COMPUTATIONAL STEROIDOGENESIS MODEL TO PREDICT BIOCHEMICAL RESPONSE TO ENDOCRINE ACTIVE CHEMICALS: MODEL DEVELOPMENT AND CROSS VALIDATION

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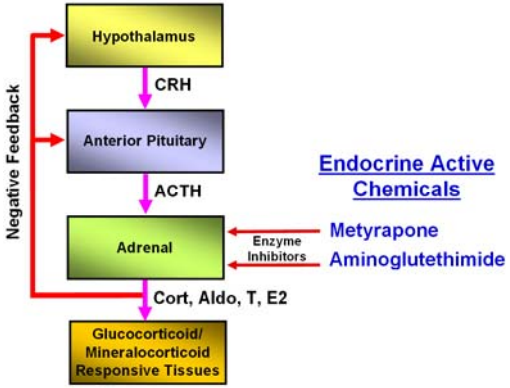
ABSTRACT

Steroids, which have an important role in a wide range of physiological processes, are synthesized primarily in the gonads and adrenal glands through a series of enzyme-mediated reactions. The activity of steroidogenic enzymes can be altered by a variety of endocrine active chemicals (EAC), some of which are therapeutics and others that are environmental contaminants. We are developing a dynamic mathematical model of the metabolic network of adrenal steroidogenesis to predict the synthesis and secretion of adrenocortical steroids (e.g. mineralocorticoids, glucocorticoids, androgens and estrogens), and their biochemical response to EAC. We previously developed a deterministic model which describes the biosynthetic pathways for the conversion of cholesterol to adrenocortical steroids, and the kinetics for enzyme inhibition by the EAC, metyrapone. In this study, we extended our model for a multiple enzyme inhibiter, aminoglutethimide. Experiments were performed using the H295R human adrenocortical cells, and concentrations of 12 steroids were simultaneously measured with a newly developed LC/MS/MS method. We performed cross validation of our model for the baseline data across multiple experimental studies. Results show that the model simulation closely corresponds to the time-course baseline data. Our study demonstrates the feasibility of using the *in silico* mechanistic computational model to predict the *in vitro* adrenocortical steroid concentrations using H296R cells. This capability could be useful to help define mechanisms of actions for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system, and to screen drug candidates based on steroidogenic effects in the early phase of drug development.

EFFECTS OF ENDOCRINE ACTIVE CHEMICALS ON HPA AXIS



Feedback control system of hypothalamus-pituitary-adrenal (HPA) axis regulates synthesis and secretion of adrenocortical steroids (aldosterone (Aldo), cortisol (Cort), testosterone (T), estradiol (E2)) by release of corticotropin releasing hormone (CRH) from hypothalamus, and adrenocorticotropic hormone (ACTH) from pituitary

H295R CELL LINE

- Established from human female adrenocortical carcinoma
- Maintains ability to secrete all major adrenocortical steroids
- Proposed as EPA screening assay for environmental chemicals capable of disrupting or modulating steroidogenesis
- Being evaluated to screen drug candidates based on steroidogenic effects in early phase of drug development

CELL PROLIFERATION EXPERIMENTS

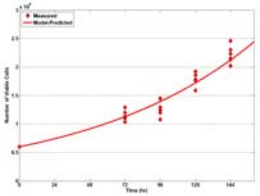
- Incubate cells in medium for 72 hr
- Incubate cells in new medium + stimuli + carrier for 72 hr
- Collect samples at 0, 72, 96, 120, 144 hr
- Measure number of viable cells using cell viability analyzer

CELL PROLIFERATION MODEL

$$\frac{dN}{dt} = k_p N \;\Rightarrow\; N = N_0 e^{k_p t}$$

N = number of viable cells
k_p = growth rate
N_0 = initial number of viable cells

Estimated: $k_p = 0.00878$



- Direct least-squares solution by linearization

STEROIDOGENESIS EXPERIMENTS

- Incubate cells in medium for 72 hr
- Start clock: Incubate cells in new medium + stimuli + carrier for 72 hr

Metyrapone Study

- Baseline and two concentrations (1, 10 μM)
- Collect samples at 0, 8, 24, 48, 72 hr
- Measure cell and medium concentrations of E1 and E2 using ELISA and remaining 12 steroids using LC/MS/MS

Aminoglutethimide Study

- Baseline and one concentration (100 μM)
- Collect samples at 24, 48, 72 hr
- Measure cell and medium concentrations of 12 steroids except E1 and E2 using LC/MS/MS

STEROIDOGENESIS MODEL

- Mathematical model based on *in vitro* experimental design
- Two compartments: medium and H295R cells
- Two pathways: transport and metabolism
- First-order metabolic reaction rates
- Assumed quasi-equilibrium for steroid transport
  - rapid & reversible steroid transport between medium and cells
- First-order cholesterol transport rate
- Endocrine active chemicals: partition coefficient for transport, competitive enzyme inhibition

Equilibrium Equations: Transport Pathway

$$C_{x,\text{med}} = q_x C_{x,\text{cell}}$$

q_x = equilibrium constant for steroid x
$C_{x,\text{cell}}$ = concentration of steroid x in cells
$C_{x,\text{med}}$ = concentration of steroid x in medium

- Yields algebraic equations
- Decouples equations for steroids in medium from equations for steroids in cells
  - simplify large inverse problem

Dynamic Mass Balances: Metabolic Pathway

$$\frac{d}{dt}\left(V_{\text{cell}} C_{x,\text{cell}} + V_{\text{med}} C_{x,\text{med}}\right) = P_{x,\text{cell}} - U_{x,\text{cell}}$$

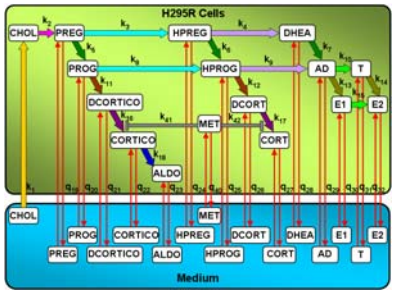
V_{cell} = volume of viable cells
V_{med} = volume of medium
$P_{x,\text{cell}}$ = production rate of steroid x in cells
$U_{x,\text{cell}}$ = utilization rate of steroid x in cells

Competitive Enzyme Inhibition Equation

$$v_i = \frac{k_i}{1 + \dfrac{C_{\text{EAC,cell}}}{k_{\text{EAC},i}}}$$

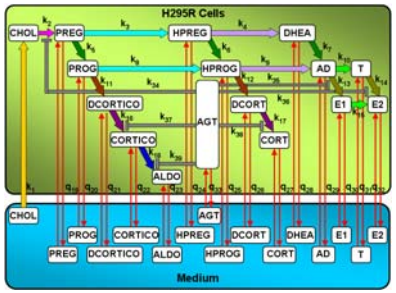
v_i = overall metabolic reaction rate for reaction i
k_i = first-order metabolic reaction rate for reaction i
$C_{\text{EAC,cell}}$ = concentration of EAC in cells
$k_{\text{EAC},i}$ = enzyme inhibition constant of EAC for reaction i

Metyrapone Model



- 17 first-order metabolic reaction rates
- 14 steroid equilibrium constants
- First-order CHOL uptake rate
- MET partition coefficient
- 2 enzyme inhibition constants

Aminoglutethimide Model



- 17 first-order metabolic reaction rates
- 14 steroid equilibrium constants
- First-order CHOL uptake rate
- AGT partition coefficient
- 6 enzyme inhibition constants

MODEL PARAMETERS

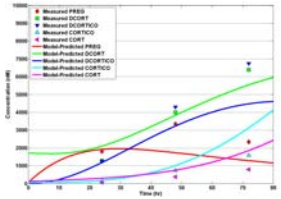
- Estimated parameters with data from metyrapone study

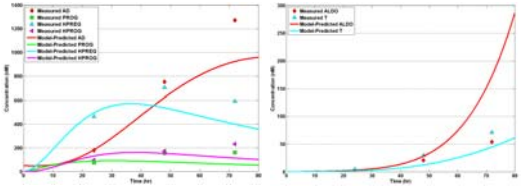
Aldosterone (ALDO) Pathway		Metyrapone (MET)		Testosterone (T) Pathway	
q_{19}	0.0129	q_{24}	0.0557	q_{28}	0.0377
q_{20}	0.0052	Cortisol (CORT) Pathway		q_{29}	0.0400
q_{21}	0.0411	q_{24}	0.0604	q_{30}	0.0266
q_{22}	0.0557	q_{25}	0.0211	q_{31}	0.0442
q_{23}	0.0910	q_{26}	0.0422	q_{32}	0.0350
		q_{27}	0.0675		

Aldosterone (ALDO) Pathway			Metyrapone (MET)			Testosterone (T) Pathway		
k_1	0.002	hr^{-1}	k_{41}	61.438	nM	k_6	$7x10^{-4}$	hr^{-1}
k_2	0.021	hr^{-1}	k_{42}	23.813	nM	k_7	$8x10^{-4}$	hr^{-1}
k_3	0.535	hr^{-1}	Cortisol (CORT) Pathway			k_9	2.177	hr^{-1}
k_{11}	4.590	hr^{-1}	k_3	0.525	hr^{-1}	k_{10}	0.028	hr^{-1}
k_{16}	0.409	hr^{-1}	k_4	8.213	hr^{-1}	k_{13}	0.380	hr^{-1}
k_{18}	0.069	hr^{-1}	k_5	$3x10^{-5}$	hr^{-1}	k_{14}	$4x10^{-4}$	hr^{-1}
			k_{12}	7.969	hr^{-1}	k_{15}	0.059	hr^{-1}
			k_{17}	0.170	hr^{-1}			

CROSS VALIDATION

- Validated model with baseline data from aminoglutethimide study





DISCUSSION

- Dynamic behavior of model simulation and baseline data in medium correspond across both studies
- Mechanistic model can improve understanding of dose-response behavior for chemicals that alter activity of steroidogenic enzymes
- This capability could help define mechanisms of action for poorly characterized chemicals and mixtures in support of the H295R steroidogenesis screening system

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