Application and Validation of an Advanced Gastrointestinal *In Vitro* Model for the Evaluation of Drug Product Performance in Pharmaceutical Development

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ABSTRACT: Methods to understand and predict the oral bioavailability of drug products are a prioritized research area within the pharmaceutical industry. Models to predict oral bioavailability have the potential to reduce risk, time, and cost in development as well as decrease the need for animal studies. The TNO intestinal model (TIM-1) is an advanced dissolution model deployed by AstraZeneca since 2008. This article presents a systematic evaluation of TIM-1 against *in vivo* data. The relative performance of compounds and formulations tested in TIM-1 and *in vivo* was compared both by a qualitative analysis and a linear regression analysis of relative exposure measures between test and reference formulations in TIM-1 and *in vivo*. The TIM-1 correctly predicted *in vivo* rank order in 84% and 79% of cases for AUC and C_{max}, respectively, when using the 3-h time point. There was only one case for C_{max} in which TIM-1 did not predict an *in vivo* difference. The correlation coefficient (R^2) between relative (test vs. reference formulations) fraction available in TIM-1 after 3 h and AUC was 0.78. Thus, this study suggests that the TIM-1 system can be used to assess the risk for significant differences in exposure between formulations and compound modifications. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3704–3712, 2014

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INTRODUCTION

Oral administration is the predominant drug delivery route for the majority of marketed pharmaceutical products worldwide. It has been estimated that approximately 90% of all new drug candidates are poorly soluble in relation to the given dose.[1] Understanding and optimizing the drug delivery of poorly soluble drug candidates is a major challenge for the pharmaceutical industry and a prioritized area of research across academia and industry.

The TNO intestinal model (TIM-1) system is a multicompartmental, dynamic system that uses *in vivo* relevant media, volumes, and hydrodynamics to simulate the upper part of the gastrointestinal (GI) tract also including an absorptive step by hollow fiber filtration. The model was initially developed by TNO Nutrition and Food Research (Zeist, The Netherlands)[2] and has mainly been used so far in the nutritional science area.[3] The TIM-1 system has been suggested as a suitable tool for developing *in vivo* relevant i*n vitro* dissolution and absorption methodologies,[4,5] with an emphasis on poorly soluble drug candidates.[6] The TIM-1 system is used by AstraZeneca as part of the development process for evaluating prepermeation absorption processes of drug candidates and their formulations. The system has widely been used in a large portion of drug projects, evaluating approximately 65 unique compounds.

The most common application of the TIM-1 system during the employment at AstraZeneca has been to evaluate relative performance of different formulations for poorly soluble drugs for the purpose of:

1. Supporting the development and optimization of formulations.
2. Supporting risk assessments related to bridging between formulations used in different stages of the clinical trial programme or commercial manufacturing.
3. Supporting selection processes between different drug substance modifications, that is, different salts or solid forms, and/or formulations.
4. Predicting food interactions.
5. Predicting dose linearity in exposure because of solubility limitations.

There are additional potential applications of TIM-1, such as investigating different populations, for example, pediatrics, or influence of disease states influencing GI conditions. However, the current study is limited to formulation comparison studies in the fasted state under normal GI conditions.

Dog studies have been commonly used to support the above assessments related to relative bioavailability (RBA). A review of the correlation of RBA between humans and dogs was published by Sutton (2004),[7] showing a good correlation. However, because of initiatives to reduce animal studies as well as the

Abbreviations used: TIM-1, TNO intestinal model; C_{max}, maximum plasma concentration; AUC, area under the plasma concentration–time curve; GI, gastrointestinal; API, active pharmaceutical ingredient; FaSSIF, fasted-state simulated intestinal fluid; BCS, biopharmaceutics classification system; PVP, peristaltic valve pump; $NaHCO_3$, sodium bicarbonate; HCl, hydrochloric acid; GES, gastric electrolyte solution; SIES, small intestine electrolyte solution; Fav, fraction available for absorption; RBA, relative bioavailability; geomean, geometric mean value; CIs, confidence intervals; PK, pharmacokinetic; OrBiTo, innovative tools for oral biopharmaceutics.

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need to reduce development costs and timelines, alternatives to dog studies for the evaluation of RBA are a prioritized area within the pharmaceutical industry as stated by the European Federation of Pharmaceutical Industries and Associations in the 3R initiative, 2012.[8]

The employment of physiologically based pharmacokinetic *in silico* models to predict RBA has been evaluated in literature.[9–11] Sjögren et al. (2013)[12] showed that the GI-Sim in silico model was useful in the assessment of relative performance between different active pharmaceutical ingredient (API) particle size distributions as well as in predicting the increased absorption from a nanosuspension formulation. However, complex combination of mechanisms such as disintegration, dissolution, and subsequent crystallization behavior of different supersaturating delivery forms are still challenging to evaluate with currently available *in silico* models.

Traditional static one-compartment dissolution systems, such as US Pharmacopeial Convention (USP) apparatus 1 and 2 used for quality control during routine manufacture of most pharmaceutical products, can be useful for developing clinically relevant dissolution methods. However, drug- and formulation-specific factors such as p*K*a, solubility, dose, and formulation type needs to be taken into account in the development of such dissolution methods. As a consequence, there is no such thing as a generic, static, one-compartment dissolution method that fits all pharmaceutical products that is reflected by the great variety of conditions used for dissolution testing of approved products. Even slightly more complex approaches with stepwise changes of media, for example, to mimic pH changes in the GI tract[13] or use of simulated GI fluids[14] is still only capturing some limited aspects of the GI physiology. TIM-1 offers a system for *in vitro* dissolution studies that has been developed to capture most of the physiological conditions in the GI tract and thereby holds the promise of a system that can evaluate most pharmaceutical compositions under one standardized experimental setting. This is compelling from a theoretical point of view, but with the complexity of the TIM-1 machine also comes an increased risk of artifacts and there are most probably still some physiological factors that are not identical in TIM-1 compared with the GI tract. Therefore, there is a need to validate the use of TIM-1 by comparisons to *in vivo* data. Initial validation work has been promising[6] but have so far only included one low-solubility compound. Therefore, further studies are welcome, especially for low-solubility drugs to further strengthen the confidence in predictions and to define potential gap areas.

The objective of this study was therefore to evaluate the predictive power of TIM-1 regarding RBA obtained in *in vivo* studies in humans and dogs. The usefulness and limitation of using TIM-1 in the evaluation of drug candidates is discussed, with a focus on drugs and formulations with potential solubility or dissolution limitations in absorption, that is, class II and IV drugs according to the biopharmaceutics classification system (BCS).

METHODS AND MATERIAL

Compounds and Formulations

Nine different compounds developed at AstraZeneca were identified as suitable for inclusion in the validation exercise based on the following criteria:

1. Recovery in TIM-1 was above 70%. A poor recovery implies method artifacts like binding of drug to surfaces or poor analysis and thereby the risk of misinterpretations of data is high. A poor recovery can also be an indication of degradation of the API in GI fluids that would be further evaluated using separate techniques.
2. Two or more formulations of the same compound were compared in both TIM-1 and *in vivo* studies.
3. All *in vivo* studies were performed in either man or dog according to a cross-over design with at least three different subjects.
4. Within a given study, compared formulations were administered at the same dose. A couple of minor exceptions to this rule were made as explained later on.

The majority of the 65 compounds evaluated to date were discarded from the analysis because of criteria 2, that is, no *in vivo* data were available for comparison against the TIM-1 data.

All evaluated compounds were developed as oral immediate-release treatments. The dose–solubility ratio, acidic/basic properties, and evaluated formulations for compounds and formulations meeting above criteria are briefly described in Table 1. All compounds were crystalline unless otherwise stated. A dose solubility ratio above 250 mL means that more than 250 mL of fasted-state simulated intestinal fluid (FaSSIF) was required to dissolve the dose that corresponds to the limit for low solubility according to BCS.[15] Of the nine compounds identified, seven were classified as poorly soluble. There were 19 unique comparisons between different formulations evaluated in both TIM-1 and *in vivo* studies among the nine identified compounds (see Table 2). In total, the *in vivo* validation set includes data from 12 unique studies; five were performed in man and seven in dogs.

TIM-1 Experiments

Design and Operation Principles of TIM-1

The TIM-1 model has been described previously.[2,6] Briefly, TIM-1 consists of four interconnected compartments designed to simulate the stomach, duodenum, jejunum, and ileum (Fig. 1). Each of these compartments has the same basic structure of two glass jackets containing a flexible silicone membrane. Heated water was pumped under computer control into the glass jackets to affect physiologically relevant heating and peristaltic mixing of the chyme via alternate compression and relaxation of the flexible silicone membrane.

These compartments are connected by peristaltic valve pumps (PVPs), comprising three glass T-shaped pieces containing a flexible silicone membrane. Computer-controlled gas flow allows the application of pressure between the glass wall and the flexible membrane to close the valve and restrict the flow of chyme between compartments. When no pressure is applied, the valve remains open. Peristalsis is achieved by regulating the sequence and timing by which these valves open and close, thus controlling the volume delivered per peristaltic cycle. By changing the frequency with which the PVP cycle occurs, the computer can regulate the delivery of chyme into the downstream compartment. The volume of the various compartments is monitored by level sensors, and the correct volume is maintained by secretion of various buffers throughout the experiment.