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Corresponding Author:
Dr. Robert Lodder,
Professor, Pharmaceutical Sciences, BPC223 Biopharmaceutical Complex, 40536 - United States of America

Submitting Author:
Dr. Robert Lodder,
Professor, Pharmaceutical Sciences, BPC223 Biopharmaceutical Complex, 40536 - United States of America

Other Authors:
Ms. Cynthia Dickerson,
research assistant, Pharmaceutical Sciences, University of Kentucky - United States of America

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None.

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Author(s): Lodder R, Dickerson C

Abstract

QBEST, a novel statistical method, can be applied to the problem of estimating the No Observed Adverse Effect Level (NOAEL) of a New Molecular Entity (NME) in order to anticipate a safe starting dose for beginning clinical trials. The NOAEL from QBEST (called the QNOAEL) can be calculated using multiple disparate studies in the literature and/or from the lab. The QNOAEL is similar in some ways to the Benchmark dose (BMD) and is superior to the BMD in others. The Benchmark Dose Method is currently widely used in toxicological research.

Results are used in a simulation based on nonparametric cluster analysis methods to calculate confidence levels on the difference between the Effect and the No Effect studies. The QNOAEL simulation generates an intuitive curve that is comparable to the dose-response curve. The NOAEL of ellagic acid (EA) will be calculated for clinical trials of its use as a component therapeutic agent (in BSN476) for treating Chikungunya infections. This will be the first published application of QBEST to the problem of NOAEL determination. The specific aims of the proposed study are to evaluate the accuracy and precision of the QBEST Simulation and QNOAEL compared to the Benchmark Dose Method, and to calculate the QNOAEL of EA for BSN476 Drug Development.

Specific Aims

Nonparametric statistics are statistics that are not based on parameterized families of probability distributions, like the normal distribution. They are important because is it common for data to follow a distribution other than a known one, like the normal distribution.

The NOAEL is an important part of the non-clinical risk assessment for new drugs like BSN476, a drug for treating Chikungunya. The NOAEL is a professional opinion based on the design of the study, indication of the drug, expected pharmacology, and spectrum of off-target effects. It is the highest dose at which there was not an observed toxic or adverse effect [1]. There are important theoretical limitations to the traditional NOAEL calculation, which led to the newer Benchmark Dose method, which also has a number of problems. In brief, the traditional NOAEL is determined by administering a few different doses of drug to a group of subjects, observing those subjects for physiological change, and assigning the dosages to the categories of "having an adverse effect" and "not having an adverse effect". The highest dosage resulting in no adverse effect is determined to be the NOAEL.

The NOAEL method is problematic because (1) dose levels are often an order of magnitude apart, and it is highly unlikely that the exact NOAEL dosage will be administered in any particular study; (2) determination of what constitutes an "effect" can be difficult when negative effects are of a highly subjective nature (for example, when mood is affected)[2]. A nonparametric simulation using QBEST (Quantile Bootstrap Error-adjusted Statistical Test) can solve the problems associated with the use of the traditional NOAEL or the Benchmark Dose (BMD) and enable accurate toxicity estimates to be made.

Specific Aim 1. Evaluate the accuracy and precision of the QBEST Simulation and QNOAEL compared to the Benchmark Dose Method.
Utilizing synthetic data with known characteristics, the BMD and QNOAEL will be calculated. The QNOAEL will then be compared to the BMD to determine which is closest to be known answer for the synthetic data.

Specific Aim 2. Calculate the QNOAEL of Ellagic Acid for BSN476 Drug Development.
Chikungunya is a rapidly spreading mosquito-borne disease that now infects over 3 million people worldwide [3]. BSN476, a drug for treating chikungunya infections, contains in part EA. QBEST will estimate a safe level of EA for the first-in-human study in order to develop a treatment for Chikungunya. An EA toxicity meta-analysis using food consumption will be completed as part of the Investigational New Drug (IND) application to the FDA. Studies will be selected from the literature and analyzed according to the Cochrane protocols, and the QNOAEL of EA will be calculated along with the NOAEL and BMD. These results will serve as the basis for the first-in-human study of EA.

Research Strategy

Method Significance.Â The NOAEL depends strongly on the dose selection, dose spacing, and sample size of a single study from which the critical effect has been identified.Â The primary goal of BMD modeling is to define a point of departure that is largely independent of study design. But while the BMD effectively enables multiple studies to be pooled to increase accuracy, it does not handle studies with conflicting results gracefully, as will be seen below [4].

The new QBEST nonparametric meta-analysis of multiple studies so far appears to be superior to BMD modeling (the current toxicological "gold standard"). Unlike BMD, the QNOAEL estimate is not limited by the format of the data presented. The QNOAEL is no more time-consuming to calculate than the BMD, and provides a simpler decision-making process. For example, the graphs below show the BMD and QBEST simulations for THC in hemp seed. Note that the same clinical studies were used for both analyses. QBEST not only more clearly demonstrates the trend of data, but also produces a correlation curve which is intuitively noncontradictory. (It is to be expected that different studies may reach contradictory results on the effects of a given dosage, as study methods and populations vary.)

Application Significance. Chikungunya is a rapidly spreading mosquito-borne disease that now infects over 3 million people worldwide [3]. The disease originated in Africa around 1700 A.D., and until recent years, reported infections were limited to the African continent and Southeast Asia [5]. The disease was first identified in 1952 during an outbreak so serious that infections were clinically indistinguishable from dengue fever [6]. Throughout the 1960s and 1970s, outbreaks were reported in Southeast Asia[6]. After decades without another Southeast Asian outbreak, a 1999 outbreak in Indonesia led to a massive outbreak reported in India in 2006, the strain responsible for this resurgence bearing 99% similarity to the strain responsible for a 1989 outbreak in Uganda [4,6]. In December, 2013, the disease made its debut in the Americas, with its first local transmission occurring on the island of St. Martin; local transmission in French Guiana on the South American continent occurred later that month [7-9]. After only two years, local transmission had been documented in 19 Caribbean countries, including Puerto Rico, as well as in nearly every country on the South American continent [7-10]. The WHO has currently issued a level-1 watch for travelers visiting South America and the Caribbean and expects Chikungunya to spread [7,8].

BSN476 contains in part EA. EA is a polyphenolic compound with antiproliferative and antiviral properties[11]. EA at 10 uM produces 99.6% inhibition of Chikungunya virus in vitro [11]. EA is found in a number of plant extracts, usually in the form of hydrolyzable ellagitannins which are complex esters of EA with glucose [12,13]. Ellagitannins are broken down in the intestine to eventually release EA [12,13]. To develop BSN476 as a treatment for Chikungunya, the PK of the drug must be studied in a first-in-human (FIH) trial, and a safe range of exposure must be determined for that trial.

QBEST is applied within the Cochrane framework for meta-analysis (the Cochrane framework provides a "Garbage-In-Garbage-Out" standard for data inputs - generally clinical studies) to determine doses for the first in human study [14]. The QNOAEL of EA will be estimated from previously published food consumption studies. Bootstrap replication and manipulation of data clusters will reveal the QNOAEL of EA with 98% confidence.

This project will use the QNOAEL to estimate a safe range of exposure to EA for the FIH study on the way to developing a treatment for Chikungunya. QBEST is a robust $O(n^1)$ algorithm that is designed for massively parallel computers, and is a very powerful meta-analysis tool (most algorithms use matrix factorization and are $O(n^3)$ in execution time). The QBEST algorithm will be translated from MATLAB into Python to make it more accessible to the scientific community. Very large sample datasets will be used to test the memory usage and reproducibility of the results of the algorithm, as well as minimum parameters for its usage. These data have permitted estimation of the maximum analysis capability of cloud computing services and the National Science Foundation XSEDE supercomputer (Comet).

Innovation

This proposal utilizes a statistical method designed for Big Data problems, QBEST, inside a new simulation to estimate the QNOAEL for a drug with 98% confidence from a set of small studies [15,15-18]. QBEST is a form of cluster analysis, which is a common analytical technique for determining chemical identity and purity [15-18]. So far, the QNOAEL appears to be superior to the NOAEL and the BMD.

QBEST is applied within the Cochrane framework for meta-analysis.Â QBEST works by analyzing clusters of studies that found an effect, and clusters of studies that found no effect (the studies can use different dose levels).Â By analyzing the quantiles of each cluster and adjusting for cluster skew, QBEST can measure the distance between clusters in probability space,

until it finds the dose that yields no adverse effects for the entire human population at a specified level of statistical significance (see Fig. 6, 98% level set by default).

Approach

This is a software development project undertaken as part of a larger drug development project. Good Engineering Practice, Standard Operating Procedures (SOPs) and working practice guidelines have been implemented for project design as well as execution (see Fig. 7). A robust change control system must be implemented in this project.

The Design SOPs and Configuration Management system will be applied to the system designed to resolve each specific aim in this project. Each specific aim will begin with a Needs Analysis to determine what the new system needs to be able to do. A requirements analysis will also be conducted to determine what is required to fill those needs. A System Requirements Review (SRR) will demonstrate understanding of the requirements documents (scope, specifications, schedule, validation plans, and budget). SRR will determine the initial design direction and describe preliminary data and progress, and how these will converge to an optimum and complete system configuration for the specific aim.The memory needed to run QBEST on large datasets and evaluate the performance of the algorithm must be quantified. (Very large sample datasets to test the memory usage and reproducibility of the results of the QBEST algorithm are being created, as well as to set ranges of parameters for its usage. Parameters include the number of bootstrap replications desired (b), the number of variables in the multivariate analysis (d), and the size of the dataset used (n). Preliminary data indicate that the typical laptop computer can process over one million bootstrap replication samples, independent variables, or sample data points when the other two parameters are minimized, or over one-thousand bootstrap replications, variables, and data points [all maximized at approximately 1000 inputs]).

As the system evolves through the development process, topic experts will be invited to later design reviews (especially CDR, TRR, and MRR). System Design Review (SDR) acts as a control gate that reviews and approves the top-level system design solution and rationale 19. It is the decision point to proceed with system specification flow down to individual physical and process configuration items [19]. System limitations will be refined at SDR.

Using the run-time and memory usage data determined in Specific Aim 1, the performance capabilities of the algorithm on an NSF supercomputer (XSEDE Comet) will be calculated. QBEST is capable of tackling immensely large datasets, and the computing capabilities of the algorithm will be stretched on a massively parallel machine to demonstrate proof-of-concept. An SDR report will be added to the Design History file for FDA.

A Preliminary Design Review (PDR) will be performed on each configuration item or group of configuration items to: (1) Evaluate the progress, technical acceptability, and risk resolution, (2) Measure its harmony with performance and engineering specialty requirements of the Configuration Item development specification, (3) Evaluate the extent of definition and evaluate the technical risk connected with the selected methods/processes, and (4) Demonstrate the existence and compatibility of the physical and functional interfaces among the configuration item and other items of equipment, facilities, computer software, and personnel [19]. Topic experts will also be invited to the review. A Blue team and a Red team are used for design and validation, respectively. A PDR report will be added to the Design History file for FDA in the annual reporting system.

Critical Design Review (CDR) is the last design review conducted before an action is taken that is irreversible. (1) CDR is a review to establish that detail design of the configuration item under review meets cost, schedule, and performance requirements. (2) CDR will establish detail design compatibility among the configuration item and other items of equipment, facilities, computer software and personnel. (3) CDR will gauge configuration item risk areas (on a technical performance, cost, and schedule basis). Topic experts will again be invited to the review. A Blue team and a Red team are used for design and validation, respectively. A CDR report will be added to the Design History file for FDA in the annual reporting system.

Deployment Readiness Review (DRR) is held to confirm readiness for deployment. This review is conducted to ensure that all deficiencies are corrected before actual use. The complete system is challenged every feasible way (conceptually, physically, cyber-, etc.). The DRR demands the review and analysis of all subsystem/unit level testing preceding the formal acceptance tests. Topic experts will be invited to the review. A Blue team and a Red team are used for design and validation, respectively. A DRR report will be added to the Design History file for FDA.

The QBEST algorithm will be translated into Python to make it more accessible to the scientific community. Once QBEST is available in Python it will run on

Amazon Web Services, Microsoft Azure, and Google Compute Engine as well as the NSF XSEDE Comet supercomputer currently being used. The REPLICA algorithm has already been translated from Matlab into Python to make it more accessible to the scientific community. However, the algorithm on which QBEST relies has yet to be translated and the entirety of the program remains to be validated.

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