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Modeling the inflammatory response in the hypothalamus ensuing heat stroke: Iterative cycle of model calibration, identifiability analysis, experimental design and data collection

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ABSTRACT

Heat Stroke (HS) is a life-threatening illness caused by prolonged exposure to heat that causes severe hyperthermia and nervous system abnormalities. The long term consequences of HS are poorly understood and deeper insight is required to find possible treatment strategies. Elevated pro- and anti-inflammatory cytokines during HS recovery suggest to play a major role in the immune response. In this study, we developed a mathematical model to understand the interactions and dynamics of cytokines in the hypothalamus, the main thermoregulatory center in the brain. Uncertainty and identifiability analysis of the calibrated model parameters revealed non-identifiable parameters due to the limited amount of data. To overcome the lack of identifiability of the parameters, an iterative cycle of optimal experimental design, data collection, re-calibration and model reduction was applied and further informative experiments were suggested. Additionally, a new method of approximating the prior distribution of the parameters for Bayesian optimal experimental design based on the profile likelihood is presented.

1. Introduction

Heat Stroke (HS) is a life-threatening illness caused by prolonged exposure to heat. It is commonly diagnosed as core temperatures (T_c) > 40 °C, profound central nervous system abnormalities and organ or tissue damage [1]. In times of global warming, HS is not only a sports and military problem [2] but becomes a public health issue, endangering not only the young and elderly [3]. In the past two decades HS had a higher death toll in the United States than tornadoes, hurricanes, earthquakes and lightning combined [4]. Despite clinical cooling therapies, HS is often followed by the systemic inflammatory response syndrome (SIRS) and multi organ dysfunction and no preventive treatments, e.g., pharmaceutics have been discovered [5,6]. Mechanisms mediating SIRS are not well understood, but concomitantly elevated pro- and anti-inflammatory cytokines during HS recovery [1,3–6] suggest that a complex network of cytokines functions as potential mediator. Furthermore, HS patients and animal models show unexplained temperature behavior during recovery consisting of immediate hypothermia and fever 24 h after heat exposure. Hypothermia is thought to be a consequence of damage to the pre-optic anterior hypothalamus (POAH) [1,3], which is considered the main thermoregulatory center in the brain [7,8], however, these effects also occur in absence of any damage [6]. Elevated pro- and anti-inflammatory cytokines are able to act on the CNS to regulate T_c during inflammation which makes them most likely to be part of the T_c response. Biedenkapp et al. [6] determined increased cytokines (heat shock protein 72 (HSP), interleukin-6 (IL-6), IL-1, tumor necrosis factor (TNF) α) and cyclooxygenase (COX) 2 gene expression changes in the hypothalamus, suggesting them to be associated with SIRS and T_c regulation.

Rodriguez-Fernandez et al. [4] developed a mathematical HS model, describing the dynamics of gene expression of HSP, IL-1α, IL-1β, IL-6, IL-10 and TNF-α in the liver during early stages of SIRS. To understand the cytokine dynamics which may regulate T_c, we extended the approach to the hypothalamus. In contrast to the complex model in [4] (65 ordinary differential equations (ODEs) and 130 free parameters) a more simple model was built to describe the dynamics of HSP, IL-6, TNF-α, IL-1, IL-10 and cox-2

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gene expression disregarding any transcription factors. A systems biology approach helps to gain insight in the important interactions and pathways during and ensuing HS as well as explains the dynamics of the observables between measurements. The mathematical model helps to identify the molecular mechanisms, which may serve as potential pharmaceutical targets in HS patients and can be utilized to perform *in silico* experiments, which saves costs, time and reduces animal testings.

In order to rely on the obtained information from the model, it is important that its predictions can be trusted. Model predictions depend on the estimated model parameters which are obtained from the fitting to the experimental data and their identifiability. After parameter estimation, some parameters may not be identifiable, due to a limited amount and quality of the experimental data. That means the confidence intervals (interval; which contains the true value with a certain probability) are infinite. Even if parameters are uniquely identifiable they can only be estimated within a finite confidence interval, in case measurement errors exist [9]. Uncertainties in the parameter estimates thus directly translate in model predictions making some biological questions not addressable [10].

Hence, it is important to resolve non-identifiabilities in a mathematical model by incorporating new data. However, the choice of new data is crucial to the information which is needed to identify parameters. Optimal experimental design (OED) can be used to find the most informative experimental conditions. In this study an iterative cycle of model calibration, identifiability analysis, OED and experiments is demonstrated to identify parameters in an inflammatory model describing the cytokine interactions in the hypothalamus. Parameter estimation is done by optimizing the likelihood and identifiability analysis as well as OED is based on the profile likelihoods of the estimates [10]. Additionally a new method of approximating the prior distribution of parameters in Bayesian OED is presented.

2. Methods

2.1. Description of the data

A detailed description of the experimental methods can be found elsewhere [6]. Briefly, male C57BL/6J mice were accustomed to standard environmental conditions (25 ± 2 °C, 12:12 h light–dark cycle, lights on at 6am). Before the experiment mice were intraperitoneally implanted with a battery-operated radiotelemetry transmitter device to record T_c. T_c was monitored in 1-min intervals with a precision of ±0.1 °C throughout the experiment. Full recovery from surgery was awaited until experiments were begun (≥1 week). A detailed version of the heat stress protocol can be found elsewhere [3]. In summary, mice were exposed to an ambient temperature (T_a) of 39.5 ± 0.2 °C without food and water until they reached a maximum temperature ($T_{c,\max}$) of 42.7 °C. Ensuing removal from the heat, mice were kept at $T_a = 25$ °C with free access to food and water for recovery. Prior to the experiment mice were randomly assigned to one of the following groups for tissue collection: (1) baseline (immediately prior to the experiment, $t = 0$), (2) $T_{c,\max}$ (T_c = 42.7 °C), (3) hypothermia depth (HD; lowest T_c value with cooling rate ≤ 0.01 °C/min) and (4) 24 h after heat exposure. RNA was isolated from micropunches of the hypothalamus and cDNA was synthesized which was used in real-time PCR (polymerase chain reaction) experiments. For each gene a threshold cycle (Ct) was defined as the PCR cycle where the emitted fluorescence signal was greater than any background noise. Data of heated and non-heated controls were normalized by calculating the difference in Ct values between the target gene of interest and the 18s internal housekeeping gene

$$\Delta\mathrm{Ct} = \mathrm{Ct}_{target} - \mathrm{Ct}_{18sRNA}. \tag{1}$$

Gene expression changes in heated mice were calculated as fold changes relative to the average of non-heated controls using the $2^{-\Delta\Delta\mathrm{Ct}}$ method [11] at the specific time points ($T_{c,\max}$, HD, 24 h) with

$$\Delta\Delta\mathrm{Ct} = \Delta\mathrm{Ct}_{heated} - \Delta\mathrm{Ct}_{av,control}. \tag{2}$$

Furthermore the mean of the fold changes and its standard error was calculated at every time point t_j ($T_{c,\max}$, HD, 24 h) for all observables i (HSP, IL-6, IL-1β, TNF-α, cox-2) according to

$$\tilde{y}_i(t_j) = \frac{1}{Z}\sum_{z=1}^{Z}\tilde{y}_{iz}(t_j) \text{ and } \sigma_i(t_j) = \frac{\sigma_{\tilde{y}_{iz}}(t_j)}{\sqrt{Z}} \tag{3}$$

with

$$\sigma_{\tilde{y}_{iz}}(t_j) = \sqrt{\frac{1}{Z-1}\sum_{z=1}^{Z}(\tilde{y}_{iz}(t_j) - \tilde{y}_i(t_j))^2}. \tag{4}$$

and Z being the number of data points obtained at t_j. A detailed description of the data is provided in [6] and summarized in Table 1.

In order to use the mean fold changes calculated in Eq. (3) an average of the temperature profiles of the heated mice has to be used. However, sampling points at $T_{c,\max}$ and HD depend on the temperature and therefore vary in the sampling times according to the individual heating and cooling rate of the mice. Rodriguez-Fernandez et al. [4] has shown that averaging along the time axis would lead to misleading results in terms of the temperature values. Thus individual temperature profiles were averaged along the temperature axis (Fig. 1). For modeling purposes sampling time points at $T_{c,\max}$ and HD were determined from the averaged temperature profile to $t_{T_{c,\max}} = 261$ min and $t_{\mathrm{HD}} = 445$ min.

2.2. Framework, modeling and assumptions

The underlying framework of the mathematical model describing the cytokine dynamics during heat stroke is presented in Fig. 2. The dynamics are modeled by ODEs

$$\frac{\mathrm{d}}{\mathrm{dt}}\mathbf{x}(t,\boldsymbol{\theta}) = \mathbf{f}(\mathbf{x},\boldsymbol{\theta},u(t)) \tag{5}$$

where $\mathbf{x}$ is a vector of the species, $\boldsymbol{\theta}$ the free model parameters and $u(t)$ an input to the system. $\mathbf{f}$ describes all reaction rates and inputs to the respective species. It will be specified in the following.

It has been assumed that the elevated T_c is the only trigger of the concomitantly elevated pro- and anti-inflammatory cytokines by increasing the concentration of denatured proteins, endotoxins (lipopolysaccharides, LPS) and reactive oxygen species (ROS) [4]. Seeing that NF-κB is not strongly elevated during heat stroke in the liver [4] and the fact of increased mortality in toll-like receptor 4 (TLR4; receptor to detect LPS and initiate an immune response) KO mice [12] suggests that LPS may not play a significant role. Furthermore we neglected the impact of ROS and tested the hypothesis that immune responses ensuing heat stroke are mainly mediated by denatured proteins. Denaturation of proteins can

Table 1
Data shows fold changes in the hypothalamus of heated relative to controls in C57BL/6J mice.

Gene	$T_{c,\max}$	HD	24 h
HSP	44 ± 12[a]	216 ± 18[a]	1.0 ± 0.5
IL-6	1.1 ± 0.3	3.6 ± 1.1[a]	0.72 ± 0.50
TNF	1.9 ± 1.3	7.8 ± 1.9[a]	2.7 ± 0.6
IL-1	3.7 ± 0.8[a]	27 ± 5.3[a]	2.5 ± 0.9
cox2	1.5 ± 0.5	3.6 ± 0.5[a]	2.0 ± 0.7

[a] Represents significant difference from controls (student's T-test: $p < 0.05$). Data obtained from [6].