# Investigation of the CYP2C9 induction profile in human hepatocytes by combining experimental and modelling approaches

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**Abstract:** The goal of the present review is to characterise the induction profile of CYP2C9, a member of the cytochrome P450 superfamily. Since the mechanism of CYP2C9 induction is fairly complex, with parallel processes triggered by various inducers, an evaluation of the experimental results is often a great challenge. At least three nuclear receptors, the glucocrticoid receptor (GR), the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR), are known to mediate the *CYP2C9* gene induction in man. However, mathematical modelling and simulation can provide an appropriate tool for the interpretation of CYP2C9 regulatory mechanisms. As an example, we present modelling and simulation approaches of the *CYP2C9* gene expression in human hepatocytes treated with well-known CYP2C9 inducers: the steroid hormone precursor dehydroepiandrosterone (DHEA) and the synthetic glucocorticoid dexamethasone (DXM). The results of the analysis suggest that in addition to the potent function of GR and the further involvement of PXR and CAR activated by DXM or DHEA, an additional factor might play a role in *CYP2C9* regulation by DHEA. The novel potential candidate for DHEA action in CYP2C9 induction is likely to be the estrogen receptor. Additionally, the balance of DHEA sulphation-desulphation processes should also be considered in any description of DHEA-induced CYP2C9 profiles.

**Key Words:** CYP2C9, estrogen receptor, dehydroepiandrosterone, dexamethasone, nuclear receptors, mathematical modelling, simulation, dynamical systems

**Abbreviations**

| | |
|---|---|
| CAR | constitutive androstane receptor |
| DHEA | dehydroepiandrosterone |
| DXM | dexamethasone |
| GR | glucocorticoid receptor |
| P450 | cytochrome P450 |
| PPAR | peroxisome proliferators activated receptor |
| PXR | pregnane X receptor |

## 1. Introduction

The induction or inhibition of drug-metabolizing enzymes as a side effect of drug therapy influences the patient's response to a drug, and is therefore of great clinical interest. Drug interactions, involving the induction of cytochromes P450 (P450) by drugs or other xenobiotics, are of pharmacokinetic types and therefore are not, a priori, predictable with classical pharmacological means, in contrast to pharmacodynamic ones [1-3]. *In vitro* studies and *in silico* estimations of the changes in P450 activities can predict, and tentatively prevent, the clinical consequences of potential metabolic drug interactions. The evaluation of experimental studies designed to investigate complex mechanisms of P450 enzyme induction is a great challenge, since the model inducers often trigger several parallel processes. The information on regulatory mechanisms thus often overlaps, and keeps unveiled, some of the processes.

This review summarizes our current knowledge of the complex mechanism of CYP2C9 induction, and presents a mathematical modelling and simulation approach as an appropriate tool for the evaluation of the results obtained from *in vitro* P450-induction experiments [4-7]. As an example, the CYP2C9 induction profiles in human hepatocytes treated with dehydroepiandrosterone (DHEA) and dexamethasone (DXM) are analysed. The modelling and simulation should describe the measured profiles with a mathematical model based on known mechanisms of *CYP2C9* induction, or, if that is not possible, suggest possible new mechanisms to be validated experimentally. The first necessary step as a basis for modelling is an extensive overview of the regulatory processes that are directly or indirectly involved in CYP2C9 induction in order to identify the key factors and to produce the scheme of the relations between them. The experimental validation of the computational model must ensure that the model behaviour is similar to the behaviour of real systems. The integration of

modelling and experimentation can contribute to a better understanding of the underlying mechanisms. Here we provide an insight into the modelling process, which combines current knowledge of the regulation of the *CYP2C9* gene expression and the results of *in vitro* CYP2C9 induction studies.

## 2. Regulation of the CYP2C9 gene expression

Although CYP2C9 is one of the major P450 isoforms expressed in the adult human liver, the mechanism of its regulation is not completely understood. The expression of the *CYP2C9* gene is inducible by many xenobiotics known to be *CYP2B6* and *CYP3A4* inducers, such as phenobarbital, DXM and rifampicin [8,9]. At least three nuclear receptors, the glucocorticoid receptor (GR, NR3C1), the pregnane X receptor (PXR, NR1I2) and/or the constitutive androstane receptor (CAR, NR1I3), have recently been shown to mediate *CYP2C9* gene induction in man [8,10]. Glucocorticoids at physiological concentrations (< 1 μM) activate GR, which dissociates from the multiprotein complex, translocates to the nucleus and forms a homodimer. The activated GR binds to its cognate sequence in DNA and triggers/blocks the expression of glucocorticoid-responsive genes. GR controls CYP2C9 expression using at least two independent mechanisms. It binds directly to the promoter region of *CYP2C9*, which contains a functional glucocorticoid-responsive element (GRE), determined as an imperfect palindrome at -1662/-1676 [11]. GR also regulates CYP2C9 indirectly via the transcriptional up-regulation of the PXR and CAR receptors [12]. Human PXR is activated by a variety of compounds, including rifampicin, phenobarbital, DXM (> 1 μM) and many others [13]. There is a high degree of ligand sharing (overlapping) between PXR and CAR. While PXR is a nuclear receptor, CAR in its inactive state resides in the cytosol and upon the activation by, e.g., phenobarbital, it translocates into the nucleus. Both activated PXR and CAR form a

heterodimer with a retinoid X receptor (RXR), and the heterodimer binds to the DR4 (direct repeat 4) motif in the CYP2C9 promoter (1803/-1818) [11].

The presence of responsive elements for GR and PXR/CAR in the CYP2C9 promoter suggests a complex regulation in response to glucocorticoids and xenobiotics. The transcriptional activation of CYP2C9 through the functional GRE may guarantee the constitutive expression of *CYP2C9* under the physiological conditions where GR is in an activated state (by natural plasma corticoids). In addition, CYP2C9 is typically an xenobiotic inducible gene via the PXR and CAR receptor. Taken together, the presence of glucocorticoids augment the CYP2C9 inducibility by xenobiotics. Finally, we have recently described a "non-classical" transcriptional mechanism with which GR regulates human CYP2A6 based on the indirect binding of GR to the CYP2A6 promoter [14]. Hence, we cannot also exclude this method of glucocorticoid-dependent CYP2C9 regulation. [11]. The schematic diagram in Fig. (**1**) presents the currently known mechanisms of CYP2C9 induction.

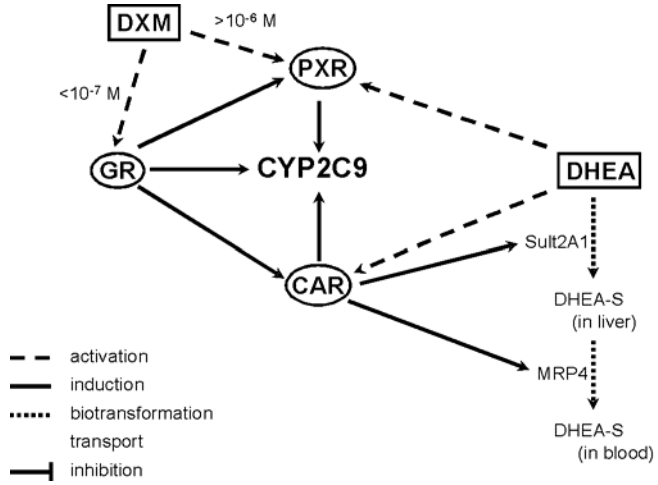


**Figure 1 Currently confirmed interactions in the regulation of *CYP2C9* expression. (DXM – dexamethasone, DHEA – dehydroepiandrosterone, DHEA-S, sulphated form of DHEA, GR – glucocorticoid receptor, PXR – pregnane X receptor, CAR – constitutive androstane receptor, Sult2A1 – sulphotransferase family 2A, subfamily 1, MRP4 - multi-drug resistance protein 4, $10^{-7}$M, $10^{-6}$M – concentrations of DXM in treatment.**

### 2.1. Model inducers of *CYP2C9*

To investigate the mechanisms of CYP2C9 regulation, several model inducers, such as glucocorticoids, rifampicin, or phenobarbital, are used in order to provoke changes in the *CYP2C9* transcription. Glucocorticoids are hormonal regulators of various physiological processes, including gluconeogenesis, cellular proliferation and differentiation, and the inhibition of inflammation. In target cells, GR, the transcription factor, mediates the effects of glucocorticoids on many glucocorticoid-responsive genes in a ligand-dependent manner by binding to GRE sequences within the regulatory DNA regions. Glucocorticoid hormones and their potent synthetic analogue, DXM strongly influence the expression of several enzymes involved in metabolism and disposition of foreign chemicals [15]. Sub-micromolar concentrations of DXM have been shown to up-regulate *CYP2C9* expression primarily through GR activation, leading to the direct control of *CYP2C9* gene transcription or by increasing the expression of PXR, CAR and RXR. DXM at higher concentrations is also a potent ligand of the human PXR, resulting in the transactivation of the *CYP2C9* gene through the CAR/PXR-responsive element [10,11,14,16].

DHEA, the major secretory product of the adrenal cortex, is the most abundant natural steroid in humans (physiological circulating level of 5-7 μM in young adults), and has multifunctional properties: it is a precursor of sex steroid hormones and a peroxisome proliferator at pharmacological dosages [17]. DHEA is derived from cholesterol *via* a series of steps catalyzed by P450 enzymes [18]. It is secreted primarily as the 3β-sulfate conjugate (DHEA:DHEA-S ratio 1:250 or 1:500 in plasma), which is taken up by target tissues and hydrolyzed by sulphatases back to DHEA [19,20]. DHEA is further metabolized to androgens and estrogens in the testes and ovaries [21]. DHEA has been demonstrated to activate several

xenosensor nuclear receptors, such as the peroxisome proliferator activated receptor (PPARα), PXR and CAR, and to consequently induce several drug-metabolizing P450s (CYP2B6, CYP2C9, CYP3A4, CYP4A1) [20,22-25].

### 2.2. *In vitro* induction of *CYP2C9* in primary human hepatocytes

A primary culture of hepatocytes can offer a simple and reliable experimental system for evaluating the potential for drugs and xenobiotics to induce drug-metabolizing P450s. Moreover, one of the main advantages of the cell culture is that the cell populations of the control and treated groups are isolated from the liver of the individual human tissue donor. The hepatocyte-based *in vitro* model also provides valuable information on the kinetics of P450 gene expression and the identification of key factors in the regulatory processes.

In several studies, DXM and DHEA treatments of the primary human hepatocytes have been used in order to investigate the regulation of the P450 expression [10,24-27]. CYP2C9 induction has been clearly displayed in human hepatocytes treated with DXM and DHEA for 24 or 48 hours [10,25]. Demonstrating the modelling process, we analyzed the datasets of the *CYP2C9* expression kinetics in human hepatocytes treated with DXM and DHEA. The experimental conditions are briefly described in the appendix. The expression of CYP2C9 was significantly induced by both compounds; however, the profiles of the *CYP2C9* induction, measured after DHEA treatment (Fig. (**2**)), represented the major motivation for the analysis. The increasing expression of CYP2C9 with a peak at 48 hours was detected in human hepatocytes from all of three donors. Such a peak of CYP2C9 mRNA levels has not been observed in DXM-, Phenobarbital- or rifampicin-treated hepatocytes (Fig. (4)) [10]. DXM, phenobarbital and rifampicin were found to be potent inducers of CYP2C9 mRNA, producing a plateau of maximum levels after 24 hours of treatment. In DHEA-treated primary hepatocytes, the rebounds in CYP2C9 induction profiles indicate complex feedback

mechanisms that were triggered by the treatment. These CYP2C9 expression profiles can be hardly evaluated by generating a combination of known mechanisms involving GR, PXR and CAR.

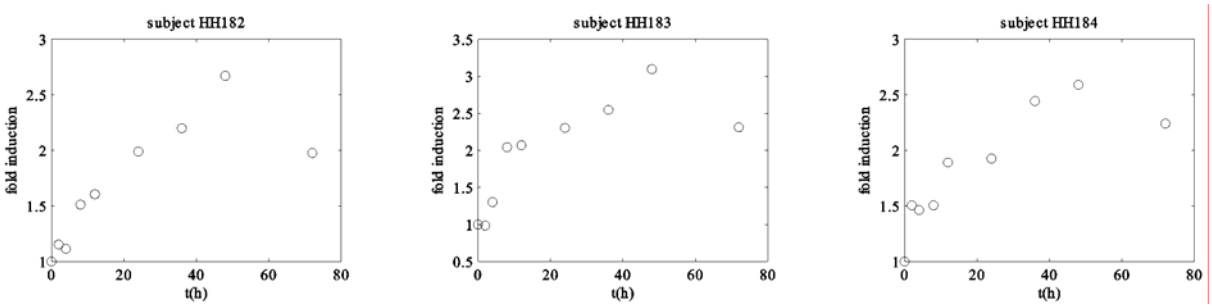


**Figure 2 CYP2C9 induction profiles (CYP2C9 mRNA) measured in DHEA-treated hepatocytes isolated from three human liver donors (HH182, HH183 and HH184).**

### 3. Modelling and simulation of the induction mechanisms of *CYP2C9*

Our current knowledge, as the basis for the modelling of CYP2C9 induction, is represented by the scheme in Fig. (**1**), while the datasets of DHEA-treated hepatocytes motivating the analysis are presented in Fig. (**2**). The datasets are relatively small, thus only simplified, basic regulatory mechanisms can be modelled. The basic model implicates the regulatory processes of *CYP2C9* expression mediated by GR, PXR and CAR; however, an additional mechanism may possibly be involved. It is well known that DHEA does not activate GR, but has the potential for PXR and CAR activation [25]. DHEA also has the capability of the physiologically relevant direct activation of the estrogen receptor (ER) [28], thus we may assume ER to be a potential candidate for the mediation of DHEA action in CYP2C9 induction. As the role of ER in CYP2C9 regulation is not confirmed experimentally, two models were designed, with and without ER influence on the CYP2C9 induction. The model without ER influence, designed in SIMULINK, is presented in Fig. (**3**).

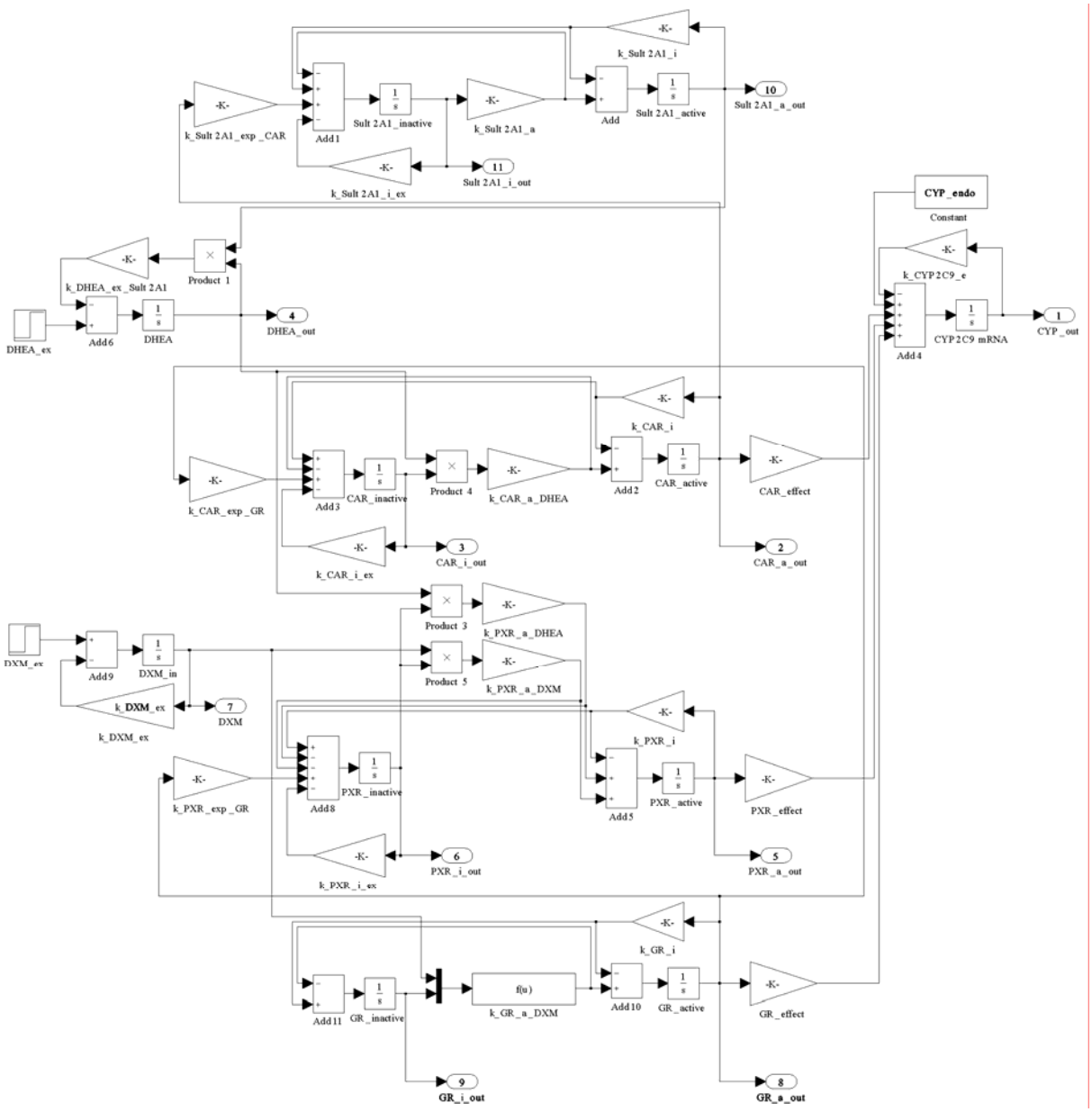


**Figure 3 The mechanisms of CYP2C9 induction by DHEA and DXM programmed in SIMULINK. Each connection represents a variable that is sent to an input of a block, where a numerical operation on the variable is performed. The arrows show the direction of the flow of the variables. Square blocks marked with $\frac{1}{s}$ represent the integration of the input variable, square blocks with + and – signs represent a summation of the input variables, square blocks marked with X represent the multiplication of the input signals, square blocks marked with f(s) represent a special user-programmed function of the input, square blocks marked with a step-like signal represent a step-change of the independent input signal, square blocks marked with variable names represent a constant input to the model, black square blocks represent multiplexers, triangular blocks represent multiplication of the input signal by a constant, oval blocks marked with number represent variables that are stored during the simulation.**

The model in Fig. (**3**) (see the appendix for the equations) describes the three known parallel pathways of CYP2C9 induction. *CYP2C9* is considered to be a primary glucocorticoid-responsive gene, which in addition is also induced through CAR and PXR activation. The

CYP2C9 induction profiles resulting from 0.1-μM and 1-μM DXM treatments are almost identical, which suggests that the activation process of GR is level- and rate-limited. Thus, the rate of GR activation ($k_a$) was modelled as

$$k_a = \frac{k[GR_i][DXM]}{[GR_i]+[DXM]},$$

where $k$ is the rate constant, $[GR_i]$ is the concentration of inactive GR, and $[DXM]$ is the concentration of DXM in the cell. The cumulative amount of active and inactive GR was constant. This is a critical mechanism of the model, since it enables the model to describe the CYP2C9 induction profiles caused by DXM. Another critical part of the model is the mechanism that can cause a rebound in the DHEA-induced CYP2C9 profile. A mathematical model that will produce a rebound when excited with a step function cannot be composed of parallel and/or serial substance transformations, since this causes only a delay and smoothening of the time course of the first substance in the chain. In a particular case, the system excited with a step function and transformations can only result in a time course of the final substance that has a delayed response followed by a slow rise to a plateau. Thus, more complex structures must be involved to recreate a rebound in CYP2C9 induction. There are two possibilities in the case of CYP2C9 induction.

i) First, the concentrations of DHEA are regulated in the cell. Since DHEA is an endogenous substance, mechanisms exist to control its levels. DHEA is known to activate CAR [25], which mediates the induction of *Sult2A1* and *MRP4* [29]. Sult2A1 and MRP4 are responsible for the sulphation (inactivation) and active transport of the DHEA out of the cell. The structure forms a feedback loop that could control DHEA levels in the cell.

ii) Another possibility is an additional parallel pathway of CYP2C9 induction that can be deactivated by other transcription factors. ER can be activated by DHEA [28],

and the ER function is substantially inhibited by CAR [30]; however, it is not clear if ER can mediate CYP2C9 induction.

The parameters of both models were set in a procedure with three consecutive steps. In each step, optimisation of the CYP2C9 induction profile with respect to the measured data was performed. First, the datasets from 0.1-μM and 1-μM DXM treatments were analyzed. In both cases, only the GR-mediated transcription of the *CYP2C9* is activated, as was also indicated by the measured profiles. In the first step, only the values of the model parameters describing the DXM, GR, and CYP2C9 dynamics were set. Next, the data obtained from the hepatocytes treated with 10-μM DXM were evaluated. At concentrations higher than 1 μM, DXM also causes the activation of PXR [31]; therefore, the values of the model parameters describing the PXR dynamics were set. In the last step, the data from DHEA-treated liver cells were used to set the values of the parameters describing the CAR and ER or Sult2A1 and MRP4 dynamics, depending on the model. Finally, all the values of the model parameters were set and the model could simulate all the described situations, while retaining some basic mechanistic properties of the system. The procedure was successfully repeated for the hepatocytes of all three donors involved in the study.

The profiles shown in Fig. (**4**) and Fig. (**5**) were obtained from the model simulation.

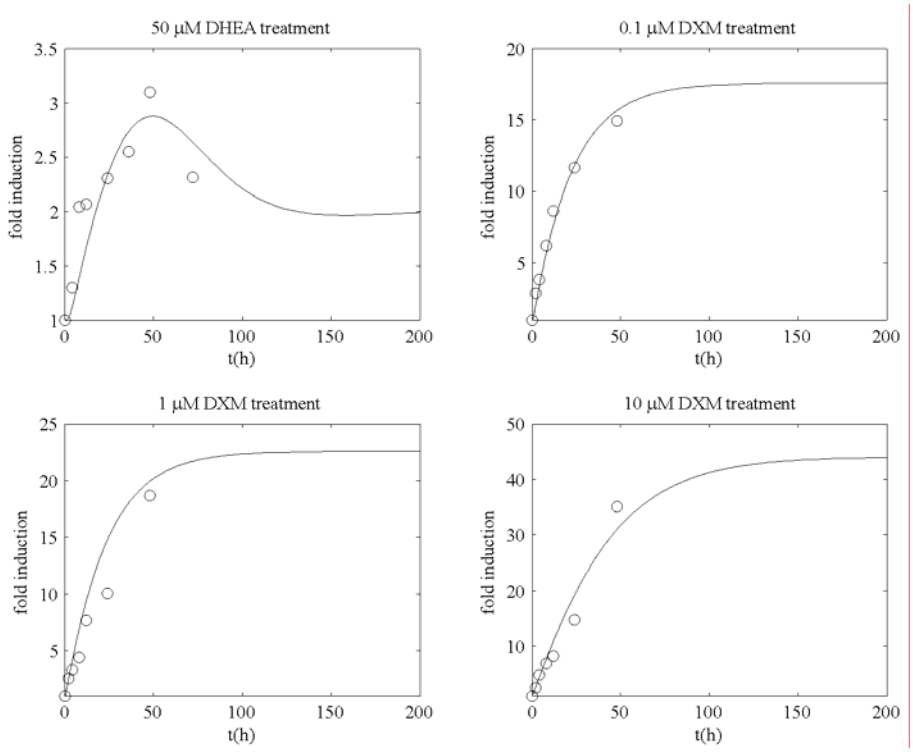


**Figure 4 Simulated and measured CYP2C9 induction profiles for subject HH182. The simulation model uses ER as an additional transcription factor involved in the CYP2C9 induction to generate a rebound in the profile after DHEA treatment, while the active DHEA-induced elimination and deactivation of DHEA is not included in the model (circles – measured data, line – simulation).**

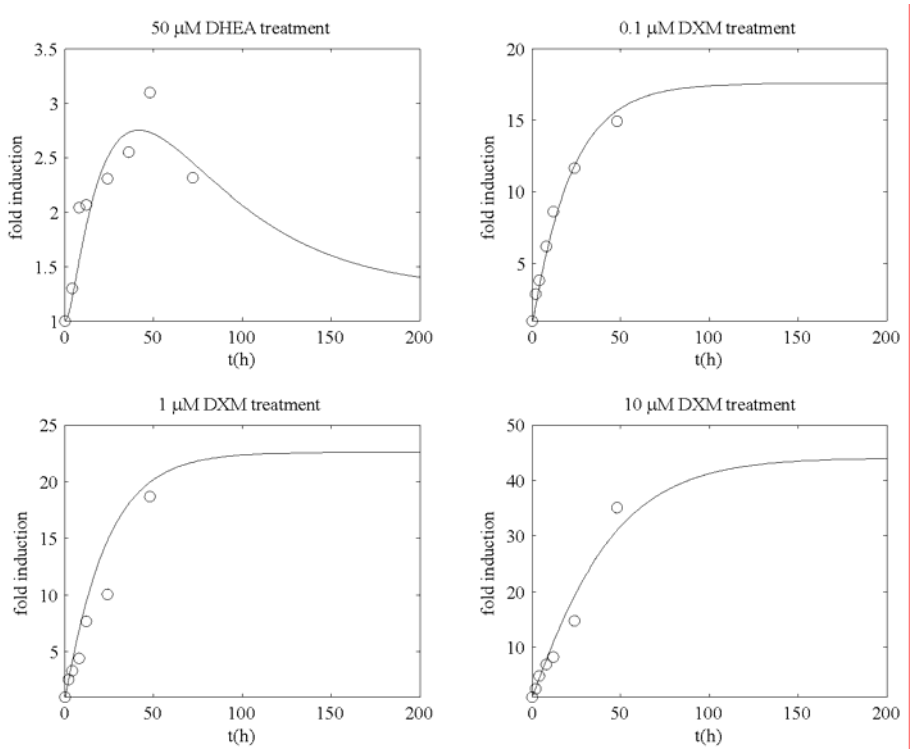


**Figure 5 Simulated and measured CYP2C9 induction profiles for the subject HH182. The simulation model uses the active DHEA-induced elimination and deactivation of DHEA to generate a rebound in the**

**profile after DHEA treatment, while ER as an additional transcription factor involved in the CYP2C9 induction is not included in the model (circles – measured data, line – simulation).**

Although the profiles of CYP2C9 induction produced by the DXM treatment presented in the article are not conclusive, whether a rebound is also present or not, the literature data on the measured profiles of CYP2C9 suggest that no rebound can be expected [10]. Both models can describe the rebound of the CYP2C9 induction profiles of DHEA-treated cells equally well. Therefore, with respect to the presented data, it is not possible to decide which model describes the situations more realistically.

## 4. Influences of the modelling and simulation results on our understanding of the mechanisms involved in *CYP2C9* induction

A comprehensive literature search was performed in order to evaluate both possible additional mechanisms involved in the CYP2C9 induction after the DHEA treatment. Several references [26,29,32-35] suggest that sulphation through Sult2A1 is an important mechanism of detoxification, which represents one of the major defence processes against xenobiotics, as well as the main mechanism of DHEA inactivation before the transportation from the adrenal glands to the rest of the body tissues in humans. In the liver it is an important mechanism involved in the metabolism of bile acids that prevents liver intoxication, especially during abnormal bile acid metabolism. Thus, the capacity of Sult2A1 should be sufficient to cause a rebound in the CYP2C9 expression profile as a consequence of reduced levels of DHEA after increased *Sult2A1* induction mediated by CAR. However, the effect of ER may still play some role in the CYP2C9 induction. None of the two models could simultaneously fit the rebound and the fast rise of the CYP2C9 mRNA levels that occurred after the DHEA treatment in the first 4 hours. In order to improve the fitting of the simulated CYP2C9 profile and the measured values, the two models were combined. The parameter values that were identified with the sulphation mechanism were used, while the values for the ER effect were identified

in such a way that the initial part of the simulated CYP2C9 profile was improved. At the same time, the ER profiles of the DHEA-treated hepatocytes from the three donors were measured and compared with the simulated profiles. From Fig. (**6**) to Fig. (**8**), the results of the comparison between the simulated and measured ER profiles are shown, accompanied by the measured and simulated CYP2C9 profiles.

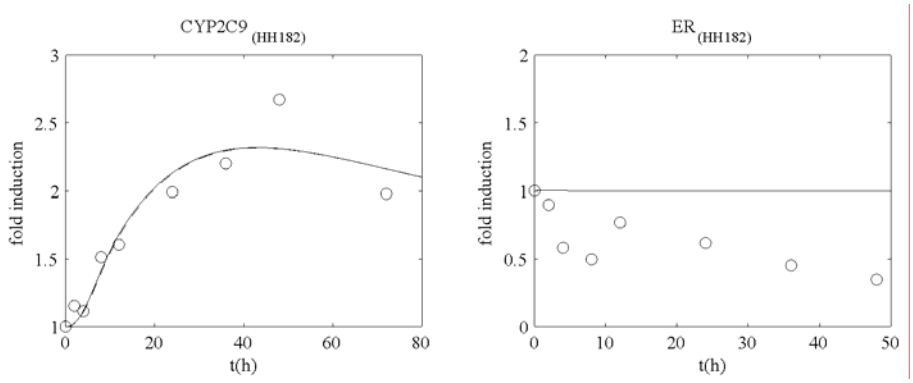


**Figure 6 The effect of ER as an additionsl transcription factor on the CYP2C9 induction profiles in DHEA-treated hepatocytes from the HH182 donor (circles – measured data, broken line – simulation of the model without ER as an additional transcription factor involved in the CYP2C9 induction, full line – simulation of the model with ER as an additional transcription factor involved in the CYP2C9 induction ER effect)**

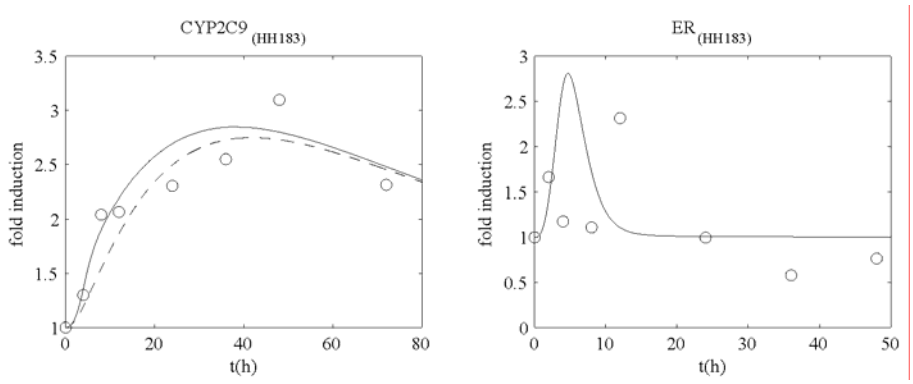


**Figure 7 The effect of ER as additional transcription factor on the CYP2C9 induction profiles in DHEA-treated hepatocytes from HH183 donor (circles – measured data, broken line – simulation of the model without ER as an additional transcription factor involved in the CYP2C9 induction, full line – simulation of the model with ER as an additional transcription factor involved in the CYP2C9 induction)**

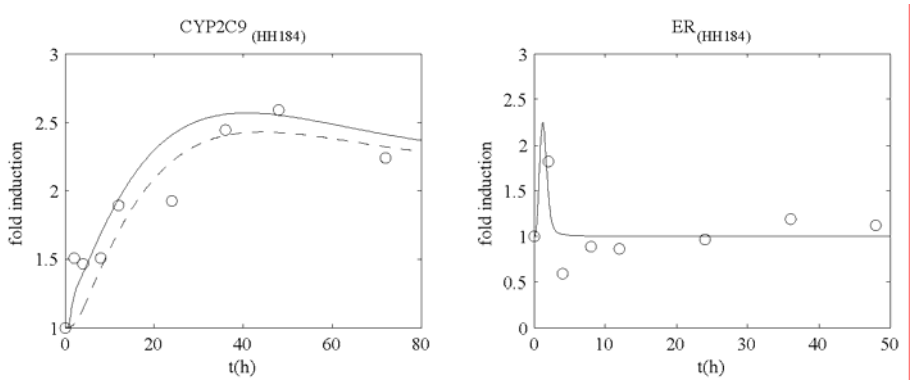


**Figure 8 The effect of ER as an additional transcriotion factor on the CYP2C9 induction profiles in DHEA-treated hepatocytes from HH184 donor (circles – measured data, broken line – simulation of the model without ER as an additional transcription factor involved in the CYP2C9 induction, full line – simulation of the model with ER as an additional transcription factor involved in the CYP2C9 induction)**

In Fig. (**6**) it is clear that the initial part of the CYP2C9 induction profile does not exhibit a faster initial elevation, as is observed in Figs. (**7**) and (**8**). Therefore, no additional induction mediated by ER is expected in the HH182 hepatocytes. This assumption is supported by the measured and simulated levels of ER, which are not significantly altered during the experiment (see the right-hand graph of Fig. (**6**)). The slope of the initial elevation in the CYP2C9 profiles also correlates with the measured and simulated profiles of ER. In Fig. (**7**), the initial slope of the CYP2C9 is less steep than the initial slope of the CYP2C9 profile in Fig. (**8**). Similarly, the induction profiles of ER (measured as well as simulated) show slower (Fig. (**7**)) or faster (Fig. (**8**)) dynamics. The ER induction profile in Fig. (**7**) does not correlate well with the simulated profile; however, all the time points up to 12 hours after the treatment show an elevated *ER* expression, which is similar to the simulated profile. The regulation of the ER expression is highly complex; hence, some other transcription factors might be the cause of the discrepancy between the measured and the simulated ER profiles.

## 5. Conclusions

Although no studies were yet reported where ER-mediated CYP2C9 was demonstrated, our modelling and simulation study indicated that ER might be involved in the regulation of

CYP2C9 expression (Fig. (**9**)). Since modelling and simulation results are never performed on real datasets, some caution is nevertheless necessary. The match of the measured and simulated profiles is not perfect; therefore, the possibility exists that some other transcription factor with a similar expression profile to ER could be the factor involved in the regulation of the *CYP2C9* profiles. Since the specificity of the modelling and simulation results is relatively poor, the current findings only suggest that there might be an additional transcription factor involved in the *CYP2C9* induction regulation. The additional transcription factor must be similarly regulated as ER.

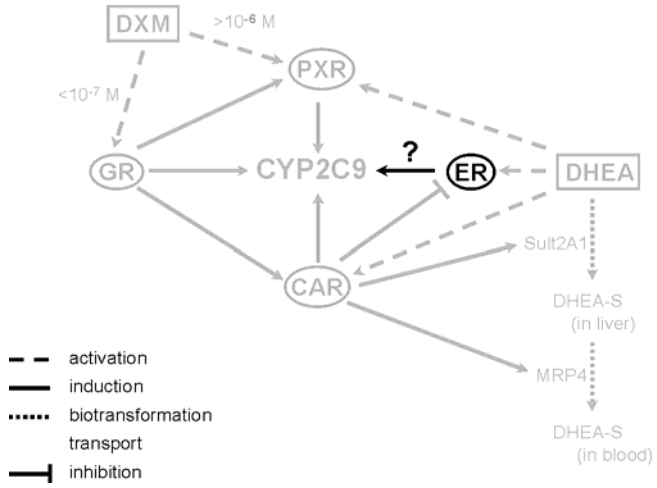


**Figure 9 Confirmed (in gray) and assumed (in black) interactions in the regulation of *CYP2C9* expression. (DXM – dexamethasone, DHEA – dehydroepiandrosterone, DHEA-S, sulphated form of DHEA, GR – glucocorticoid receptor, PXR – pregnane X receptor, CAR – constitutive androstane receptor, Sult2A1 – sulphotransferase family 2A, subfamily 1, MRP4 - multi-drug resistance protein 4, $10^{-7}$M, $10^{-6}$M – concentrations of DXM in treatment.**

As the effect of the additional transcription factor is significant only for a short time after the administration of DHEA, it would have been overlooked if only one time-point was measured in this early period. The complexity of the biochemical regulatory mechanisms and their indisputably dynamic nature requires measurements of detailed time-profiles of the involved substances in order to capture reliable information on their relations.

Modelling and simulation is a "soft" science that bridges the gap between the physical and mathematical worlds. Each of the two worlds has its own set of governing rules and they are

related only through the interpretation by the user. Thus, modelling and simulation can generate misleading results when they are not used under a set of strict interpretation rules that must be set during the modelling and used during the analysis of model's simulation results. The modelling results of *in vitro* experiments cannot be directly applied to *in vivo* systems where several other mechanisms of induction may be triggered as well; however, *in vitro* conditions reduce the complexity of the system and therefore reduce the problem of identifying several simultaneously active mechanisms. Recognizing the limitations of the modelling and simulation processes, the combination of *in vitro* experimental systems and mathematical tools can contribute to the evaluation of experimental results and to a better understanding of complex induction mechanisms.

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**Appendix:**

**Isolation and culture of human hepatocytes.** The human livers were obtained from kidney-transplant donors at the Transplantation and Surgical Clinic, Semmelweis University Budapest (Hungary). The permission of the Hungarian Regional Committee of Science and Research Ethics was obtained to use human tissues. The clinical histories of the donors are shown in Table 1. The liver cells were isolated by the method of Bayliss and Skett [36]. Hepatocytes having a viability of better than 90%, as determined by trypan blue exclusion, were used in the experiments. The cells were plated at a density of $1.7x10^5$ cells/$cm^2$ in plastic dishes precoated with collagen in a medium described by Ferrini et al. [37]. After overnight culture, the medium was replaced by a serum-free medium. Twenty-four hours after serum deprivation, the cells were cultured in the presence or absence of inducers for 2, 4, 8, 12, 24, 36, 48 and 72 hours. The hepatocytes were treated with dexamethasone (0.1, 1 and 10 μM), DHEA (50 μM).

Table 1. Clinical histories of the human donors

| Donor | Age (year) | Sex | Race | Cause of death | Medication |
|---|---|---|---|---|---|
| HH-182 | 38 | male | Caucasian | Subdural hemorrhage | noradrenaline |
| HH-183 | 50 | female | Caucasian | Subarachnoidal hemorrhage | dopamine, noradrenaline |
| HH-184 | 56 | male | Caucasian | Subarachnoidal hemorrhage | mannitol, noradrenaline |

**RNA extraction and quantitative RT-PCR.** The total RNA was isolated from the human hepatocytes using the TRIzol reagent (Invitrogen, Carlsbad, CA). Ten million liver cells were homogenized in 1 ml of TRIzol reagent, and the total RNA was extracted according to the manufacturer's instructions. The RNA was precipitated using ethanol and stored at -80˚C for further analyses. The RNA (3 μg) was reverse transcribed into a single-stranded cDNA using a Transcriptor First Strand cDNA synthesis kit (Roche Diagnostics GmbH, Mannheim,

Germany) and then real-time PCR with human cDNA was performed using FastStart Taq DNA polymerase (LightCycler TaqMan Master, Roche Diagnostics GmbH, Mannheim, Germany) and UPL probes and primers for CYP2C9 (Roche Diagnostics GmbH, Mannheim, Germany). The quantity of CYP2C9 mRNA relative to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was determined.

**Equations of mathematical model**

$$\frac{d[Sult2A1_i]}{dt} = -[Sult2A1_i]k_{Sult2A1\_a} + [Sult2A1_a]k_{Sult2A1\_i} + [CAR_a]k_{Sult2A1\_\exp\_CAR}$$
$$-[Sult2A1_i]k_{Sult2A1\_ex}$$
$$\frac{d[Sult2A1_a]}{dt} = -[Sult2A1_a]k_{Sult2A1\_i} + [Sult2A1]k_{Sult2A1\_a}$$

$$\frac{d[DHEA]}{dt} = DHEA_{in} - [DHEA][Sult2A1_a]k_{DHEA\_ex\_Sult2A1}$$

$$\frac{d[CAR_i]}{dt} = [CAR_a]k_{CAR\_i} - [CAR_i][DHEA]k_{CAR\_a\_DHEA} + [GR_a]k_{CAR\_\exp\_GR} - [CAR_i]k_{CAR\_ex}$$

$$\frac{d[CAR_a]}{dt} = -[CAR_a]k_{CAR\_i} + [CAR_i][DHEA]k_{CAR\_a\_DHEA}$$

$$\frac{d[DXM]}{dt} = DXM_{in} - [DXM]k_{DXM\_ex}$$

$$\frac{d[PXR_i]}{dt} = [PXR_a]k_{PXR\_i} - [PXR_i][DHEA]k_{PXR\_a\_DHEA} - [PXR_i][DXM]k_{PXR\_a\_DXM}$$
$$+[GR_a]k_{PXR\_\exp\_GR} - [PXR_i]k_{PXR\_i\_ex}$$
$$\frac{d[PXR_a]}{dt} = -[PXR_a]k_{PXR\_i} + [PXR_i][DHEA]k_{PXR\_a\_DHEA} + [PXR_i][DXM]k_{PXR\_a\_DXM}$$

$$\frac{d[GR_i]}{dt} = -\frac{k[GR_i][DXM]}{[GR_i]+[DXM]} + [GR_a]k_{GR\_i}$$

$$\frac{d[GR_a]}{dt} = \frac{k[GR_i][DXM]}{[GR_i]+[DXM]} - [GR_a]k_{GR\_i}$$

$$\frac{d[CYP2C9_{mRNA}]}{dt} = CYP2C9_{const} - [CYP2C9_{mRNA}]k_{CYP2C9\_ex} + [GR_a]k_{GR\_effect} + [PXR_a]k_{PXR\_effect} + [CAR_a]k_{CAR\_effect}$$

The variables in brackets represent the concentrations of substances. The indexes *i* and *a* designate inactive and active substances, respectively. The name of the substance that is not within brackets represents the inflow of the substance to the cell. The coefficients begin with *k* and are followed by an index. The meaning of an index is as follows: the first part of the index is the name of the substance the coefficient is associated with, after the underline is the function of the coefficient either as an activation (a), inactivation (i), degradation (ex) or expression (exp), and the last part of the index is used when the function is mediated by a third substance.