ANALYSIS OF A COMPLEX PHYSIOLOGY-DIRECTED MODEL FOR INHIBITION OF PLATELET AGGREGATION BY CLOPIDOGREL

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In memory of Paul Fife, friend and much admired colleague

Abstract. Clopidogrel is an anti-platelet compound that is widely used with aspirin to reduce the risk of cardiovascular incidents. In itself it is inactive; only after a biotransformation into its active metabolite clop-AM, does it inhibit platelet aggregation. Recently a system-pharmacological model has been proposed for the network of processes leading to reduced platelet aggregation. In this paper we present a mathematical analysis of this model and demonstrate how the complex pharmacokinetic model can be reduced to two simple coupled models, one for clopidogrel and one for clop-AM, yielding insight into the dynamics of clop-AM and the impact of inter-individual differences on the level of inhibition.

1. Introduction. Cardiovascular diseases are the leading cause of death in the United States and worldwide [27], [15]. Acute coronary syndrome (ACS), a life threatening disorder with an estimated 780,000 incidences and about 1.2 million hospitalisations in the United States each year, is associated with high morbidity and mortality [2]. Platelet activation and aggregation play a major role in thrombus formation and the pathophysiology of atherothrombosis, atherosclerosis and their coronary manifestations, necessitating the use of antiplatelet therapy to minimise the risk of adverse cardiovascular events [22].

Clopidogrel (Plavix) is a second generation thienopyridine antiplatelet agent approved by the U.S. Food and Drug Administration (FDA) in 1997, and is widely prescribed as a dual antiplatelet regimen along with aspirin to reduce the risk of cardiovascular death, recurrent stroke or myocardial infarction (MI) in patients with ACS [2], [21].

Although clinically effective in many patients, substantial inter-individual variability in platelet aggregation and clinical response to clopidogrel’s one size fits all
dosing regimen (300 mg loading dose, 75 mg maintenance dose) has been a long-standing clinical issue \cite{2}, \cite{21}. High on-treatment platelet reactivity and recurrent adverse cardiac events are observed in 5-40\% patients, while life-threatening and fatal bleeding incidences due to excessive platelet inhibition have also been observed \cite{7}, \cite{1}.

Multiple genetic and non-genetic factors that have the potential to affect the pharmacokinetics (PK) and/or pharmacodynamics (PD) of clopidogrel have been investigated as sources of between-subject variability in clopidogrel antiplatelet therapy \cite{3}, \cite{11}. Of these factors, genetic polymorphisms in metabolizing enzymes as well as drug-drug interactions are thought to be most important for response to clopidogrel antiplatelet therapy as well as the associated between subject variability \cite{13}, \cite{8}.

Clopidogrel is an orally administered prodrug that requires a two-step biotransformation to its pharmacologically active metabolite, which is mediated by five cytochrome P450 (CYP) enzymes \cite{13}, \cite{8}. In addition, clopidogrel as well as its active metabolite are inactivated by carboxyl esterase 1 (CES1) \cite{30}. Of these enzymes, CYP2C19 and associated genetic polymorphisms have received the biggest attention so far. This is highlighted by the fact that the FDA has issued a boxed warning for CYP2C19 mutation carriers, in particular for homozygous carriers of the CYP2C19 loss-of-function alleles, due to their inability to convert clopidogrel to its active metabolite to the same extent as CYP2C19 wild-type carriers \cite{6}, \cite{26}.

Clinical evidence suggests, however, that only 10-15\% of the between subject variability in response to clopidogrel treatment are explained by CYP2C19 polymorphisms \cite{26}. This highlights the need to also identify and consider other clinically important factors \cite{11}.

In order to account for the combined impact of multiple factors on the dose-concentration-response relationship, Jiang et al. \cite{10} developed a new system-pharmacological model that, in contrast to earlier models (cf. \cite{5}, \cite{29}), exhibits the dynamics of clopidogrel as well as of clop-AM, and integrates the action of multiple enzymes and their genetic polymorphisms.

The present paper focusses on the mathematical structure of the complex model introduced by Jiang et al. \cite{10}. Very recently this model was refined to include, for instance, plasma-protein binding \cite{12}. The new model still has the same structure as the basic model. Hence, for the sake of transparency, we analyse the simpler basic model proposed in \cite{10}; the results are easily extended to the more recent model. For the same reason, we also adopt the notation of the basic model.

It is shown how, thanks to the different orders of magnitude of the parameters found by fitting the model to data, the pharmacokinetic model can effectively be reduced to two simple coupled models, one for clopidogrel and one for it’s active metabolite clop-AM, making it possible to localise the impact of inter-individual differences of CYP enzyme-activity and assess their importance.

2. The model. In this paper we are using an approach to modeling that combines principles from conventional population PK/PD analysis and true physiologically-based pharmacokinetic modeling as we borrow physiological information on e.g. liver size and liver blood flow and use them as structural parameters in our population model. This is also the reason these models are often referred to as minimal PBPK models (For further background, see \cite{15}, \cite{17}, \cite{18}, \cite{28}).

Clopidogrel reaches the liver via a series of metabolic steps where - through enzymatic action - it is converted into an active metabolite, clop-AM. This active
form is exchanged with plasma, where it binds the P2Y12 receptor on the platelets, inhibiting aggregation. However, in the liver a great deal (85 to 90%) of clopidogrel is also hydrolysed and thereby lost for platelet inhibition. In the end, only about 2% of the drug that is administered finally reaches the platelets.

In Figure 1 we present a schematic outline of the structure of the model: a pharmacokinetic (PK) model involving clopidogrel and its active metabolite clop-AM, and a pharmacodynamic (PD) model which describes the action of clop-AM on platelet aggregation.

![Figure 1. Schematic model of clopidogrel action on platelet aggregation: clopidogrel travels from the gut to the liver, where one fraction (ECES1) is hydrolysed into an inactive metabolite, one fraction (ECYP) is transformed into the active metabolite clop-AM and one fraction FH goes into systemic circulation. Clop-AM also goes into systemic circulation where it binds to receptors situated on the platelets and thus inhibits platelet aggregation. The compartments are numbered 1 to 6; the amounts of clopidogrel in the gut, the liver and plasma are denoted by, respectively, A1, A2 and A3 and the amounts of clop-AM in the liver and in plasma by A4 and A5. The platelet reactivity in compartment 6 is denoted by P.](image)

In the following two subsections we introduce the PK and the PD model.

2.1. The pharmacokinetic model. The PK model describes the dynamics of clopidogrel and its active metabolite, clop-AM, in the various locations: the gut, the liver and in plasma. The amount of clopidogrel in these locations is denoted by A1 (in the gut), A2 (in the liver) and A3 (in plasma), and the amount of clop-AM by A4 (in the liver) and A5 (in plasma). The quantities A1, . . . , A5 are measured in µmol.

Clopidorel is administered orally through a bolus dose. If its amount is D µmol, then a fraction Fa × D will enter the gut, where 0 < Fa ≤ 1 is called the fraction of absorption. It is found that clopidogrel reaches systemic circulation after a certain delay. This process is modelled by inserting three transit compartments. Denoting the amount of clopidogrel in these transit compartments by T1, T2 and T3, drug
transport to the liver is described by the following system of equations, involving the rate constant $k_a$:

$$
\begin{align*}
\frac{dA_1}{dt} &= -k_a A_1, \\
\frac{dT_1}{dt} &= k_a (A_1 - T_1), \\
\frac{dT_2}{dt} &= k_a (T_1 - T_2), \\
\frac{dT_3}{dt} &= k_a (T_2 - T_3).
\end{align*}
$$

(1)

The system (1) can be solved sequentially. Plainly, $A_1(t) = A_1(0) e^{-k_a t}$. When this solution is used in the second equation one obtains an expression for $T_1(t)$, which in turn yields $T_2(t)$ and subsequently $T_3(t)$ (see also Savic et al. [23]). Since $A_1(0) = F_a \times D$ and the transit compartments are assumed to be empty prior to drug administration, i.e., $T_i(0) = 0$ ($i = 1, 2, 3$), the solution of the system (1) is found to be

$$
T_n(t) = \frac{1}{n!} (k_a t)^n e^{-k_a t} \cdot F_a \cdot D, \quad n = 1, 2, 3.
$$

(2)

2.1.1. Clopidogrel. As explained above, drug administered orally is eventually deposited into the liver according to the time-dependent infusion $I_n(t) = k_a T_3(t)$. Thus, by the expression for $T_3(t)$ from (2) we obtain

$$
I_n(t) = F_a \cdot D \cdot f(t) \quad \text{where} \quad f(t) \overset{\text{def}}{=} \frac{1}{3!} k_a (k_a t)^3 e^{-k_a t}.
$$

(3)

For the amount of clopidogrel in the liver ($A_2$) we then have the following turnover equation:

$$
\frac{dA_2}{dt} = I_n(t) - Q_H \left( \frac{A_2}{V_H} - \frac{A_3}{V_3} \right),
$$

(4)

where $A_3$ denotes the amount of clopidogrel in plasma. Here $Q_H$ denotes the flux of the hepatic plasma flow, $V_H$ the volume of the liver and $V_3$ the volume of distribution of clopidogrel in plasma.

In the liver, clopidogrel is involved in two processes: (i) it is converted into an active metabolite R-130964 (clop-AM) through enzymatic processes involving CYP-enzymes, and (ii) it is hydrolysed by liver-specific carboxyl esterase 1 (CES1) into an inactive form. Thus, the loss term in equation (4) is composed of three terms: one for the fraction $E_{H,CYP}$ that is converted to clop-AM, one for the fraction $E_{H,CES}$ that is inactivated through hydrolysis and the remaining fraction, $F_H = 1 - (E_{H,CYP} + E_{H,CES})$ that goes into systemic circulation. Thus, we can write equation (4) as

$$
\frac{dA_2}{dt} = I_n(t) - Q_H E_{H,CYP} \frac{A_2}{V_H} - Q_H E_{H,CES} \frac{A_2}{V_H} - Q_H F_H \frac{A_2}{V_H} + Q_H \frac{A_3}{V_3}. \quad (5)
$$

The amount of clopidogrel in systemic circulation ($A_3$) thus evolves according to the turnover equation

$$
\frac{dA_3}{dt} = Q_H F_H \frac{A_2}{V_H} - Q_H \frac{A_3}{V_3}. \quad (6)
$$

The clearance through CYP-enzymes is saturable at therapeutic dose levels and is modelled by the nonlinear Michaelis-Menten function

$$
CL_{int,CYP} = \frac{V_{\text{max,CYP}}}{K_{m,CYP} + (A_2/V_H)}.
$$

(7)
where $V_{\text{max,CYP}}$ is the maximal clearing and $K_{m,CYP}$ the Michaelis-Menten constant. On the other hand the clearance by the process involving carboxyl esterase 1 (CES1), is approximately linear because of the high capacity of this pathway. Therefore, it is modelled by a constant, $CL_{\text{int,CES1}}$.

Thus, the fraction of clopidogrel which is converted into clop-AM ($E_{H,CYP}$) is given by

$$E_{H,CYP} = \frac{CL_{\text{int,CYP}}}{Q_H + CL_{\text{int,CES1}} + CL_{\text{int,CYP}}} \quad (8)$$

and the fraction of clopidogrel which is hydrolysed ($E_{H,CES1}$) is given by

$$E_{H,CES1} = \frac{CL_{\text{int,CES1}}}{Q_H + CL_{\text{int,CES1}} + CL_{\text{int,CYP}}} \quad (9)$$

The remaining fraction $F_H$, which goes into systemic circulation, i.e., into blood plasma, is then given by

$$F_H = \frac{Q_H}{Q_H + CL_{\text{int,CES1}} + CL_{\text{int,CYP}}} \quad (10)$$

**Remark.** We conclude from equations (5) and (6) that the total amount of clopidogrel in the liver, $A_2$ and in plasma $A_3$, evolve according to the equation

$$\frac{d}{dt}(A_2 + A_3) = In(t) - Q_H (E_{H,CYP} + E_{H,CES1}) \frac{A_2}{V_H}, \quad (11)$$

i.e., a balance between supply from the gut and loss through hydrolysation and conversion into the active metabolite clop-AM.

### 2.1.2. The active metabolite clop-AM.

The dynamics of active metabolite in the liver (amount $A_4$) is described by a turnover equation in which clop-AM is supplied through the action of CYP-enzymes, as described by equations (7) and (8), and lost to systemic circulation, from which some comes back again:

$$\frac{dA_4}{dt} = Q_H E_{H,CYP} \frac{A_2}{V_H} - Q_H \frac{A_4}{V_H} + Q_H \frac{A_5}{V_5} \quad (12)$$

The temporal behaviour of clop-AM in systemic circulation (amount $A_5$) is modelled by a turnover equation involving the exchange with the liver as well as direct clearance at the rate $CL_{50}$:

$$\frac{dA_5}{dt} = Q_H \frac{A_4}{V_H} - Q_H \frac{A_5}{V_5} - CL_{50} \frac{A_5}{V_5} \quad (13)$$

**Remark.** The total amount of clop-AM in the liver and plasma is therefore given by

$$\frac{d}{dt}(A_4 + A_5) = Q_H E_{H,CYP} \frac{A_2}{V_H} - CL_{50} \frac{A_5}{V_5} \quad (14)$$

i.e., supply from the liver and loss due to direct elimination.

**Remark.** It is seen that, although the full PK system involves 5 turnover equations for the quantities $A_1, \ldots, A_5$, it can be divided into two sub-models, one for clopidogrel and one for clop-AM:

1. The system pertaining to clopidogrel, which involves the quantities $A_1, T_1, T_2, T_3$ and $A_2 \& A_3$, is independent of the system pertaining to the active metabolite clop-AM involving $A_4$ and $A_5$.  

(2) The clopidogrel system is *nonlinear* because of the enzymatic conversion through CYP enzymes, and the clop-AM system is *linear* in \(A_4\) and \(A_5\), with a forcing term driven by the amount of clopidogrel in the liver \((A_2)\).

**Steady state concentrations:** We conclude from equation (14) that at steady state the clop-AM concentration \((C_5)\) in plasma is given by

\[
C_5 = \frac{Q_H}{C \cdot L_{50}} E_{H,CYP} \cdot C_2,
\]

where \(E_{H,CYP}\) is determined by the clopidogrel concentration in the liver \(C_2\). In order to make the dependence on \(C_2\) transparent we use the expression for \(E_{H,CYP}\) in (8) and for \(CL_{int,CYP}\) in (7). This yields the function

\[
C_5 = F(C_2) \defeq \frac{Q_H}{C \cdot L_{50}} \frac{V_{max:CYP} \cdot C_2}{V_{max:CYP} \cdot C_2 + (Q_H + CL_{int:CES1})(K_{m:CYP} + C_2)}.
\]

Plainly \(F(0) = 0\) and

\[
\lim_{C_2 \to \infty} F(C_2) = \frac{Q_H}{C \cdot L_{50}} \frac{V_{max:CYP}}{Q_H + CL_{int:CES1}}.
\]

Thus, at steady state the clop-AM concentration increases with the clopidogrel concentration in a sigmoidal manner, as shown in Figure 2 for the parameter values given in Table 1 that are used for the simulations in Section 3.

![Graph of the clop-AM concentration in plasma \((C_5)\) versus the clopidogrel concentration in the liver \((C_2)\) based on equation (15) and parameter values given in Table 1.](image)

According to the simulations shown in Figure 2, the amount clopidogrel in plasma ranges over an interval \(0 < A_2 < 30 \mu\text{mol}\). Since \(V_H = 1.5 \text{ L}\) by Table 1, this implies that \(0 < C_2 < 2.0 \mu\text{M}\). Similarly, we see that the amount clop-AM in plasma \(A_5\) ranges over an interval \(0 < A_5 < 1.2 \mu\text{mol}\), and, since \(V_5 = 3 \text{ L}\) by Table 1, it follows that \(0 < C_5 < 0.4 \mu\text{M}\).
2.2. The pharmacodynamic model. In systemic circulation, clop-AM binds irreversibly to the P2Y12 receptor on platelets. This results in inactivation of the receptor and thus a decrease in the platelet reactivity. This reactivity is measured by what is called the maximal platelet aggregation \((MPA)\). Comparing this quantity with its baseline value \(MPA_0\) we introduce the relative platelet reactivity

\[
P = \frac{MPA}{MPA_0}.
\]  

(16)

The dynamics of this fraction \(P\) is modelled by a classical turnover equation (cf. [4]) in which clop-AM in plasma stimulates elimination and so reduces the platelet aggregation. Specifically, the impact of clop-AM is modelled by an additional irreversible elimination term which is linear in \(C_5\) and \(P\):

\[
dP/dt = k_{in} - k_{out}P - k_{irre}C_5 \cdot P, \quad C_5 = \frac{A_5}{V_5}.
\]  

(17)

Thus, the steady state value of the relative platelet reactivity \(P\) is given by

\[
P_{ss} = \frac{k_{in}}{k_{out} + k_{irre}C_5} \quad \text{and} \quad P_0 = \frac{k_{in}}{k_{out}} = 1.
\]  

(18)

Note that depending on the clop-AM concentration, the relative importance of the two elimination terms varies. They become equal when \(k_{out} = k_{irre}C_5\), i.e., when \(C_5 = k_{out}/k_{irre}\).

In Figure 3 we show a graph of this concentration-response relationship for the parameter values given in Table 2 with \(C_5\) on a logarithmic scale.

![Graph of the response \(P_{ss}\) versus the clop-AM concentration in plasma \((C_5)\) based on equation (18) and parameter values given in Table 2 with \(C_5\) on a logarithmic scale.](image)

Note that \(P_{ss}\) has dropped down to 50% of \(P_0\) (the dotted line) when \(C_5 = k_{out}/k_{irre} = 0.0019 \mu\text{M}\) (cf. Table 2).

3. Simulations. In order to acquire a first impression of the dynamics of clopidogrel and its active metabolite in the body we carry out simulations of the PK model and the PD model. This is done for realistic parameter values taken from Jiang et.
The PK parameters are listed in Table 1 and the PD parameters in Table 2.

Table 1: PK parameter estimates

| Parameter       | Unit | Estimate | CV % |
|-----------------|------|----------|------|
| $Q_H$           | L/h  | 50       | 0    |
| $V_H$           | L    | 1.5      | 0    |
| $F_a$           | –    | 0.5      | 0    |
| $k_a$           | 1/h  | 9.28     | 7.63 |
| $V_3$           | L    | 61.3     | 24.3 |
| $V_{\text{max:CYP}}$ | µmol/h | 314     | 23.2 |
| $K_m:CYP$       | µM   | 4.95     | 27.7 |
| $CL_{\text{int:CES1}}$ | L/h | 19400   | 19.5 |
| $CL_{50}$       | L/h  | 3.86     | 11.5 |
| $V_5$           | L    | 3        | 0    |

In Figure 4 we show how the amounts $A_1, \ldots, A_5$ evolve with time after an iv bolus dose of 300 mg, which corresponds to an amount of 931 µmol.

![Figure 4](image-url)

**Figure 4.** Temporal behaviour of $A_1(t), \ldots, A_5(t)$ according to the PK model with parameter values given by Table 1 after an iv bolus dose of 300 mg i.e., 931 µmol. In the left two panels values of $A_1 - A_5$ are given on a linear scale, and in the right panel they are given on a logarithmic scale.

We make the following observations:

1. The amount of clopidogrel in the liver ($A_2$) is significantly larger than that in plasma ($A_3$) (by a factor of $O(10)$).
2. The amount of clopidogrel in plasma ($A_3$) and the amount of clop-AM in the liver ($A_4$) and plasma ($A_5$) are comparable.
3. The half-life of the four compounds ($A_2, \ldots, A_5$) is the same.
4. The elimination of clopidogrel from the liver ($A_2$) proceeds in two phases: (i) In the first phase, which is relatively short, most of the clopidogrel is removed from the liver. (ii) In the second phase clopidogrel is eliminated much more slowly; in fact in this phase the half-life is the same as that of the $A_3, A_4$ and $A_5$.

In Figure 5 we show the corresponding behaviour of the relative maximal platelet activity $P$ according to the turnover model (17) with an irreversible inhibition term which is linear in the clop-AM plasma concentration $C_5$. 

![Figure 5](image-url)
Table 2: PD parameter estimates

| Parameter | Unit     | Estimate | CV % |
|-----------|----------|----------|------|
| $k_{in}$  | 1/h      | 0.00783  | 5.54 |
| $k_{out}$ | 1/h      | 0.00783  | 5.54 |
| $k_{irre}$| 1/$\mu$M/h | 4.06   | 4.14 |

Figure 5 shows two graphs of $P(t)$ and $C_5(t)$ versus time. The left one on a time scale corresponds to that used in Figure 4 and the right one on a much longer time scale in order to capture the return to baseline.

Observations:

(1) Inhibition of platelet aggregation, characterised by $P$, follows the clop-AM concentration in plasma $C_5$ after the initial peak inhibition takes a long time to return to baseline. Specifically, the time of maximal response of $P$ is about 4 h, whilst the time of maximal clop-AM concentration in plasma is about 0.3 h.

(2) The period over which platelet aggregation is inhibited is much longer than that of clop-AM in plasma and appears to be $O(10^2)$ h.

(3) According to Table 2, the reversible elimination rate $k_{out}$ is much smaller than the irreversible rate $k_{irre}$. Therefore, it takes only a small amount of clop-AM, i.e., a small value of $C_5$, for the irreversible elimination to dominate over the reversible elimination. In fact, the threshold lies at $C_5 = 1.93$ nM.

In the next section we present a mathematical analysis of the PK and PD models in order to obtain qualitative explanations and quantitative estimates for the observations made about the simulations, and find ways to reduce the full model into simpler models which yield insight into the dynamics of the system and make it possible to determine the way in which inter-individual differences may affect platelet activity and how to compensate for these differences.
4. **Mathematical analysis.** As we have seen in the previous section, after delivery of clopidogrel into the liver, it is possible to divide the model into two sub-models:

1. A model for the dynamics of clopidogrel in liver and plasma.
2. A model for the dynamics of clop-AM in liver and plasma.

As shown in (2) and (3), an initial iv bolus dose of \(D \mu\text{mol}\), results in the following drug input \(I_n(t)\) into the liver:

\[
I_n(t) = F_a \cdot D \cdot f(t) \quad \text{where} \quad f(t) = \frac{1}{3!} k_a (k_a t)^3 e^{-k_a t}.
\]

4.1. **Clopidogrel in liver and plasma.** In order to analyse the clopidogrel model, we transform the amounts \(A_2\) and \(A_3\) to dimensionless variables. We thus relate these amounts to relevant quantities. For \(A_2\) the dose \(D\) is a natural candidate. In light of the simulations shown in Figure 4 we relate \(A_3\) to a fraction of the drug dose \(\varepsilon D\), where \(\varepsilon\) is a small quantity that is yet to be selected. Thus, we put

\[
x_2 = \frac{A_2}{D} \quad \text{and} \quad x_3 = \frac{A_3}{\varepsilon D}.
\]

Introducing these variables into the equation (4) for \(A_2\) we obtain

\[
\frac{dx_2}{dt} = F_a f(t) - \frac{Q_H}{V_H} (x_2 - \varepsilon a x_3), \quad a = \frac{V_H}{V_3}.
\]

Similarly, the equation for \(A_3\) becomes

\[
\frac{dx_3}{dt} = \frac{Q_H}{V_H} (\varepsilon^{-1} F_H x_2 - a x_3).
\]

We now wish to choose \(\varepsilon\) so that it is comparable to \(F_H\). Recall from (10) that

\[
F_H = \frac{Q_H}{Q_H + CL_{int:CES1} + CL_{int:CYP}}.
\]

For the parameter values given in Table 1, we have

\[
CL_{int:CYP} < \frac{V_{max:CYP}}{K_m:CYP} \ll CL_{int:CES1}.
\]

Therefore, the function \(CL_{int:CYP}\) in the denominator of the expression for \(F_H\) may be neglected, so that

\[
F_H \approx \frac{Q_H}{Q_H + CL_{int:CES1}}.
\]

This suggests we define the constant \(\varepsilon\) by:

\[
\varepsilon = \frac{Q_H}{Q_H + CL_{int:CES1}}.
\]

With this choice of \(\varepsilon\) we may approximate equation (22) by

\[
\frac{dx_3}{dt} = \frac{Q_H}{V_H} (x_2 - a x_3).
\]

It remains to choose a dimensionless time. Since the factor \(Q_H/V_H\) appears as a factor in the equations for \(x_2\) and \(x_3\), a natural choice is

\[
\tau = \frac{Q_H}{V_H} \times t.
\]
Introducing this dimensionless time into the equations for \( x_2 \) and \( x_3 \) then yields the system
\[
\begin{align*}
\frac{dx_2}{d\tau} &= \phi(\tau) - x_2 + \varepsilon a x_3 \\
\frac{dx_3}{d\tau} &= x_2 - a x_3
\end{align*}
\] (28)

For the constants listed in Table 1, the dimensionless parameters \( \varepsilon, a \) and \( \frac{V_H}{Q_H} \) become:
\[
\varepsilon = 0.026, \quad a = 0.0245, \quad \frac{V_H}{Q_H} = 0.030.
\] (29)

Since \( \varepsilon \ll 1 \), we may to good approximation omit the term involving \( x_3 \) from the first equation in (28) and thus compute \( x_2(\tau) \) from the simple equation:
\[
\frac{dx_2}{d\tau} = \phi(\tau) - x_2 \quad \text{with} \quad x_2(0) = 0.
\] (30)

This problem can be solved explicitly. Having obtained \( x_2(\tau) \), it can be used in the second equation of (28) to derive an explicit expression for \( x_3(\tau) \).

In terms of the original variables, equation (30) translates back into
\[
\frac{dA_2}{dt} = F_a \cdot D \cdot f(t) - \frac{Q_H}{V_H} A_2 \quad \text{with} \quad A_2(0) = 0.
\] (31)

Since, \( \frac{Q_H}{V_H} = 33.3 \text{ h}^{-1} \) is larger than \( k_a = 9.28 \text{ h}^{-1} \), it follows that the terminal slope \( \lambda_z \) is given by \( k_a \) and the elimination half-life is
\[
t_{1/2} = \frac{\ln(2)}{k_a} = 0.075 \text{ h},
\] (32)

which agrees with the simulations shown in the left-panel of Figure 1.

4.2. Clop-AM in liver and plasma. The dynamics of clop-AM in the liver (\( A_4 \)) and in plasma (\( A_5 \)) is driven by \( A_2 \) and described by the equations (12) and (13). Since for the parameter values of Table 1, \( E_{H,CYP} = O(\varepsilon) \), it is reasonable to expect that \( A_4 \) and \( A_5 \) are \( O(\varepsilon D) \). Hence we put
\[
x_4 = A_4 \frac{1}{\varepsilon D} \quad \text{and} \quad x_5 = A_5 \frac{1}{\varepsilon D}.
\] (33)

Introducing these variables into equations for \( A_4 \) and \( A_5 \) we obtain to good approximation
\[
\frac{dx_4}{dt} = \frac{CL_{int:CYP}}{V_H} x_2 - \frac{Q_H}{V_H} x_4 + \frac{Q_H}{V_5} x_5.
\] (34)

Transforming to the dimensionless time \( \tau \) defined in (27) we obtain
\[
\frac{dx_4}{d\tau} = \frac{CL_{int:CYP}}{Q_H} x_2 - x_4 + bx_5, \quad b = \frac{V_H}{V_5}.
\] (35)

Observe that
\[
CL_{int:CYP} = \frac{V_{max:CYP}}{K_m + (A_2/V_H)} = \frac{V_H}{D} \times \frac{V_{max:CYP}}{K_m + x_2}, \quad K_m = K_m \cdot \frac{V_H}{D}
\] (36)

so that we can write equation (35) as
\[
\frac{dx_4}{d\tau} = \frac{1}{K_m + x_2} x_2 - x_4 + b x_5, \quad b = \frac{V_{max:CYP}}{D} \cdot \frac{V_H}{Q_H}.
\] (37)
For the parameter values of Table 1 we obtain

\[ \vartheta = \frac{9.42}{D}, \quad \kappa_m = \frac{7.43}{D}, \quad b = 0.5. \]  

(38)

Proceeding along similar lines, we obtain for \( A_5 \) the dimensionless equation

\[ \frac{dx_5}{d\tau} = x_4 - (b + c) x_5, \quad c = \frac{CL_{SO}}{Q_H \cdot V_H} \cdot \frac{V_L}{V_5} = 0.0386. \]  

(39)

Summarising, in dimensionless variables, the clop-AM system is given by

\[
\begin{aligned}
\frac{dx_4}{d\tau} &= \vartheta \frac{x_2}{\kappa_m + x_2} - \frac{x_4}{\kappa_m} + b x_5, \\
\frac{dx_5}{d\tau} &= x_4 - (b + c) x_5.
\end{aligned}
\]  

(40)

It is instructive to view the solution \((x_4(\tau), x_5(\tau))\) as an orbit in the \((x_4, x_5)\)-plane (the phase plane). Since \((x_4(0), x_5(0)) = (0, 0)\), the orbit starts at the origin, describes a loop and eventually returns to the origin. This orbit in the phase plane is shown in Figure 6.

In Figure 2 we have seen that the forcing term involving \( x_2 \) has disappeared after about 2 hours. Thus, after about 2 hours the system has become approximately autonomous. This autonomous system has two null-clines: \( \Gamma_4 \), along which \( \frac{dx_4}{d\tau} = 0 \), and \( \Gamma_5 \) along which \( \frac{dx_5}{d\tau} = 0 \). They are given by

\[
\begin{aligned}
\Gamma_4 & : \ x_5 = \frac{1}{b} x_4, \\
\Gamma_5 & : \ x_5 = \frac{1}{b + c} x_4.
\end{aligned}
\]  

(41)

Note that because \( c \ll b \) the two null clines together form a very thin wedge

\[ \Omega = \{(x_4, x_5) : x_4 > 0 \& (b + c)^{-1} \cdot x_4 < x_5 < b^{-1} \cdot x_4\}. \]

Thus, after about 2 hours, when the system has become approximately autonomous, the orbit can only enter the wedge \( \Omega \), but not leave it.

![Figure 6. Orbit in the \((x_4, x_5)\)-plane (red) together with the null clines \( \Gamma_4 \) (blue) and \( \Gamma_5 \) (green) for PK parameter values from Table 1 and an iv bolus dose of 300 mg i.e., 931 µmol.](image)

We see in Figure 6 that, once inside the wedge \( \Omega \), the orbit hugs the null cline \( \Gamma_5 \), and hence that the amounts of the two compounds are approximately proportional:

\[ A_4(t) \approx \mu \times A_5(t) \quad \text{for} \quad t > t_0 \quad \text{where} \quad \mu \in (b, b + c). \]  

(42)
An elementary computation shows that because \( c \ll b \), the terminal slope \( \lambda_z \) of the two compounds is given by
\[
\lambda_z \approx \frac{c}{1 + b + c} = 0.0251,
\]
so that the half-life is given by
\[
\tau_{1/2} = \frac{\ln(2)}{\lambda_z} = 27.6 \quad \Rightarrow \quad t_{1/2} = \frac{\frac{V_H}{Q_H} \times \tau_{1/2}}{0.0251} = 0.829 \text{ h}.
\]
This value corresponds well with the graphs shown in Figure 4.

4.3. Platelet aggregation. As we see in Table 2, \( k_{out} = 0.00783 \text{ 1/h} \). Therefore, after the infusion of clop-AM has ceased, i.e. \( C_5 = 0 \), the half-life of the inhibition of platelet aggregation becomes
\[
t_{1/2} = \frac{\ln(2)}{0.00783} = 88.5 \text{ h},
\]
considerably longer than that of the clop-AM concentration \( C_5 \). We see this demonstrated in Figure 5 where \( C_5(t) \approx 0 \) for \( t > 5 \text{ h} \), which corresponds with 5 times the half-life of \( C_5 \) together with a brief interval during which \( C_5 \) reaches its maximum value.

Thus, for the first 5 h, we may neglect the \( k_{out} \)-term and so approximate the platelet equation by
\[
\frac{dP}{dt} = -k_{irre} C_5(t) P.
\]
At \( t = 5 \) the graph of \( P(t) \) levels off. Thus, integrating equation (46) over the interval \( 0 < t < 5 \) we obtain for the maximum response \( P_{max} \) (the minimum of \( P(t) \) on the interval \( 0 < t < \infty \)) the following expression:
\[
P_{max} \approx e^{-k_{irre} \text{AUC}_5} \quad \text{since} \quad \text{AUC}_5 \overset{\text{def}}{=} \int_0^\infty C_5(t) dt \approx \int_0^{T_{max}} C_5(t) dt,
\]
where the integral could be extended to \( t = \infty \) because \( C_5(t) \approx 0 \) for \( t > T_{max} \) h.

For \( t > 5 \), the graph of \( P(t) \) is approximately mono-exponential with a constant rate \( k_{out} \) and half-life 88.5 h, as explained above.

5. Results. We have shown that the full model, proposed in [10] can be divided into two simple sub-models: (i) a model describing the dynamics of the clopidogrel and (ii) a model for the dynamics of the active metabolite clop-AM, which is driven by the clopidogrel concentration obtained from the first model.

Clopidogrel: The amount of clopidogrel in the liver \( (A_2) \) and in plasma \( (A_3) \) is described by a simple linear equation for \( A_2 \) (cf. equation (31)):
\[
\frac{dA_2}{dt} = F_a \cdot D \cdot f(t) - \frac{Q_H}{V_H} A_2,
\]
whilst the amount of clopidogrel in plasma is very small and may be neglected.

Equation (48) only involves two physiological parameters, the hepatic blood flow \( Q_H \) and the apparent volume of distribution \( V_H \) for the liver. The values of these parameters generally do not differ a great deal between individuals.

Because equation (48) is linear, the amount of clopidogrel in the liver varies linearly with the drug dose \( D \).

Thus, assuming that \( k_a \) and \( F_a \) are also comparable between individuals, we may conclude that individual differences are restricted to the clop-AM dynamics.
Clop-AM: Translating the dimensionless model (40) for clop-AM back to physiological variables we obtain as a very good approximation, the simple two-compartment model which is linear in the variables $A_4$ and $A_5$:

$$\begin{align*}
\frac{dA_4}{dt} &= Q_H \frac{V_{\text{max:CY}P}}{CL_{\text{int:CES1}}} \cdot \frac{C_2}{K_m + C_2} - Q_H \frac{A_4}{V_H} + Q_H \frac{A_5}{V_5}, \\
\frac{dA_5}{dt} &= Q_H \frac{A_4}{V_H} - (Q_H + CL_{50}) \frac{A_5}{V_5},
\end{align*}$$

where we recall that, usually, $CL_{\text{int:CES1}}$ is a very large parameter (cf. Table 1), so that the amount of clop-AM is much smaller than the amount of clopidogrel in the liver and in plasma (compare left- and middle-panel in Figure 4).

Individual differences between genetic factors are known to be associated with CYP enzymes and CES1 enzymes. Genetic differences in the genes encoding for these enzymes can lead to an altered enzyme activity (cf. [10]). These critical activities are reflected in the parameters $V_{\text{max:CY}P}$ and $CL_{\text{int:CES1}}$ in the source term of the equation for clop-AM in the liver. With $A_2 -$ and hence $C_2 -$ determined by equation (48) and hence not very different between individuals, the genetic differences are localised in the fraction $V_{\text{max:CY}P}/CL_{\text{int:CES1}}$ and the Michaelis-Menten constant $K_m$ in the source term, i.e., effectively in two parameters of which one is a simple multiplicative constant.

6. Discussion. Variability in response to clopidogrel treatment has become an increasingly important clinical issue, with potentially severe consequences in recent years [3]. This is in part due to the fact that the impact of multiple enzymes involved in the biotransformation of clopidogrel and its active metabolite as well as the impact of genetic and non-genetic factors on the response to clopidogrel treatment is not yet fully understood. In order to identify the factors that contribute to the high inter-individual variability and their relative importance for response to clopidogrel treatment, it is important to understand the dynamic interplay between systems components and how they relate to clinical outcome. In addition, identification of the relative importance of the various model components as well as the relative speeds of the processes involved allows for a more rational experimental design because processes driving the system can be identified during model reduction. In addition, the relative speed of these processes will also help determining for when to experimentally measure associated factors.

To date, multiple clinical studies have been conducted to address this question [11]. However, the predictive value of these studies is frequently limited due to the fact that they often investigate specific aspects of clopidogrel’s dose-concentration-response-covariate relationship rather than the dynamic interplay between them. A concerted effort that allows for an integrated use of all available data is consequently needed to identify clinically important pharmacogenetic and demographic factors that drive the PK/PD relationship of clopidogrel.

The use of clinical data in conjunction with mechanism-based quantitative analysis techniques provides an opportunity for integrating data from head-to-head clinical trials as well as information on genetic and non-genetic factors into an overarching framework that allows for the simultaneous consideration of all available evidence in light of the underlying (patho)physiology. Once developed and qualified, these frameworks can be used in silico to explore the cause-effect relationship
and to assist the formulation of new hypotheses as well as the design of new experimental studies (cf. Schmidt et al. [24]). However, as these physiology-directed frameworks become more complex, problems with identifying the key mechanisms that cause a system to undergo (patho)physiological changes as well as the key factors that are responsible for inter-individual variability in treatment response may arise (cf. Post et al. [19] and Schmidt et al. [24]).

One way of exploring a system’s dynamic properties is to mathematically reduce the physiology-directed model framework in order to determine: (i) the relative importance of the various model components and (ii) the relative speed of the processes involved for the overall performance of the system [25]. At the same time, the degrees of freedom freed by reducing the number of structural model parameters can be allocated for estimating associated variance parameters.

In this paper we have successfully reduced the full system of Section 2, involving 10 PK parameters, to two small systems, one for clopidogrel (48) and one for clop-AM (49), with only 3 sensitive parameters $V_{\text{max,CYP}}$, $CL_{\text{int,CES}}$, and $K_m$, all of them located in the clop-AM-system. The structure thus obtained will also be applicable to more complex models involving additional metabolites and enzyme reactions [11], [20]. The results of this analysis also indicate that factors contributing to inter-individual differences in $V_{\text{max,CYP}}$, $CL_{\text{int,CES}}$, and $K_m$, such as genetic polymorphisms in cytochrome P450 and CES1 enzymes or drug-drug interactions are clinically important sources of variability as reflected by the black boxed warning for CYP2C19 poor metabolizers.

The proposed model was used to characterize clopidogrel’s dose-concentration-response relationship in healthy adults using non-linear mixed effects modeling (Jiang et al. [12]). This made it possible to also identify and quantify the contribution of covariates like CYP2C19 and CES1 enzyme genetic polymorphisms and demographic covariates like body mass index and age that significantly impacted clopidogrel’s dose-concentration-response relationship.

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