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RESEARCH ARTICLE

Results from a Validated *in vitro* Gastrointestinal Model (TIM) used as input Data for *in silico* Modeling Give Highly Predictive Information for the Human Situation

Robert Havenaar, DVM, PhD*[1], Susann Bellmann, MSc[2]

[1] TNO, Zeist, The Netherlands (retired)
[2]The TIM Company, Thijsseweg 11, 2629JA Delft, The Netherlands.

* rbhvnr.zeist@gmail.com

ABSTRACT

The aim of this review paper is to evaluate the predictive quality of a combination of *in vitro* dynamic gastrointestinal models, mucosal transit models and *in silico* kinetic modeling. The TNO gastro-Intestinal Model (TIM) is a computer-controlled system, mimicking essential gastrointestinal parameters of the stomach, small intestine and large intestine. The systems have dialysis or filtration units connected to the intestinal compartments. TIM settings are adapted to the condition that has to be simulated, such as fasted and fed state, age, and co-medication. In this way the transit and digestibility of food, release, dissolution, and bioaccessibility of nutrients, drugs, and metabolites can be studied. The TIM Systems have been validated in comparison to human studies for various food products and oral drugs, published in peer-reviewed journals. The results show the potential availability for absorption, called 'bioaccessibility'. Combining TIM with mucosal transit assays, it is possible to also analyze the intestinal absorption. But for predicting bioavailability and plasma concentrations in time it needs additional kinetic data, such as distribution, metabolism, and excretion. TIM bioaccessibility data and (published) kinetic data can be used as input in commercial *in silico* models or specifically developed *in silico* modeling. Validation studies show a high predictive quality for human nutrient and drug bioavailability and plasma concentrations. Maybe not (yet) in all cases the predictions will cover for 100% the human data, so there is room for improvement. However, the reviewed studies clearly show the strength of combining a validated gastrointestinal model with physiological kinetic data in *in silico* modeling. It certainly will replace animal experiments and will strongly increase the success rate of follow-up human studies, saving time and costs.

1. Introduction

For the development of foods and oral drugs there is a increasing demand on relatively fast and less expensive, but reliable and predictive research methodologies. The reason is that human studies are progressively expensive and time consuming. On the other hand, animal experiments have ethical constrains and limited predictive quality, due to a different anatomy and physiology than humans.[1] This means there is an urgent need for alternative methodologies, such as *in vitro* laboratory models of the gastrointestinal (GI) tract, intestinal cell-line and tissue assays, and *in silico* modeling. For these reasons a number of European collaboration projects are or were focused on the development of *in vitro* and *in silico* GI models. For example 'Infogest' for food and nutrition research *(www.cost-infogest.eu) and IMI 'Orbito' for pharma research (www.imi.europe/projects*-orbito).

In this review we describe the steps to come to the combination of *in vitro* and *in silico* modeling with the aim to evaluate the predictive quality of this combined methodology for pharma and nutritional research. In the first steps we describe *in vitro* GI models in general (section 2) and more specifically the TNO gastro-Intestinal Models (section 3.1), including recent developments (section 3.2), repeatability and reproducibility (section 3.3), and the validation versus human studies (section 3.4). Realizing the lack of mucosal absorption of these GI models, we evaluate the combination of TIM with intestinal cell- and tissue-assays (section 3.5). The following steps describe *in silico* models in general (section 4.1) to come to the evaluation of using TIM data, mucosal transit data and additional kinetic data as input in *in silico* modeling (section 4.2). The results of this combined technology are summarized in the conclusions (section 5).

2. GI tract models

Many different GI tract models have been developed and tested over the last 20 years. Roughly they can be divided in static models and dynamic models. Knowing the limitations of static models (actually 'static methods'), due to their lack of recreating the complex dynamic (digestive) conditions in the GI tract, we should focus on more multifaceted dynamic systems.[2,3] A number of these GI models are described in review papers. For example for food digestion studies in general, digestion of plant metabolites, bioavailability of nutrients and nutraceuticals.[3,4,5,6] For pharma studies GI models are reviewed for oral drug studies and more specifically on dissolution of immediate release solid oral drugs during fed conditions[7,8,9]. Williams et al. published a review on colon models, from batch cultures, three-compartmental vessel models to SHIME and TIM.[10] Although the given information is not always complete and correct, these reviews give a good insight in the different GI tract simulation methods and models.

The GI models that simulate most accurately the dynamics in the GI tract and are most broadly validated versus clinical nutritional and drug studies, are the so-called TIM Systems.

3. TIM Systems

3.1 Short description of TIM Systems

The TNO gastro-Intestinal Model (TIM) is a computer-controlled multi-compartmental system. It mimics the essential kinetic GI parameters of the stomach and the different segments of the small intestine (TIM-1 System), and of the large intestine (TIM-2 System).[11,12]

TIM-1 has four compartments, respectively for the stomach, duodenum, jejunum and ileum, connected by peristaltic valves. In these compartments the successive dynamic conditions are simulated, such as body temperature, realistic pH values or pH curves, peristaltic mixing, gastric and intestinal transit times of liquids and solids, physiological amounts and concentrations of electrolytes and bile salts, and digestive enzyme activities. The large-intestinal system is inoculated with microbiota of human origin.[13] This can also be from volunteers on a specific diet, from lean and obese persons, or patients with GI disorders.[14,15,16,17] The density, composition and metabolic activity of the microbiota are rather comparable with the human fecal microbiota.[18,19]

The TIM computer-controlled settings and composition of the secretion fluids are adapted to the situation that has to be simulated, such as fasted and fed conditions, type of drink and/or meal, age, co-medication, and health status. This can be based on the average GI conditions or on the GI inter-individual biological variation.

The TIM-1 and TIM-2 Systems have dialysis or filtration units connected to the intestinal compartments. Dissolved low molecular weight compounds are continuously dialyzed or filtrated. Dialysate and/or filtrate are collected per time period (e.g. per 10, 30, or 60 min) during the TIM experiments for analysis on the concentration of the compound(s) of interest. If multiplied with the collected volume, the amount per time period can be calculated. Also gastric and intestinal lumen samples can be taken during GI passage to analyze the dissolved and total concentrations of compounds.

In this way the digestibility of food (e.g. in meal matrix), release, dissolution, and bioaccessibility of nutrients, drugs, and metabolites can be studied

during transit through the GI tract under various prandial conditions.

3.2 Continuous developments

The 'historic' publications of Minekus et al. in 1995 and 1999 describe the first developments of the TIM Systems.[11,12] Over the past 27 years these systems are strongly modernized with the latest techniques and software. This includes the development of the tiny-TIM system and its successor, the tiny-TIMsmartificialgut (tiny-TIMsg), with one small-intestinal compartment.[20,21] In addition there are developments of new TIM applications, such as for the absorption of fatty acids and fat-soluble compounds.[22,23,24,25] These developments resulted in important knowledge for the testing of lipophilic drugs in TIM. New settings and secretion fluid compositions were developed for different age groups. For example mimicking the maturation of the infant GI tract after birth, which is important to study the behavior of oral drugs or the digestion of breast milk and various formula milks under infant conditions.[26,27,28] Denis et al. describe the GI conditions in elderly in relation to the digestion of meat proteins.[29]

The latest insight in the GI physiology and new technological possibilities supported optimization of the system. The advanced gastric compartment (TIMagc) mimics more accurately the human gastric physiology, especially the time dependent fluctuation in shear rate turbulence.[30,31]

Although the focus in this paper is on the human GI tract, TIM Systems are also adapted to simulate the GI tract of dogs, upper and lower GI tract of pigs, and even that of badgers to study the survival of a *Mycobacterium* oral vaccine.[32,33,34,35]

3.3 Repeatability and reproducibility of TIM Systems

The TIM parameters are continuously computer-controlled via several sensors. The secretion fluids are prepared according to Standard Operating Procedures and secreted with accurate pumps. Due to these circumstances the TIM experiments are highly controlled and reproducible. Therefore, nutritional as well as pharma TIM experiments are in general performed in duplicate and show small variations between the duplicate experiments (see references in section 3.4).

In a pharma study by Brito-de la Fuente et al. ketosteril film-coated tablets were tested in eight experimental runs in TIM-1.[36] The GI conditions of patients with chronic kidney disease (CKD) were simulated under fasted conditions. The release, dissolution and bioaccessibility of ketoanalogues of amino acids from ketosteril were evaluated. The total recovery (95.2±4.0%; RSD: 4.2) as well as the jejunum and ileum bioaccessibility (68.2-68.7% and 15.9-17.9%, resp.) of ketoanalogues demonstrated a high repeatability and explain the clinical performance of ketosteril in studies with CKD patients.

The repeatability and reproducibility of TIM-1 and tiny-TIMsg under fasted and fed conditions were tested in an international study with seven pharmaceutical laboratories.[37] In one lab each condition was performed in 6-fold, in the other labs in duplicate. The bioaccessibility in time of paracetamol as model compound (*in vivo* used as marker for the rate of gastric emptying) was analyzed. Also all crucial TIM parameters were continuously monitored. The differences for total bioaccessibility of paracetamol were never higher than 2.5% between the six individual experiments and never higher than 3.5% between the seven different laboratories. Also the paracetamol bioaccessibility time curves were identical, showing the reproducibility of the rate of gastric emptying, intestinal passage and rate of filtration. The monitored GI parameters were similar in the various laboratories; for both fasted state and fed state. This confirms a high repeatability and inter-laboratory reproducibility of TIM experiments.

3.4 Validation of the TIM Systems

Accurate and reproducible simulation of the key dynamic parameters is a first step to get reliable results in food digestion and in pharma dissolution and bioaccessibility studies. However, this should be demonstrated in validation studies in comparison to human clinical data. TIM-1's predictive quality in food studies has been presented for a broad range of nutrients, from protein digestion and the bioaccessibility of amino acids, lipid digestion and carbohydrate digestion to the bioaccessibility of minerals and of water soluble and fat soluble vitamins.[27,38,39,40,41,42,43,44] The TIM Systems are also used and partly validated for the bioaccessibility of functional food compounds, such as anti-oxidants.[45,46]

For pharma studies TIM experiments showed predictive results for the luminal dissolution and supersaturation of drugs, such as the food dependent disintegration of fosamprenavir calcium in immediate release and HPMC film coated tablets.[47] A TIM study with diclofenac (weak acidic, BCS Class II) under fasted and fed conditions and with an amorphous solid suspension of ritonavir (weakly basic, BCS Class IV) simulating fasted state and fasted state plus PPI co-medication (high gastric pH) were published by Van den Abeele et al.[48,49] Both studies showed that the pH dependent luminal behavior and supersaturation of the APIs in TIM were comparable with *in vivo*. They concluded:

"investigation of dynamic processes affecting drug disposition requires more dynamic *in vitro* models".[48]

The oral bioaccessibility of diverse active ingredients (APIs), including poorly soluble drugs, from many different oral dosage forms were accurately predicted, up to level A IVIVC.[50,51,52,53,54] A comparison study between TIM-1 and tiny-TIM showed the predictive quality of both systems for ciprofloxacin (BCS Class IV), posaconazole (BCS Class II), nifedipine (BCS Class II), and fenofibrate (BCS Class II) in different formulations.[20] It also disclosed that tiny-TIM was more accurate than TIM-1 for determining the t_{max} for immediate release formulations under fasted state. When in tiny-TIMsg the inhibiting effects of acid reducing agents on the bioaccessibility of API's were investigated, it was concluded by the authors that the results were in "excellent agreement with reported clinical findings for all twelve compounds".[21]

López Mármol et al. investigated in tiny-TIMsg the effects of different clinically relevant prandial GI conditions on itraconazole (BCS Class II; extremely low pH dependent solubility) as amorphous solid dispersion in capsules and as solution based on cyclodextrin.[55] In this TIM System equipped with the advanced gastric compartment (TIMagc), experiments were performed under fasted conditions (intake with water), with a low fat and high fat meal, with high gastric pH, and with intake of acidic drinks. Positive food effects, especially with a high fat meal, and the pH effects on dissolution and bioaccessibility were correctly predicted. The negative food effect for the oral solution, however, could not be detected.[55]

Effinger et al. describe TIM experiments while simulating Crohn's Disease (CD) conditions in the upper GI tract (reduced secretion of pancreatic juice and bile) on the effect of ciprofloxacin (BCS Class II) in a lipid based oral suspension.[56] The bioaccessibility curves of ciprofloxacin were similar under healthy and CD conditions. The total bioaccessibility was 82.6% and 86,4%, respectively. This was similar to previous TIM ciprofloxacin experiments with different formulations and in agreement with the high bioavailability in humans.[20,57]

For colon-targeted formulations TIM-1 or tiny-TIMsg can be combined with TIM-2 (colon model), such as in a study with 5-ASA (mesalamine) in pH-dependent gastroresistant-coated multi-matrix tablets.[58] The results showed that 1% of 5-ASA was released in the upper GI tract and most was releases in the colon: 78% in fasted state and 69% in fed state during the 18 h colon sampling period. This was in good comparison with clinical findings.

In a number of the above references, the TIM results were also compared with USP dissolution devices.[31,48,49,50,51] In a study published by Schilderink et al. the bioaccessibility of compound A6197 (BCS Class I) in tiny-TIM was compared with the dissolution in USP compendial apparatus I, II and IV and with clinical data.[59] Four different dosage forms were tested: immediate release, extended release and modified release tablets and extended release pellets. Only the tiny-TIM experiments correctly ranked all four formulations. The tiny-TIM A6197 bioaccessibility data highly correlated with clinical AUC values (r^2=0,989) and C_{max} values (r^2=0,962).[59]

3.5 TIM Systems and mucosal transit assays

Nonetheless the accurate GI simulation, the TIM Systems, just as any other simulation model, has its limitations. One of them is the way of removal of the digested and dissolved compounds via dialysis (cut-off 5-10 KDa) or filtration (cut-off 0.05 µm / 2 MDa). This means the systems lack the presence of the intestinal mucosa with their active transporters. For this reason, TIM studies have been combined with intestinal cell lines, such as Caco-2 cells, for absorption of bioaccessible vitamins and minerals.[41,42,60,61,62] Maybe even more reliable are porcine intestinal tissue segments in the InTESTine system for testing oral absorption.[63,64] These assays give additional information on the uptake of TIM-bioaccessible compounds from the intestine to the portal vein. An example of when combining TIM with intestinal transit experiments is important is given by Kubbinga et al.[65] Chitosan showed to inhibit the bioavailability of acyclovir in humans. However that was not found in a TIM-1 experiments. The bioaccessibilities of acyclovir without and with two levels of chitosan (1.6 g/L and 4.0 g/L) were similar (93.6±0.9%, 90.6±6.0% and 93.2±3.2%, resp.). It appeared that the clinically found inhibiting effect of chitosan was related to decreased mucosal transit, as shown in experiments with porcine intestinal segments.[65]

Thus, combining TIM with intestinal mucosa assays is an important step to bioavailability. The final goal, however, is the prediction of plasma concentrations in time after consumption of food or after intake of a drug. Therefore, we need the next step.

4 *In silico* modeling

4.1 Different *in silico* models

An *in silico* model means a computational model. It refers to experimental techniques performed by

computers. The term is most likely used for the first time in 1989 by Pedro Miramontes and in a publication in 1990 by Sieburg. In the years thereafter many different types of *in silico* models have been developed and applied, including in relation to health and disease, pharmacology and nutrition. For the development and use of *in silico* models you need reliable information as input data, using databases, data mining, data analysis tools, homology models etc. These data may come from various (published) *in vitro* and *in vivo* studies.

In nutrition research for example an *in silico* model for mass transfer and absorption in the human intestine has been published by Moxon et al. and a gut protein digestion model has been described by Del Rio et al.[66,67] However, these models are not validated versus clinical studies. The EU project Infogest organized an international webinar in 2021 on *in silico* modeling for food digestion, which can still be seen on Youtube.com.

In silico models in pharmacy are developed for, among others, drug discovery and drug development.[68,69] For prediction of the bioavailability of drugs, determining the AUC, C_{max} and t_{max}, different *in silico* models are widely used, such as GastroPlus, Simcyp and GI-Sim. Comparison of these models, using input for twelve drugs, showed some different outcomes between the models.[70] For specific purposes explicit *in silico* models can be developed (section 4.2).

The strength of the outcome, in other words the predictive quality of *in silico* models is directly dependent on the completeness and reliability of the input data. In order to get reliable input data on the behavior, digestion, release, dissolution and availability for absorption of nutrients and active drug compounds, detailed information from validated *in vitro* models will significantly increase the predictive quality of the *in silico* model.

4.2 TIM Systems in combination with *in silico* modeling

TIM data might be an important source for input data in *in silico* modeling for the following reasons:

(i) accurate simulation of dynamic GI conditions in the TIM Systems (sections 3.1, 3.2);

(ii) high repeatability and reproducibility of these simulated GI conditions and the bioaccessibility results (section 3.3);

(iii) high predictive quality of TIM results in comparison to clinical data (section 3.4).

One of the first studies in which a combination of TIM-1, intestinal cell line and a specifically developed *in silico* model was used has been described by Verwei et al.[71] In this study the long-term effect of additional folate intake on the human plasma folate concentration was studied. Different folate fortified milk products were tested in TIM-1 on the stability and bioaccessibility profile of folate and folic acid during upper GI passage, whether or not in the presence of folate binding protein.[43,72] The mucosal transit of folate was studies in Caco-2 cell line assays, knowing that deconjugation of natural folate in the small intestine is not a limiting factor for folate absorption. These data were used as input for a specifically developed *in silico* model to predict human plasma concentrations after four weeks intake of folate fortified milk. The *in silico* prediction was compared with the results of a clinical study. The results showed an accurate prediction of the increase in folate plasma concentrations over the four weeks period.[71]

Later studies showed that it is possible to develop specific *in silico* modeling with TIM-1 or tiny-TIM digestion and bioaccessibility data as input for prediction of the glycemic response and appetite rating.[73,74] For the development and calibration of these *in silico* models the *in vivo* data of a limited number of products were used. After calibration the TIM data of the different food products were used as input. Comparing the predicted plasma glucose concentration curves with human clinical data showed an accurate prediction. The correlation coefficient for glucose C_{max} and glucose $iAUC_{0\text{-}120}$ between *in silico* prediction and clinical data was 0.94 and 0.89 (n=22), respectively.[73] The prediction for human satiation and satiety after meal intake was good, but most likely can be improved by using more TIM digestion parameters.[74]

The first pharma TIM-1-*in silico* study has been published by Naylor et al.[75] The TIM-1 bioaccessibility versus time data for paroxitine-HCl were used as input in GastroPlus. The predicted plasma concentration curves were similar to the clinical data.

Ojala et al. tested two formulations of DDM-204, a lipophilic weak-base drug, in TIM-1 and used the results in GastroPlus software with standard fasted and fed physiology.[76] From one formulation clinical data were available, which showed the predictive quality of the TIM-*in silico* combination. The authors concluded that the results gave "powerful information for decision making".[76]

Another tiny-TIMsg-*in silico* study was performed with compound 'A', a weak base BCS Class II compound, in a film-coated tablet in a dose range of 10 to 80 mg under fasted and fed conditions.[77] In the *in silico* model the PK data from compartmental and PK analysis using PKPlus were incorporated in the ACAT model of GastroPlus. The results were compared with a clinical study with the use of IVIVCPlus in GastroPlus. The authors found a level A IVIVC between the *in vivo* fraction absorbed

and the bioaccessibility in tiny-TIMsg for both fasted and fed state at 40 mg dose.[77] The correlation between the amounts dissolved *in vitro* and *in vivo* was r^2=0.97 and 0.99 for fasted and fed state, respectively (level A IVIVC) and for C_{max} it was r^2=0.87 (level C IVIVC). The positive food effect was persistent with the clinical data.

A different approach to use *in silico* modeling has been described by Chiang et al.[78] The bioaccessibility of ibuprofen from three different formulations (free acid IR tablets, liquid gel fast release capsules, and film-coated fast release tablets) was determined in tiny-TIMsg experiments under fasted and fed conditions. Ibuprofen absorption versus time was calculated with a two-compartmental *in silico* model using IR tablets *in vivo* data. Based on this the IVIVC was calculated between the *in silico* absorption and the TIM bioaccessibility of ibuprofen. The tiny-TIM-based IVIVC was able to successfully predict the behaviors of the different (fast release) ibuprofen formulations where supersaturation is truly notable.[78] Fasted state predicted C_{max} was lower than the plasma C_{max}. Maybe this needs some improvement. A slight negative food effect was seen in the TIM data; the fasted versus fed state total bioaccessibility was 95-96% versus 88-89%. This is in agreement with slightly reduced AUCs in clinical data. The authors stated that "this approach was found to be effective and predictive".[78]

5 Conclusions

The results published in peer-reviewed journals showed that the TIM Systems (i) accurately mimics the key dynamic physico-chemical conditions in the human GI tract under various prandial states, age related states, and health states, (ii) that these simulated conditions are highly reproducible within a lab and between different labs, (iii) that the nutrition and pharma validation results for luminal concentrations and oral bioaccessibility of compounds prove a high predictive quality for humans, (iv) that these results are valuable as input data in various *in silico* models to (better) predict human oral bioavailability and plasma concentration curves of nutritional and pharmaceutical compounds.

Maybe not in all cases the predictions will cover for 100% the human data, so there is space for improvement. However, this review shows the strength of combining results from a validated GI model with physiological kinetic data in *in silico* modeling. It certainly will replace animal experiments, will strongly improve the confidence in the selection of products for clinical testing, and will increase the success rate of follow-up human studies. This might considerably reduce development time and costs.

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