the other hand, for a patient treated chronically with low dose aspirin, addition of celecoxib at the recommended therapeutic doses is not expected to mitigate the aspirin antiplatelet effects. At high doses or overdose, however, celecoxib will be able to compete efficiently with aspirin for COX-1 binding *in vivo*, and can interfere with the antiplatelet effects of low dose aspirin. Temporary increase in aspirin dose can be used to prevent increased platelet aggregation after addition of celecoxib. However, decisions regarding these aspirin dose adjustments should take into account celecoxib dose and administration time (i.e. higher increase in aspirin dose for higher doses of celecoxib, especially if it is taken simultaneously with aspirin, Figure 5).

The mechanistic PK-PD modelling approach that was applied in this study allows detailed and systematic analysis of the COX-1-mediated platelet aggregation pathway and its modulation by specific drugs. However, the PK-PD modelling based predictions should be verified in carefully planned clinical studies to determine the antiplatelet effects in patients taking different doses of aspirin and celecoxib. The consequences of this interaction as a risk factor for vascular thrombotic events (heart attacks and strokes) in patients concomitantly treated with aspirin and celecoxib also require clarification. Due to the complexity of thrombosis mechanisms [2, 23, 24], reduced platelet aggregation has a limited effect on the risk of vascular thrombotic events. Hence, specially designed clinical studies (long duration prospective or retrospective studies with high numbers of participants) are required to establish the effect of the aspirin–celecoxib interaction on the incidence of vascular thrombotic events.

It is possible that some categories of patients are especially prone to the celecoxib-induced inhibition of aspirin function and are at higher risk of vascular thrombotic events than others. Specifically, patients with low exposure to aspirin (due to reduced bioavailability, reduced absorption due to food–drug or drug–drug interactions, or low adherence to aspirin treatment) and high exposure to celecoxib (due to enhanced bioavailability or overdose) will maintain efficient platelet aggregation activity and may be at risk of heart attacks and strokes. Reduced sensitivity to aspirin was found in patients with type two diabetes [23, 25–27], metabolic syndrome [28] and other conditions [29], and patients with these pathologies may have increased risk of vascular thrombotic events. Twice daily dosing of low dose aspirin was demonstrated in several studies to maintain efficient antiplatelet effects in diabetes two patients and to prevent diabetes complications [30–32]. Interaction of celecoxib with aspirin at these dosing schedules and its effects on platelet aggregation and on risk of heart attacks and strokes in diabetes patients should be investigated in future clinical studies. It is possible that the interaction of aspirin with celecoxib will pose substantial risks for the above-mentioned or other patient groups, and will require changes in doses of these drugs or switching to other pharmacological agents. In addition, interaction of aspirin with other coxibs (e.g. etoricoxib, valdecoxib, parecoxib, lumiracoxib) and NSAIDs requires detailed investigation in an *in vitro*, *ex vivo* and *in vivo* setting to reveal the risks of this interaction in different groups of patients with and without cardiovascular risk factors (such as diabetes, metabolic diseases, etc.). Currently, we are performing experiments to determine competition of other NSAIDs (rofecoxib, etoricoxib, ibuprofen, naproxen, NS-398) with aspirin for COX-1 binding. Results of these studies (obtained in *ex vivo* experimental settings) and their clinical implications will be reported in our future publications.

In conclusion, celecoxib, a selective inhibitor of the COX-2 enzyme, interacts with the COX-1 enzyme and can potentially interfere with its inhibition by aspirin. We measured *ex vivo* the interaction between aspirin and celecoxib and applied a mechanism-based PK-PD model to analyze these data and to predict the interaction between aspirin and celecoxib at therapeutic doses and different administration schedules of these drugs. The modelling results indicate that celecoxib can attenuate to a limited extent the *in vivo* antiplatelet effects of aspirin. The extent of this interaction can be substantial during the first days of aspirin administration in patients who are already treated with celecoxib, and it cannot be prevented by separate administration of the interacting drugs. Implications of an interaction between low dose aspirin and celecoxib for the risk of cardiovascular and cerebrovascular events in patients who receive these drugs warrant detailed analysis. Moreover, interaction between low dose aspirin and other COX-2 inhibitors and its clinical consequences require further investigation.

Competing Interests

There was no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

We wish to thank the volunteers who participated in this study.

We thank Professor Tim Warner (The William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK) for his valuable recommendations on ex vivo measurement of antiplatelet effects of the studied drugs.

Contributors

All authors provided critical review of all drafts of the manuscript, had full editorial control of the manuscript and provided their final approval of all content. MR and DS contributed to the design and execution of the study, and analysis and interpretation of data. GR contributed to the design of the study, and interpretation of data. OP contributed to the design and execution of the study, and interpretation of data.

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