COMPUTATIONAL MODELING OF ESTROGEN METABOLISM

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ABSTRACT
Oxidative estrogen metabolites have been implicated in the development of breast cancer. An assessment of breast cancer risk due to varying patterns of lifetime estrogen exposure and genetic variations depends on the ability to estimate the concentration dynamics of oxidative metabolites. This requires modeling the system of metabolic reactions implicated in the formation of DNA adducts that are linked to mutagenesis and cancer. The proposed computational model of metabolism relies on a first order approximation of reaction kinetics combined with an experimentally driven estimation of its parameters. The performance of the model is satisfactory in the sense that it replicates the experimentally known profiles of metabolite concentrations. However, the prediction capacity of the model would greatly benefit from the inclusion of new experimental data as they become available.

KEY WORDS
Enzymatic Reaction Kinetics, Metabolism, Computational Modeling.

1. Introduction

The risk for female breast cancer has been associated with oxidative metabolites such as catechol estrogens and estrogen quinones believed to be implicated in the formation of DNA adducts leading to mutagenesis and cancer. The enhanced proliferation of cancer in cells expressing estrogen receptors is another source of breast cancer risk putatively associated with prolonged exposure to increased levels of estrogen. Genetic variation of the detoxification enzymes, independently or in interaction with environmental exposures, has been shown to have an impact on the metabolite concentrations [1, 2]. It has also been shown that the pathways leading to DNA adducts can be modulated by antioxidants [3]. Studies have exposed statistical correlations between the modulating effect of genetic variations on estrogen detoxification as well as the susceptibility to breast cancer. However, the development of preventive therapies and pharmaceutical drugs requires quantitative models of the enzymatic pathways of estrogen metabolism. These models would be instrumental in providing quantitative estimates of the effect of various patterns of endogenous and exogenous estrogen exposure on the distributions of oxidative metabolites, allowing hence an improved assessment of breast cancer risk. Modeling the effect of SNP (Single Nucleotide Polymorphism) – based genetic variations of the key metabolizing enzymes such as the Cytochrome P450 would further enhance the utility of quantitative models of estrogen metabolism. Furthermore, given the importance of P450 in drug metabolism, these models could be instrumental in the prediction of cancer drug effectiveness based on individual SNP profiles.

The established link between breast cancer and estrogen metabolites has sustained a significant research interest in the study of estrogen metabolism and the development of associated cancer risk models [4-9]. Multiple iterations of one particular estrogen metabolic model has been widely reported with proposed improvements related to reaction kinetics, lifetime estrogen exposure and enzyme polymorphism [4, 7, 8, 10, 11]. Considering these advancements in the broader context of computational modeling of metabolism, the knowledge of rate constants of enzymatic reactions still represent a challenge to the development of reliable quantitative models of metabolic processes. Such models are needed to assist not only in the assessment of individual cancer risk but also to help predict the effectiveness of new cancer drugs for different population subgroups based on genetic variations. In this respect, the paper explores the development of a computational model of estrogen metabolism based on an approximation of reaction kinetics to circumvent the need for a prior knowledge of the rate constants. The full specification of the model is driven by experimental data through an iterative process of parameter tuning from initial values that are judiciously chosen based on insight and data curated from the literature.

2. Approximation of Enzyme Kinetics

Enzymatic reactions between a substrate such as Estrogen and enzymes such as Cytchrome P450 (CYP) 1A1 take place in two phases as illustrated in figure 1.

Figure 1. Enzymatic Reaction

\[
S + E \xrightleftharpoons[k_2]{k_1} ES \rightarrow ES \xrightarrow{k_3} S + P
\]
The Michaelis-Menton equation of enzyme kinetics for
the above system of reactions provides an approximation
of the time-dependent dynamics of product concentration
as a function of the substrate and enzyme concentrations.
This approximation is given as follows:
\[
\frac{d[p]}{dt} = k_3[E_0][S] \frac{[S]}{k_m+[S]}
\]
\[= V_{\text{max}} \frac{[S]}{k_m+[S]} \quad (1.1)
\]
\[k_m = (k_2 + k_3)/k_1 \quad (1.2)
\]
Where \([S]\) and \([P]\) are the concentrations of the substrate
and reaction product respectively. \([E_0]\) is the enzyme
concentration and \(k_1, k_2, \text{ and } k_3\) are the kinetics rate
constants. \(V_{\text{max}}\) is the maximum rate of the above reaction
system under saturating substrate concentration, and \(k_m\)
is the Michaelis-Menton constant. Equation (1) can be
rewritten as follows:
\[
\frac{1}{V_{\text{max}}} \frac{dp}{dt} = x(1 + x) - 1
\]
\[= x - x^2 + x^3 \quad (3)
\]
Let us assume that the depletion of the substrate, in this
case \(x\), can be approximated with a vanishing exponential function \(A_0 e^{-\alpha t}\), from an initial concentration \(A_0\) with a
rate \(\alpha > 1\). As a result, the integration of (3) with further
term rearrangement will yield the following relation:
\[
\frac{p(t)}{V_{\text{max}}} = - \frac{1}{\alpha} A_0 e^{-\alpha t} + \frac{1}{2\alpha} A_0^2 e^{-2\alpha t} - \frac{1}{3\alpha} A_0^3 e^{-3\alpha t}
\]
\[+ A_0^4 e^{-4\alpha t} - \frac{1}{5\alpha} A_0^5 e^{-5\alpha t} + \ldots
\]
\[= - \frac{1}{\alpha} x + \frac{1}{2\alpha} x^2 - \frac{1}{3\alpha} x^3 + \frac{1}{4\alpha} x^4 - \frac{1}{5\alpha} x^5 + \ldots \quad (4)
\]
By factoring out the rate \(\alpha\), the above relation can be
rewritten as follows:
\[
\frac{\alpha p(t)}{V_{\text{max}}} = - \left( x + x^2 - x^3 - \frac{3}{2} x^2 + \frac{4}{3} x^3 + O(x^4) \right)
\]
\[= - \left( x + x^2 - x^3 - \frac{3}{2} x^2 + \frac{4}{3} x^3 + O(x^4) \right) \quad (5)
\]
Given the assumption \(x(t) \ll 1\), it can readily be shown
that the cumulative contribution of the 4th and subsequent
terms of the right hand side of relation (5) is negligible
compared to that of the first three terms. The following
approximation can therefore be made:
\[
\frac{\alpha p(t)}{V_{\text{max}}} \approx -(x + x^2 - x^3) \quad (6)
\]
Furthermore, equation (3) can be rewritten as follows:
\[
\frac{1}{V_{\text{max}}} \frac{dp}{dt} \approx 2x - (x + x^2 - x^3) \quad (7)
\]
Using (6) and (7) the product concentration kinetics can
then be approximated using the following relation:
\[
\frac{d[p]}{dt} \approx 2V_{\text{max}} \frac{[S]}{k_m} - \alpha [P]
\]
\[= k_3 [S] - k_p [P] \quad (8)
\]
The computational model associated with the derived first
order approximation of enzyme kinetics is illustrated by
the Matlab® model of figure 2. This will make up the unit
reaction subsystem in the proposed computational model
of estrogen metabolism. The performance of the proposed
approximation will be illustrated by its capacity to model
the dynamics of product formation compared to
experimental data.

Figure 2. Approximation Model of Enzyme Kinetics

3. Estrogen Metabolism

The pathways of estrogen metabolism are illustrated in
figure 3, [5, 6, 12]. The activating enzymes CYP1A1 and
CYP1B1 oxidize E₂ (17β-Estradiol – form of estrogen)
into catechol estrogens 2-OHE₂ and 4-OHE₄. These
catechol estrogens are further oxidized into semi-quinones
(E₂,3-SQ, E₂-3,4-SQ) and ultimately to the quinones
E₂-2,3-Q and E₂-3,4-Q. These quinones are known to
react with DNA and lead to the formation of depurinated
DNA adducts. Potential errors associated with the repair
of the apurinic DNA sites can lead to mutations and may
ultimately trigger the initiation of cancer [5] [13].
Molecular oxygen can also mediate the oxidation of semi-
quinones and results in the formation of hydroxyl radicals in the presence of \( \text{Fe}^{2+} \). Lipid hydroperoxides generated by these hydroxyl radicals can detrimentally modulate CYP towards higher oxidation of catechol estrogens to their quinones \([5]\). The phase II deactivating enzyme COMT catalyzes the methylation of catechol estrogens into methoxyestrogens limiting hence their transformation to semi-quinones and quinones. The homeostatic balance between the activating and deactivating pathways of estrogen metabolism is further protected by the quinones oxido reductase 1 and 2 (NQO1 and NQO2) which catalyze the reductive conversion of quinones to their catechol estrogens \([6]\) \([5]\). The glutathione-S-transferase (GST) enzyme provides an additional protection against the DNA damaging quinones by catalyzing their conjugation into GSH-conjugates.

Figure 3. Estrogen Metabolic Pathways

4. An Adaptive Model of Metabolism

The above summarized pathways of estrogen metabolism have been modeled as shown in figures 4 and 5. The model is a computational representation of the integrated effects of activating and deactivating enzymes involved in the metabolic pathway. A full quantitative knowledge of the kinetic rates is lacking for many of the enzymatic reactions making up the estrogen metabolizing system. The proposed approximation of enzyme kinetics is used to circumvent the need for explicit knowledge of the kinetics rates. The approximation is accompanied with a parameter tuning process guided by experimental data available from the literature. In the case of insufficient availability of experimental data, such as is the case for the redox cycle involving catechol estrogens, and their quinones, qualitative knowledge about the metabolites and the catalyzing reactions is used to inform the parameter tuning process. The subsystem illustrated in figure 2 represents the unit computational module used to internalize the approximation of reaction kinetics. The conservation of mass across a chain of tandem reactions is mediated through the provision of an additional negative feedback as a means to model the depletion of products serving as substrates to the next reactions in a pathway.

The parameter \( k_s \) of the kinetics approximation of a given reaction is included in the reaction module that precedes it in the pathway. This helps avoid what would otherwise be a convoluted graphical illustration of the model. Figure 4 provides the detailed structure of the redox cycle involving the catechol estrogen 4-OHE\(_2\) and its quinones.

The model parameters \( k_p \) and \( k_s \) are tuned starting from initial values that are heuristically set based on experimental data. In particular, consider the metabolite concentration profile shown in figure 6. Assuming a product concentration equal to zero at \( t=0 \), equation (8) can be rewritten as follows:

\[
\frac{dp}{dt}|_{t=0} \approx \theta = k_s [S]_{\text{max}}
\]
As a result the following approximation can be written:

\[ k_s = \frac{\theta}{[S]_{\text{max}}} \]  \hspace{1cm} (10)

The derivative \( \theta \) of the concentration at time \( t=0 \) can be estimated from the experimental data to be \( \frac{P_{\text{max}}}{t_h} \), where \( t_h \) is the time at which the product reaches its maximum value \( P_{\text{max}} = p(t_h) \). Consequently, using (10) the following estimate of \( k_s \) follows:

\[ k_s = \frac{P_{\text{max}}}{t_h[S]_{\text{max}}} \]  \hspace{1cm} (11)

Since \( p(t) \) reaches its maximum value for \( t = t_h \), its derivative will be equal to zero. It follows that the evaluation of both sides of (8) for \( t = t_h \) will yield the following estimate:

\[ k_p = \frac{k_s[S]_{t_h}}{P_{\text{max}}} \]  \hspace{1cm} (12)

Where \( [S]_{t_h} \) is the substrate concentration at \( t = t_h \).
5. Simulation Results

The experimental data used for parameter tuning and model validation are sourced from the dataset used in [14]. The corresponding in-vitro experiment of estrogen metabolism is run for 30 minutes with an initial 10 μmol of E₂. The concentrations of the metabolites of interest are recorded for the time points 0, 2, 5, 10, 20, and 30 min respectively (see Tables 1). The metabolite concentrations are expressed in μmol. It should be noted that since the experimental data were extracted from the graphical illustrations reported in [14], they match their original values only within the margin of the unavoidable parallax error.

Table 1. Metabolite Concentrations.

<table>
<thead>
<tr>
<th>Time</th>
<th>E₂</th>
<th>4-OHE₂</th>
<th>4-MeOE₂</th>
<th>4-OHE₂-2-SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1.5</td>
<td>0.038</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1.25</td>
<td>1.2</td>
<td>0.15</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>0.5</td>
<td>1.75</td>
<td>0.3</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0.125</td>
<td>1.75</td>
<td>0.4</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>0.1</td>
<td>1.55</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The initial values of the model parameters $k_p$ and $k_s$ were estimated using equations (11) and (12) applied to the data summarized in Table 1. The estrogen metabolites of interest are the DNA depurinating quinones E₂-2,3-Q and E₂-3,4-Q. However, it was reported that the latter’s contribution to DNA adducts dominates with 97% compared to 3% for the first quinone [5]. For this reason, the model simulation analysis is limited to the pathways associated with the production of the quinones E₂-3,4-Q. The initial values of the model parameters were either chosen based on insight about relevant reaction kinetics or curated from literature. The $k_p$ parameters associated with the reduction of E₂-3,4-Q were estimated using the values of Km and Vmax for the oxidoreductases NQO1 and NQO2 as reported in [6]. On the other hand the initial values of KpNQO1 and KpNQO2, which are associated with the depletion rates of E₂-3,4-Q, have been set to the rate of estrogen depletion. Furthermore, given the lack of kinetics information about the chain of reactions transforming the catechol estrogens 4-OHE₂ into quinones E₂-3,4-Q, the parameters for these reactions are initially tuned to satisfy the assumption that the quinones have concentration profiles similar to those of the substrates (catechol estrogens) from which they form. The modulating feedback of the hydroxyl radicals on CYP is another component of the model for which there isn’t sufficient knowledge of kinetics. In this case, the parameters associated with the relevant reactions are arbitrarily initialized so as not to impact the concentration of the catechol estrogen 4-OHE₂. Multiple iterations of parameter tuning were applied to replicate the concentration time-profile of the various metabolites for which experimental data is available (see Table1). The model prediction of metabolite concentrations are illustrated in Figure 6 along with the associated experimental data curatred from the literature.

The simulation results illustrate the structural capacity of the model to internalize the dynamics of estrogen metabolism as informed by the available experimental data. In particular, the model predictions of metabolite concentrations of interest matches closely published experimental results. However, given the lack of sufficient experimental data, it is not possible to assert the plausibility of the estimated concentrations for the various metabolites of interest, including the carcinogenic quinones E₂-3,4-Q. Nevertheless, given the overall encouraging performance of the model, it is expected that the confidence in its predictions will be significantly enhanced as newly available experimental data are used to further tune its parameters. Indeed, the improvement in metabolic modeling is indispensable to furthering our understanding of cancer risk and initiation associated with carcinogenic metabolites that often defy direct experimental measurements.
For future works, the model may be extended to include the effect of genetic variations and antioxidants on the activities of the metabolizing enzymes. In particular, the variability of the model parameters may be used to capture the modulating effect of enzymatic gene expression known to be caused by SNPs. Similarly, the potential effect of antioxidants may be modelled using added unit reaction subsystems (see figure 2) to neutralize oxidizing species and restore estrogen homeostasis [5]. Another aspect to be addressed in future works is the framing of the parameter tuning process as an optimization problem. In particular, the model can be conveniently specified in the S-domain as a multi-dimensional transfer function representing a MIMO (multi-input and multi-output) system. With such representation, the parametric model may be tuned to minimize an error function using a variety of classic parameter estimation algorithms [15].

6. Conclusion

Computational modeling of estrogen metabolism is explored using a proposed approximation model of enzymatic reaction kinetics. The model parameters are determined through multiple iterations of tuning applied to initially specified values. The tuning process is guided by the available experimental data about metabolite concentrations and reaction kinetics. The model is shown to reproduce the experimental time profiles of metabolite concentrations of interest. However, more experimental data is needed to enhance the overall confidence in its predictions.

References