Accelerating the acceptance of mathematical models as evidence in safety and efficacy decision making

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Beyond Heart Rate Variability: Using attractor reconstruction with all the data in a blood pressure signal for feature extraction

Professor Philip Aston
University of Surrey

The multitude of heart rate variability (HRV) methods have dominated the analysis of heart data (blood pressure, ECG, etc.) for over two decades. All these methods start by extracting the heart beats from the data and this reduced time series is then analysed in many ways. However, this approach discards most of the data before commencing any analysis and so cannot detect any changes in the waveform shape. In contrast, our approach analyses all of the data from a physiological signal using attractor reconstruction. The naturally occurring baseline variation is removed by projecting the attractor onto a plane. Quantitative measures can be derived from the reconstructed attractor and traced out as a time window moves through the data. This approach can detect changes in the shape of the waveform that HRV methods cannot detect. We have applied this approach to two problems:

1. Detection of contractility changes using blood pressure data. Excellent agreement has been obtained when compared with changes in left ventricular pressure.

2. The early detection of sepsis for which our results are significantly better than those obtained using systolic or diastolic blood pressure.
Introduction
- Blood pressure (BP) and ECG data at high sampling frequency is available over long periods of time
- Detection of subtle changes in the data can indicate early onset of disease offering the opportunity for early clinical intervention
- Can we extract diagnostic information from the data?

Attractor Reconstruction Method
- We use all the data, not the beat-to-beat (RR) intervals
- So we can detect changes in the shape of the waveform

Our method consists of four steps:
1. Reconstruct the attractor using all the data
   - Little information can be gleaned from a compressed plot of blood pressure against time
   - It would be better to plot the data in phase space
   - Problem: We only have one variable!
   - Solution: Use Takens’ method for reconstructing attractors using delay coordinates (1)
   - If the blood pressure signal is $x(t)$, we define $y(t) = x(t - \tau)$, $z(t) = x(t - 2\tau)$ where $\tau > 0$ is a fixed time delay
   - We can now plot the trajectory $(x(t), y(t), z(t))$ in a three-dimensional phase space (see Fig. 2)

2. Remove baseline variation
   - There is natural variation in the blood pressure which we want to remove
   - If $x(t) \rightarrow x(t) + c$ then there is a similar shift in $y$ and $z$
   - In the phase space, we then have
     $(x(t), y(t), z(t)) \rightarrow (x(t) + c, y(t) + c, z(t) + c) = (x(t), y(t), z(t)) + c(1, 1, 1)$
   - We define the new variables $u = \frac{1}{c}(x + y + z)$, $v = \frac{1}{c}(x + y - 2z)$, $w = \frac{1}{c}(x - y)$
   - The $(v, w)$ plane is perpendicular to the vector $(1, 1, 1)$, which is the direction associated with vertical movement in the signal (see Fig. 3)

3. Construct a density
   - We construct a density in the $(v, w)$ plane since this is more useful than a blur of lines (see Fig. 4)

4. Generate time traces
   - The density is constructed using a 10s time window
   - Various scalar measures are derived from the density
   - The time window is moved through the data to generate a collection of time traces of these measures

Comparison with Heart Rate Variability
- HRV reduces the large volume of data by considering only the beat-to-beat (RR) intervals
- There are many ways of analysing this reduced time series and using it to diagnose various diseases [3]
- All the data regarding the shape of the waveform is discarded which may contain useful diagnostic information
- Accurately determining the peaks in the data can be difficult

Attractor Reconstruction:
- Our method uses all the available data
- It can detect changes in the shape of the waveform that HRV cannot detect
- We can detect both cardiac and vascular changes
- The method is robust as artefacts in the data have little effect on the density
- Removal of baseline variation is very simple
- This approach can be used for any approximately periodic signal e.g. ECG, PPG, respiratory waveform

Example
For this data, there is no significant change in heart rate over the 15 minute time interval

Conclusions
- There is a significant change in the data at 13 minutes
- This change is not detected by the HRV parameters
- The change is very clearly detected by the AR maximum density parameter
- The AR pulse pressure measures also show a significant change in the variability and the magnitude of the pulse pressure (amplitude) at the same point
- The blood pressure data changes from quite variable to almost periodic. This variability (or lack of it) is often associated with health (or disease)
- This significant change in the data is detected by the AR parameters, but is not detected by the HRV parameters

Contractility
- Drugs can cause unwanted changes in the contractility of the heart which can result in failure of a new drug
- Changes in contractility can be characterised by changes in the left ventricular pressure (LVP) [4] measured with a probe in the heart

Q: Can drug induced changes in LVP be detected using a peripheral blood pressure (BP) signal?

Results
Average correlation coefficients for the 4 animals are:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Itraconazole</th>
<th>Pimobendan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>0.9784</td>
<td>0.9762</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

A linear transformation can be used to compare our results with the IVP data

Conclusion
- Changes in LV dP/dt max have good correlation with our measure extracted from blood pressure data for both a positive and a negative inotrope

3Rs Impact
- Replacement: Blood pressure data from past experiments can be re-analysed to obtain new results
- Reduction: Blood pressure data is inherently noisy and so extracted features are often noisy too. Our traces are less variable and show a larger drug related change than traditional measures and so a drug effect can be determined using fewer animals
- Refinement: One aim of our work is to provide an early diagnosis of disease. Early detection means that animal experiments can be run for a shorter time and terminated at a less severe time point

Acknowledgements
We are grateful to Data Sciences International for providing the IVP data and for their support of the contractility work

References
Cardiovascular Modelling to Support the 3Rs

Dr Michael Chappell

University of Warwick

Mean arterial pressure (MAP) and heart rate (HR) are important factors in assessing the safety of novel compounds. While MAP and HR are measured simultaneously, and are known to be interrelated; the impact on each variable is typically quantified with two separate drug effects.

Hemodynamic modelling incorporates the feedback from MAP on other cardiovascular variables, and thus captures the impact on both endpoints through a single mechanism of action. This can potentially improve the predictivity of safety testing, by capturing the behaviour of homeostasis with physiological parameters.

The complexity of such models places a greater burden on the need for a priori testing of structural identifiability, and the robustness of parameter estimates in practice.

We present structural identifiability analysis and parameter estimation results for three hemodynamic models. The analysis clarifies the suitability of models for different experimental conditions, and the parameters that must be fixed to ensure identifiability. Appropriate use of hemodynamic models has the potential to reduce animal use in cardiovascular safety testing, and in developing effective strategies to manage undesired effects.
Structural Identifiability analysis of Cardiovascular Feedback Models

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Introduction

Drugs influence the cardiovascular system in a wide variety of ways; either as part of an intended treatment or as an undesired side effect. Mean arterial pressure (MAP) and heart rate (HR) are important factors in assessing the safety of novel compounds. While MAP and HR are usually measured simultaneously, and are known to be interrelated; a drug’s effect on each variable is typically quantified separately. There have been a few attempts to model the combined drug effect on hemodynamic variables by using homeostasis models with MAP negative feedback (Fancheteau et al. 1993; Snelder et al. 2014).

The advantage of these models is an improved understanding of effects on the cardiovascular system earlier in preclinical development, and the potential to better anticipate the magnitude effects in humans. However, the complexity of such models places a greater burden on the need for a priori testing of structural identifiability, and the robustness of parameter estimates in practice.

Structural identifiability analysis determines whether it is possible to uniquely estimate model parameters, based on the structure of the model and experimental observations available; assuming noise-free data.

Models of the Cardiovascular System

Haemodynamic variables are fundamentally related as follows:

\[ CO = HR.SV \]
\[ MAP = TPR.CO \]

Where HR is Heart Rate, CO is Cardiac Output (blood flow), SV is Stroke Volume (volume of blood ejected in each heart beat), TPR is Total Peripheral Resistance and MAP is Mean Arterial Pressure.

Several feedback mechanisms respond to changes in MAP and act to maintain blood pressure homeostasis over the short and medium term. Within their own limits, heart rate responds to maintain blood pressure, and TPR responds via blood vessels dilating and contracting. There is a need for simplified models to represent this complex feedback in aggregate terms.

\[ \frac{dHR}{dt} = k_{in}HR \times (1 - FB \times MAP) \times (1 + E) - k_{out}HR \times HR \]
\[ k_{in}HR = k_{out}HR \times HReq, \ E = drug \ effect \]


- Proportional and derivative feedback
- Baseline MAP as a setpoint
- External control state U. E.g.

\[ \frac{dHR}{dt} = \frac{HReq \times (1 - \alpha \times U - E - HR)}{\tau_R} - \frac{dE}{dt} \]
\[ \frac{dE}{dt} = \frac{(MAP - MAP_e) + \frac{dMAP}{dt} - U}{\tau_U} \]

Reduced Model

- Based on Cheung et al. (2012).
- Proportional feedback
- Baseline MAP as a setpoint
- No external control state. E.g.

\[ \frac{dHR}{dt} = \frac{HReq \times (1 - \alpha \times (MAP - MAP_e) - E - HR)}{\tau_R} - \frac{dE}{dt} \]

Results

Structural identifiability analysis of the four models was performed using a differential algebra approach (Evans et al. 2013) and the exact algorithm rank approach (Karlsön and Anguelova 2012). The model of Snelder et al. (2013) requires measurement of cardiac output to be structurally globally identifiable (SGI). Fancheteau et al. (1993) was confirmed to be SU, and Cheung et al. (2012) to be SGI, conditional on fixing the value of stroke volume. The model should be used with an awareness of this assumption.

<table>
<thead>
<tr>
<th>Model</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fancheteau 1993</td>
<td>HR &amp; MAP</td>
</tr>
<tr>
<td>Cheung 2012</td>
<td>SGI*</td>
</tr>
<tr>
<td>Reduced Model</td>
<td>SGI</td>
</tr>
<tr>
<td>Snelder 2013</td>
<td>SU &amp; SGI</td>
</tr>
<tr>
<td>Snelder 2014</td>
<td>SGI</td>
</tr>
</tbody>
</table>

*SGI with a fixed value of SV or TPR, otherwise SU
SGI: Structurally globally identifiable, SU: Structurally unidentifiable

Parameter estimation was performed for SGI models on HR and MAP data from rat telemetry (n=8) Nifidipine (0, 3 and 30mg/kg) and Amphetamine (0, 0.3 and 3mg/kg) studies.

All three SGI models could be fitted to the data. Some system parameters could not be robustly determined in the Snelder 2014 model, without fixing some parameters (baseline SV and TPR were fixed due to their clear physiological meaning). This suggests that it could be difficult to distinguish effects on SV and TPR in practice from HR and MAP data alone.

Discussion

The reduced model had the fewest parameters and state equations, and was able to adequately describe the available MAP and HR data. It could potentially offer an improved choice of model in terms of identifiability and parameter estimation. The inclusion of rate-sensitive feedback, as in the Cheung et al. (2012) model may provide a more accurate physiological representation, and be preferred in certain situations.

However, both Cheung et al. (2012) and the reduced model require a priori the value of SV to be fixed, meaning that they cannot represent or estimate the effects on SV. (Alternatively, if a drug is known to effect SV, the value of TPR could be fixed instead of SV). The Snelder et al. (2014) model is more ambitious in including SV and TPR simultaneously. Well parameterised, this model could serve a range of applications in quantifying, translating and predicting hemodynamic effects.

Different strategies may be preferred in the models to implement circadian baseline functions in MAP and/or HR. For example, a cosine function can be used on the MAP equilibrium set point in Cheung et al. (2012) and the reduced model, and in Snelder et al. (2014) this instead amplifies the production rate of HR. The circadian rhythm could not be distinguished from background variability in this case. This is not an ideal scenario for parameter estimation, but reflects a common situation, with MAP and HR evaluated at hourly timepoints.

The feedback models provided better fits than using two separate drug effects on MAP and HR.

3Rs Impact

Cardiovascular safety is one of the leading reasons for drug attrition and withdrawal. By improving confidence in hemodynamic models, this work has the potential to improve their predictive power. Making better use of experimental data enables the reduction and refinement of animal use in cardiovascular safety testing, and in developing treatments to manage hemodynamic effects.

References:
Development and application of evidencing and argumentation tools in the NC3Rs CRACK IT Virtual Infectious Disease Laboratory and Virtual Fish Ecotoxicology Laboratory Projects

Professor Mark Coles
SimOmics Ltd / University of York

Mathematical and computational models have the potential to accelerate medical research and reduce and replace the use of animals in therapeutic discovery and development and toxicology testing. This is because simulations provide a platform to rapidly predict outcomes of complex phenomena (e.g. immune function) without the limitations inherent in animal experiments. Models permit testing of a large number of variables and capacity to provide key evidence for clinical trial design and regulatory approval. However, simulations are inherently dependent on processes and assumptions that underpin their design, these are frequently unclear and leads to a loss of confidence in the intended users. One method to address this challenge is to utilise a robust methodology to evidence the models in an open and transparent format that is accessible to all potential users, from basic research scientists, regulators and modeller to corporate management. This mitigates risk, provides a platform to understand the model as scientific understanding of complex biological systems evolve. Through taking a robust approach to the evidencing and argumentation process it is possible to convince key stakeholders that a given computational / mathematical model is appropriate and have confidence in the relationship between simulation outcomes and real-world data.
Evidenced-based Virtual Laboratories to Support the 3Rs

Paul Andrews, Adam Nellis, Ed Clark, Mark Coles, Jon Timmis

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Background: SimOmics is funded by NC3Rs and InnovateUK to develop virtual laboratories to reduce and replace animals in pharmaceutical development and testing. SimOmics is developing software tools and modelling technologies to assist in evidence-based decision making in areas including infectious disease and ecotoxicology risk assessment. SimOmics technologies include software to generate argumentation structures, automated generation of diagrams to reveal model structures and automated tools for the detailed analysis of computational and mathematical models, all of which can be linked via a novel web-based user interfaces for high degrees of transparency.

2. Virtual Fish Ecotoxicology Laboratory (VFETL)

3Rs and fish toxicology: All new active pharmaceutical ingredients (API) must undergo an environmental risk assessment (ERA) before being authorised. Currently tens of thousands of fish are used worldwide as part of API ERAs. Development of predictive in silico models already has the potential to significantly reduce animal use (3Rs) and reduce R&D costs around the ERA of pharmaceuticals. VFETL: Our approach will characterise the movement of an API from the patient, through the wastewater and river systems, into fish tissues and predict the apical and non-apical effects on individuals and populations. By understanding the pathway from patient to effect, it will be possible to develop an optimum experimental testing strategy for an API. Working closely with AstraZeneca and University of York to deliver commercial product by 2018.

3. Virtual Infectious Disease Laboratory (VIDL)

LeishSim: Part of a wider team developing a virtual platform that models infection and the host response to the Leishmania pathogen for basic research and enhances new target development in infectious diseases. Leishmania, one of the most neglected tropical diseases, yet ranked 9th in analysis of global burden of disease, with an associated mortality amongst parasitic infections second only to malaria. A free virtual lab, focussed on Leishmania, will be available to the community by mid 2017.

5. Evidence and Transparency Tools

Underpinning our virtual laboratories is a clear evidence base that can be summarised using a variant of Goal Structuring Notation (GSN) adapted from critical systems engineering. This allows for the construction of logical arguments underpinning evidence relating to data, assumptions in models and assessment of risk. Users will be able to develop their own evidence base, and critical, assess those of others.

6. Summary

- We are developing an integrated web-based system for the intelligent analysis of APIs in the environment and assessment of drug therapeutics for infectious disease
- Focus is on developing commercially viable 3Rs technologies that can be translated to a wide variety of 3Rs challenges

Fund: Innovate UK
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Investigating the role of lactate and proton efflux in extracellular acidification using a pH dependent in silico model of hepatic glycolysis

Ross Kelly
Liverpool John Moores University

Extracellular flux analysis (EFA) is gaining momentum as a versatile high throughput method of assessing cellular bioenergetics for a plethora of biological points of interest, boasting inclusion in over 1500 peer reviewed publications. Commonly used as a method of investigating drug induced mitochondrial toxicity/dysfunction in a wide variety of different cellular systems, EFA using any of the XF analysers (Seahorse machines) is accomplished by measuring rates of oxygen consumption and proton efflux. The majority of investigations centre on changes cellular respiration, measured via changes oxygen consumption rate (OCR), with the concomitant extracellular acidification rate (ECAR) measurements, indicative of glycolytic flux, often considered a secondary measurement likely due to the ambiguous nature of ECAR.

In order to investigate lactic acid as the predominant driving force of ECAR during extracellular flux analysis, a mathematical model of hepatic glycolysis that is pH-dependent with respect to reaction equilibria and enzyme kinetics, capable of computing a dynamic pH time course as a function of glycolytic flux was constructed. Recognising that lactic acid exists as its lactate anion and a proton at physiological pH prompted ECAR measurements to be simulated using the liver specific monocarboxylate transporter 1 (MCT1) flux. The model was then aligned with in vitro EFA data using HepG2 cells, assessing the changes in ECAR as a function of extracellular glucose concentrations illustrating a relationship between the GLUT2 transporter $V_{\text{max}}$ parameter and ECAR. Finally, the model was used to simulate the effects of extracellular pH on ECAR highlighting the need for care when using compounds that may alter extracellular pH.
Glucose metabolism (glycolytic flux) accounts for a significant portion of cellular bioenergetics as a direct source of ATP, and as a molecular precursor source for other energy producing systems such as the mitochondria via the TCA cycle. As a result, perturbations of cellular respiration, often as a result of “off-target” drug induced mitochondrial toxicity, may be observed by compensatory effects on glycolytic flux [1].

- In vitro, extracellular acidification rate (ECAR) is the predominant method of measuring glycolytic flux, with lactic acid postulated to be the source main of acidification [1].
- As a biological system of high importance, particularly for drug toxicity, in silico models of hepatic glucose metabolism already exist [2].
- However, a comprehensive pH-dependent model that includes dynamic proton buffering and cellular cation binding within hepatic cellular respiration and glucose metabolism has yet to be presented.

**Aims**

- Construct pH-dependent mathematical model that includes dynamic proton buffering and cellular cation binding.
- Illustrate the models ability to replicate in vitro hepatic glucose metabolism.

**Hepatic Glucose Model**

- Illustration of model components. The model includes the major metabolic pathways glycolysis, gluconeogenesis and glycogenolysis, as well as additional reactions for ATP synthesis and production by oxidative cellular respiration (OCR).
- Model was constructed using Matlab (2019a).

**Biochemical Reactions**

**Biochemical Species**

**Discussion**

- The pH-dependent hepatic glucose metabolism model simulates the fundamental characteristics of cellular hepatic bioenergetics.
- This model is able to simulate ECAR via the MCT1 flux (Figure 1), lending credibility to lactic acid efflux as the driving factor of ECAR.
- Readjusting the MCT1 flux by fitting to in vitro data allows the model to be used synergistically with extracellular flux analysis, providing an enhanced platform from which to predict and assess drug induced bioenergetics impairment, reducing the usage of animal models.
- A pH-dependent in silico model of human hepatic glucose metabolism that accounts for proton buffering and rapid metal cation binding was successfully constructed.
- Coupling detailed thermodynamically driven, charge and mass balanced mathematical models with in vitro extracellular flux analysis has the potential to yield mechanistic insight into “off-target” toxicities such as drug induced mitochondrial dysfunction.

**Conclusions**

- In silico methods combined with in vitro techniques allow intelligent prediction and assessment of drug toxicity, facilitating the reduction of animal models.

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**References**


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**In Vitro Glucose Metabolism**

- In vitro hepatic glucose metabolism was measured using extracellular flux analysis ( Seahorse) in order to validate the model. HepG2 cells were deprived of extracellular glucose for 3 hour before analysis, glucose injection at t = 16 min (black line (a,c)) with 10 different concentrations (0.1 mM – 25 mM).

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**Proton & Ion Binding**

The state of the proton bound to reactant A is referred to as [AH]. Considering only proton binding, the total concentration of A is given by (1.1) and the concentration of A with a single protonation is given by (1.1). Here, K_d denotes the dissociation constant.

**Thermodynamically Derived Equilibrium Constant Keq**

The equilibrium constant for a reaction may be derived using the ∆G° for the Gibbs free energy change for the reaction. For reaction (2.0), the expression for Keq may be written in terms of ∆G° (2.1), in terms of reactant concentrations (2.2) using their binding polynomials, allowing inclusion of respective proton and ion binding (5).

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**Sensitivity Analysis**

- Model parameter sensitivity is expressed as the % mean change of variable time course solutions altering all 228 model parameters within the range of -90% to +400% of their original values.
- Parameters that induce >10% sensitivity are classified as sensitive.

**ECAR Sensitivity**

- Changes in model ECAR after parameter perturbations in the form of sensitivity analysis. Sirius GLK, Km DHAP and Km PYR were changed from -90% to 400% and Changes in ECAR were measured.

**ECAR Simulation**

- Experimental design: A) Cell seeding incubation -720 min, B) Media removal - 60 min, C) glucose injection (5 mM), D) ECAR measurement / simulation – 90 min.

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**References**

New possibilities in Mathematical Toxicology

Marko Raseta
University of York

Bayesian Networks have recently been advocated to predict hazard and potency class of chemicals in the context of skin sensitization by using only animal-free assays. We have used developments in probability and statistics to both simplify and generalize these initial results. Our new approach provides robust procedures for constructing Bayesian Networks, avoiding earlier pitfalls involving data imputation, loss of information and cross-validation while maintaining (or improving) the accuracy of hazard and potency prediction.

Importantly, our methods use Machine learning, avoiding need for mechanistic knowledge of the biological processes in question. This gives us hope that Bayesian Networks may be successful in general toxicity prediction using only Machine Learning and animal-free assays. In this spirit preliminary results for liver carcinogenicity, where the underlying biological mechanisms are much less understood, will be discussed.

By extending this probabilistic thinking, we have also developed a rigorous framework to derive optimal integrated testing strategies for toxicity assessment using animal-free test alone. We combine a population model (accounting for individual-level differences in exposure and in reaction to that exposure) with an explicit cost structure (including both testing and misclassification costs) to derive optimal integrated testing strategies based on the powerful mathematical machinery of Markov Decision Problems. It turns out that, even in the simplest set-ups, optimal policies turn are typically adaptive. In other words, our mathematics demonstrates that one-size-fits-all testing policies cannot possibly be optimal.
Exploring the use of Bayesian statistical models to reduce the number of animals in control groups

Ros Walley and John Sherington

UCB

Many of the *in-vivo* models used in pre-clinical pharmaceutical research are run repeatedly over a period of time with a consistent protocol and control group(s) to test different compounds. Typically, historic data from the control group of previous studies are ignored when running a new study. We have used Bayesian statistical models to incorporate information from the historical controls into a new study, down-weighting this information to allow for variation between studies. This Bayesian methodology can allow the number of control animals in the current study to be reduced or, in some cases, a whole control group to be excluded. Alternatively, more statistical precision can be obtained with the same number of animals. We have successfully piloted this approach and present two case studies from immunology and CNS research areas.

Exploring the use of Bayesian statistical models to reduce the number of animals in control groups

Ros Walley, John Sherington, Joe Rastrick, Alex Vugler, Gill Watt

Introduction

- Many of the in-vivo models used in pre-clinical pharmaceutical research are run repeatedly over a period of time with a consistent protocol and control group(s) to test different compounds.
- Typically, historic data from the control group of previous studies are ignored when running a new study.
- We have used Bayesian statistical models to incorporate information from the historical controls into a new study, down-weighting this information to allow for variation between studies.
- This Bayesian methodology can allow the number of control animals in the current study to be reduced or, in some cases, a whole control group to be excluded. Alternatively, more statistical precision can be obtained with the same number of animals.
- We have successfully piloted this approach and present two case studies from immunology and CNS research areas.

How useful is the historical information?

Imagine we have 10 historic experiments and are about to run experiment 11.

Intuitively, the relevance of the historic controls depends on the size of the study to study variation.

Overview of Bayesian methods used

1. Analyse historic control data, excluding the last study.
   - Bayesian meta-analysis, based on methodology in Neuenschwander et al., Clin Trials 2010 7: 5
2. Analyse the last study
   - Show what would have happened if we had “bought into” the Bayesian approach; omitting animals if necessary
3. Possible options for future studies:
   - Omit all/some animals from all/some control groups.
   - Use historic data as prior information combined with observed data in a Bayesian analysis.
   - Use historic data to give a predictive distribution for control group. i.e. don’t include that treatment group in current study.

Two approaches for different controls

1. Control group not used in formal comparisons.
   - Examples of uses: To ensure challenge is working in a robust reproducible manner; to establish a “window”; to check consistency with previous studies; to convert values to %.
   - Approach: Replace control group with a range from a predictive distribution. This is illustrated for a ‘No challenge’ group in Case study 1.

2. Control group used for formal comparison vs. test compounds/doses
   - Examples of uses: Comparison in t-tests; confidence intervals of differences between control vs. treated etc.
   - Approach: Combine down-weighted historic data with the current experiment. This is illustrated for the Vehicle treated group in Case study 2.

“Using a Bayesian approach will lead to more rapid and more economical drug development without sacrificing good science”


Case study 1: Lipopolysaccaride (LPS)-induced endotoxaemia

- The LPS-induced model of endotoxaemia is a short term in vivo pharmacology model used for the initial assessment of novel inhibitors on pro-inflammatory pathways.
- Mice were pre-treated with either an anti-inflammatory reference antibody; test compound(s); or vehicle - 5 and 30mins prior to LPS challenge.
- Mice were challenged with LPS (i.p; 25mg/mouse).
- There was an additional vehicle treated group that was not challenged
- At 3.5 hours post LPS challenge, mice were terminally anaesthetized; blood was collected and then plasma IL-6 levels determined using an ELISA-based multiplex platform (Mesoscale Discovery; NT50128-1).
- All studies were performed using male Balb/c mice (20-25g) obtained from Harlan (UK). All mice were housed in specific pathogen free conditions in standard cages with food and water supplied ad libitum. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986.

Conclusions

Bayesian methods for the statistical analysis of in-vivo studies have two potentially useful roles, both of which can reduce the use of animals in research:

- Predictive distributions can be used to replace a control group (one not used in formal statistical comparisons), as in the LPS model.
- A full Bayesian approach can be used to augment a control group with historical data, appropriately down-weighted, as in the NOR model.

In terms of the 3Rs, Bayesian statistical models can reduce the numbers of control animals used in studies.

- While the reduction of numbers of animals in a single study may be modest, for repeated assays the reductions significantly accumulate over time.
- For example, if the number of control animals is reduced from 12 to 6 in an assay run 25 times per year, this amounts to 150 animals per year.

Case study 2: Novel Object Recognition model

- Each experiment is performed in two trials:-
  - Acquisition trial – the rat is allowed to explore an arena containing two identical objects.
  - Retention trial – this is run a fixed time after the acquisition trial, and one of the two objects presented during the acquisition trial is replaced by an unknown (novel) object. With this delay, untreated animals poorly distinguish familiar and novel objects.
  - An index of differential exploration is calculated as the difference in time spent exploring the new and familiar objects divided by the sum of both times, and is regarded as representative of the functionality of recognition.
- Data from Vehicle group from 17 previous studies were analysed to give prior information for the mean of this group.

- Data from Vehicle group from 17 previous studies were analysed to give prior information for the mean of this group.
- This was then incorporated into a full Bayesian analysis of the latest study.
- The prior information was equivalent to have an additional 90 vehicle animals.

The figure below shows interval estimates of the vehicle means and differences from vehicle, comparing a conventional ANOVA vs. the full Bayesian approach.

Acknowledgements: Rafeah Alam, Katie Burrows, Eric Detrait, Anne-Catherine Michaux and Jozef Tarrant.