**Teaching of Drug Disposition using Physiologically Based Pharmacokinetic Modeling Software: GastroPlus as an Educational Tool**

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Supporting Information

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Supplementary Figure 1: GastroPlus screenshots of example project with midazolam with scientific explanations. This is a 25-part figure labeled A-Y.

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| Graphical user interface  Description automatically generated with medium confidence | Graphical user interface  Description automatically generated |
| A. Cp-time profile and summary of results from the midazolam model based on properties predicted in silico by ADMET Predictor® (AP) v10.0. This simulation used a dose of a single 15 mg oral tablet. | B. Dissolution and absorption curves from the midazolam model based on properties predicted in silico by ADMET Predictor (AP) v10.0. Note that while some precipitation is occurring, absorption is limited by permeation. |

Chart, waterfall chart

Description automatically generated

C. Regional absorption plot for midazolam based on properties predicted in silico by ADMET Predictor (AP) v10.0.

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| Histogram  Description automatically generated with medium confidence  D. Predicted pH-solubility profile of midazolam. The AP-predicted pKa values are: 4.57 (base) and 0.84 (base), with a predicted solubility factor of 2488.5. Note that midazolam is ionized and increasingly soluble at low pH, such as that in the fasted stomach (~1.2). As the pH approaches that of the small intestine (~6), solubility decreases substantially and precipitation may occur, as seen in B. | | A picture containing chart  Description automatically generated  E. pH-LogD profile of midazolam based on predicted pKa (D) and logP (3.56) values. Note that logD has increased to a maximum value by pH 6, implying that most molecules are in the neutral lipophilic form that can be absorbed via passive diffusion. This explains why most of the compound absorbs in the duodenum (C), and almost all remaining molecules (i.e. those that transit through the duodenum before being absorbed) are absorbed quickly in the jejunum. This also explains the relatively early Tmax of ~1.5 h (A). |
| Chart  Description automatically generated with medium confidence  F. pH-solubility profile of midazolam fit to *in vitro* solubility data (pKa = 6.04, Solubility Factor = 487). Observed data are shown as blue circles. | | Graphical user interface, chart  Description automatically generated with medium confidence  G. Dissolution and absorption plot for midazolam using the new pH-solubility and pKa inputs (F). Note that dissolution is slightly slower now that reference solubility and solubility factor are reduced, but no precipitation occurs as the drug enters the higher pH of the small intestine, as the higher pKa of ~6 (F) results in sufficient drug being ionized at the duodenal pH of ~6. |
| Chart  Description automatically generated with medium confidence  H. pH-LogD profile of midazolam using the aforementioned pKa and experimentally measured LogP of 2.7. The profile is shifted slightly to right, and has a lower maximum LogD, than the predicted profile in C. | Chart, waterfall chart  Description automatically generated  I. Regional absorption plot for midazolam model based on the new pH-LogD profile (H). The drug is still predicted to be completely absorbed, but absorption is occurring slightly later in the small intestine (as pH increases further and more time passes), reflective of both the lower LogP value used and the shift of the pH-LogD profile (H) to the right. | |

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| Graphical user interface  Description automatically generated with low confidence  J. Cp-time profile and summary results for midazolam 5 mg IV, using a compartmental pharmacokinetic model fit to the IV data. As may be expected, the model that is fit to these data describes it well. Blue squares represent observed data | Graphical user interface, table  Description automatically generated  K. Compartmental PK parameters for model in J. Values were fit using the PKPlus™ module in GastroPlus. |

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| Graphical user interface  Description automatically generated with medium confidence  L. Cp-time profile and summary results for midazolam 7.5 mg PO solution, using the compartmental model from J-K and the oral absorption model that uses experimental data (F-I). For this dose, the compartmental model and current oral absorption model describe the observed data relatively well. Note that a fixed first pass extraction (FPE) of 67% (fit in PKPlus from oral and IV data) is used in this model. | Graphical user interface  Description automatically generated with medium confidence  M. Cp-time profile and summary results for midazolam 30 mg PO solution, using the compartmental model from J-K and the oral absorption model that uses experimental data (F-I). For this dose, the compartmental model (including FPE from L) underpredicts the observed data. This is likely due to the fact that the compartmental model used here cannot capture saturable processes, such as enzymatic FPE in the gut wall and/or liver. |
| Chart  Description automatically generated with medium confidence | N. Dissolution, absorption, and amount reaching the portal vein and systemic circulation profiles for midazolam 30 mg PO solution (corresponds to Cp-time profile in M). We note that minor precipitation occurs with this dose, but absorption is still permeability limited. Amount entering the portal vein closely follows amount absorbed, reflective of having no FPE or trapping in the gut wall in this model. The amount entering systemic circulation is markedly lower than that in the portal vein, due to the fixed FPE of 67% assigned to the liver (L). As seen in M, the Tmax of this model aligns closely with that of the observed data, indicating that permeation, as the rate limiting step, is likely adequate in this model and does not need to be further adjusted at this time. Similarly, given that the terminal phase in M matches the observed data, volume of distribution and systemic clearance are also adequate. This leaves FPE as the most likely explanation for the differences in the simulated and observed data for this dose. A review of the literature indicates that CYP3A4 is the enzyme responsible for midazolam metabolism, and its presence in both the gut wall and the liver can explain the saturable FPE process. |

Table

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O. *in vivo* kinetic parameters for CYP3A4 metabolism of midazolam added to the model, replacing systemic CL and fixed FPE. *in vitro* metabolic data were taken from the literature, and converted to *in vivo* inputs using the Metabolism and Transporter module in GastroPlus. For compartmental models with nonlinear processes, the “Gut” and “Liver” locations will be used (GastroPlus handles this by attaching a fourth ‘liver’ compartment capable of calculating nonlinear metabolism to the existing 3-compartment model), while a whole-body PBPK model will use the “Gut” and “PBPK” locations (the PBPK model includes its own Liver, separate from the ‘compartmental’ liver seen in the second row). Enzyme kinetic data shown were used for all doses.

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| Graphical user interface  Description automatically generated  P. Cp-time profile and summary results for 7.5 mg midazolam PO solution, using the compartmental model with nonlinear CYP3A4 metabolism (O) included. This model is commensurate to the results seen in L. | Graphical user interface  Description automatically generated  Q. Cp-time profile and summary results for 30 mg midazolam PO solution, using the compartmental model with nonlinear CYP3A4 metabolism (O) included. This model is significantly improved compared to M. |

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R. Physiology used in PBPK model for midazolam, based on the subject demographics from the clinical study. Note that the same oral absorption model as from previous iterations is used, delivering absorbed drug to portal circulation, which then feeds into the whole body model.

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| --- | --- |
| Table  Description automatically generated  S. Tissue/plasma partition coefficients (Kp), tissue clearance, and fraction unbound in tissue (Fut) parameters used in the midazolam PBPK model. Kps and Futs were calculated using the Lukacova equation, which uses the molecular properties of ionization, charge, lipophilicity, protein binding, and phospholipid binding, as well as the aqueous, neutral lipid, acidic phospholipid, and protein composition of individual tissues, to estimate partitioning. Renal clearance estimated from fraction unbound in plasma × glomerular filtration rate. This model also includes the enzymatic metabolism (not shown) from O. | A picture containing graphical user interface  Description automatically generated  T. Performance of completed PBPK model for midazolam 5 mg IV. |

Graphical user interface

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U. Performance of completed PBPK model for midazolam, for both 15 and 30 mg oral solutions. Note that the same model is used in both instances, only the dose and corresponding observed data are different.

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V. Dissolution, absorption, and mass entering portal and systemic circulation plots for PBPK model of midazolam at 15 and 30 mg PO solution doses.

Graphical user interface

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W. Systemic distribution plot for PBPK model of midazolam at a 15 mg PO solution dose.

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X. Systemic distribution plot for PBPK model of midazolam at a 30 mg PO solution dose.

Chart

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Y. Clearance plots for completed PBPK model of midazolam at 15 and 30 mg PO solution doses. Almost all clearance is through CYP3A4 metabolism, with approximately 55% and 45%, respectively, occurring in the gut wall and the remainder in the liver.

Supplementary Figure 2. Final presentation instructions

**Presentation Outline**

1. Overview of PBPK model (1-2 slides, 5 min, preferred 1 person)
   1. Review structure and basic physicochemical properties + known enzyme/transporter involvement and other clearance routes (brief recap)
   2. Model Setup
      1. PEAR Physiology
      2. PBPK settings (include calculated Vss on slide)
      3. Enzyme/transporter kinetics, dissolution settings, etc.
   3. Model fit
      1. Plot comparing predicted to observed for final PBPK model
      2. Include oral and IV if applicable
2. Application of model (Per prompt: 1 person, 1-2 slides, 5-7 min)
   1. For each prompt, specify (2 min):
      1. The prompt
      2. Your approach to modeling the scenario in question, what simulation inputs were shown
      3. The Cp-time profile(s) and summary statistics of results (see example below)
   2. Discuss the results (3-5min)
      1. How do you interpret the results?
      2. What specific processes do you identify?
      3. Is the drug predicted to be safe and effective?
      4. Include additional figures demonstrating how you reached these conclusions

**Other Requirements**

1. Always give Age, Weight, BMI, and Sex when showing results and/or observed data
   1. Helpful to include PK params as well (Vss, CL, enzyme/transporter kinetics)
2. Always give units
3. Create figures that are legible and consistent. This includes:
   1. Same Y-axis units and scale when comparing results
   2. Clear colors, markers, and line styles for plot series
   3. Chart title, axis labels, legend
   4. Legible font sizes and colors

Supplementary Table 1: Drugs assigned in course project

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Group | 1 | 2 | 3 | 4 | 5 | Instructors |
| Drug | Metformin | Atorvastatin | Ibuprofen | Amoxicillin | Digoxin | Midazolam |
| Structure | Shape  Description automatically generated with medium confidence | Shape  Description automatically generated with medium confidence | A picture containing text  Description automatically generated | Shape  Description automatically generated with medium confidence | Shape  Description automatically generated with medium confidence | Shape  Description automatically generated with medium confidence |
| Physicochemical properties | Highly ionized, soluble base | Highly lipophilic acid | Moderately lipophilic acid, low solubility | Hydrophilic zwitterion, formulated as salt | Nonionizable, high molecular weight, amphoteric | Lipophilic, weak base |
| Absorption | Paracellular | Passive, transporter mediated | Passive | Transporter mediated | Passive | Passive |
| Protein Binding | <1% | 98-99% | 99% | 20% | 25% | 95% |
| Tissue Distribution | High, mainly liver | Liver and muscle | Limited | Soft tissues | Limited | Fatty tissues |
| Primary Clearance Mechanism | Renal | Hepatic | Hepatic | Renal | Renal | Hepatic |
| Metabolism |  | Gut, Liver (CYP3A4) | Liver (CYP) |  |  | Liver, Gut (CYP3A4) |
| Transport | Kidney (OCT2, MATE1), Liver (OCT1), Gut (OCT1) | Liver (OATP1B1), Gut (BCRP) |  | Gut, Kidney (PEPT1, PEPT2) | Gut, Liver, Kidney (Pgp) |  |
| IV Cp-time Data | yes | no | yes | yes | yes | yes |
| Oral Cp-time Data | yes | yes | yes | yes | yes | yes |

Supplementary Table 2: Experimental data used in the midazolam model

A) Physicochemical properties and structure of midazolam

|  |  |  |
| --- | --- | --- |
| **Property** | **Value** | **Reference** |
| logP | 2.7 | Roche FH-L: Midazolam (base) Safety Data Sheet. Basel, Switzerland, 2000 |
| Human Ussing Chamber (cm/s) | 3.80×10-5 | Sjöberg Å, Lutz M, Tannergren C, Wingolf C, Borde A, Ungell AL. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. *Eur J Pharm Sci*. 2013;48(1-2):166-180. doi:10.1016/j.ejps.2012.10.007 |
| pKa (Base) | 6.04 | Andersin R. Solubility and acid-base behaviour of midazolam in media of different pH, studied by ultraviolet spectrophotometry with multicomponent software. *J Pharm Biomed Anal*. 1991;9(6):451-455. doi:10.1016/0731-7085(91)80246-6 |
| Human fup (%) | 4.4 | de Vries JX, Rudi J, Walter-Sack I, Conradi R. The determination of total and unbound midazolam in human plasma. A comparison of high performance liquid chromatography, gas chromatography and gas chromatography/mass spectrometry. *Biomed Chromatogr*. 1990;4(1):28-33. doi:10.1002/bmc.1130040105 |
| Human Blood/Plasma Concentration Ratio | 0.55 | Gertz M, Houston JB, Galetin A. Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab Dispos*. 2011;39(9):1633-1642. doi:10.1124/dmd.111.039248 |

B-F) Experimental human pharmacokinetics (Cp-time data) of midazolam (all mean age = 25 yr, mean weight = 70 kg)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| B | | C | | D | | E | | F | |
| IV 5 mg Midazolam | | 15 mg PO Tablet Midazolam | | Midazolam 7.5 mg PO Solution | | Midazolam 15 mg PO Solution | | Midazolam 30 mg PO Solution | |
| Time (h) | ng/mL | Time (h) | ng/mL | Time (h) | ng/mL | Time (h) | ng/mL | Time (h) | ng/mL |
| 0.083 | 95.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.167 | 90.00 | 0.25 | 8.28 | 0.25 | 13.50 | 0.25 | 33.60 | 0.25 | 129.00 |
| 0.330 | 82.10 | 0.45 | 45.20 | 0.50 | 27.00 | 0.30 | 41.80 | 0.50 | 137.00 |
| 0.670 | 67.50 | 0.70 | 48.60 | 0.75 | 24.90 | 0.40 | 51.90 | 0.75 | 130.50 |
| 1.000 | 53.90 | 1.00 | 40.30 | 1.00 | 24.80 | 0.50 | 57.40 | 1.00 | 127.00 |
| 1.500 | 41.90 | 1.50 | 36.10 | 1.25 | 19.30 | 0.75 | 58.50 | 1.25 | 113.00 |
| 2.000 | 32.00 | 2.00 | 31.90 | 1.50 | 16.10 | 1.00 | 51.90 | 1.50 | 102.00 |
| 2.500 | 23.50 | 2.50 | 24.70 | 2.00 | 12.90 | 1.25 | 42.60 | 2.00 | 77.30 |
| 3.500 | 17.80 | 3.50 | 15.20 | 3.00 | 10.20 | 1.50 | 35.60 | 3.00 | 51.90 |
| 5.000 | 11.40 | 5.00 | 6.81 | 4.00 | 6.46 | 2.00 | 31.00 | 4.00 | 37.80 |
| 7.000 | 6.90 | 7.00 | 3.65 | 5.00 | 5.30 | 3.00 | 18.10 | 5.00 | 31.60 |
| 9.000 | 3.70 | 9.00 | 2.23 | 6.00 | 4.43 | 4.00 | 16.10 | 6.00 | 20.80 |
|  |  |  |  | 7.00 | 3.10 | 5.00 | 10.00 | 7.00 | 16.40 |
|  |  |  |  | 8.00 | 2.44 | 6.00 | 8.36 | 8.00 | 13.20 |
|  |  |  |  |  |  | 7.00 | 7.73 | 10.00 | 9.61 |
|  |  |  |  |  |  | 8.00 | 5.74 | 12.00 | 6.33 |
|  |  |  |  |  |  | 10.00 | 3.86 | 16.00 | 4.43 |

B, C) Mean 5 mg IV bolus (B) and mean 15 mg PO tablet (C) Digitized from: Kupferschmidt HH, Ha HR, Ziegler WH, Meier PJ, Krähenbühl S. Interaction between grapefruit juice and midazolam in humans. *Clin Pharmacol Ther*. 1995;58(1):20-28. doi:10.1016/0009-9236(95)90068-3

D - F) Mean 7.5 mg PO solution (D), mean 15 mg PO solution (E), and mean 30 mg PO solution (F). Digitized from: Bornemann LD, Min BH, Crews T, et al. Dose dependent pharmacokinetics of midazolam. *Eur J Clin Pharmacol*. 1985;29(1):91-95. doi:10.1007/BF00547375

Supplementary Table 3: Prompts selected by students for each project, with reference to Table 3. The same prompts for ibuprofen and metformin are given in Table 4.

|  |  |  |
| --- | --- | --- |
| Group | Drug | Prompts |
| 1 | Metformin | 1D. Is your drug expected to be safe and effective in a special population of renally impaired patients?  1E. Is your drug expected to be safe and effective in a special population of MATE1 and/or OCT2 variant patients?  2C. How would your drug’s ADME be affected by changes in dosing schedule (qDay vs BID vs TID)? |
| 2 | Atorvastatin | 1A. Is your drug expected to be safe and effective in a special population of elderly patients?  1E. Is your drug expected to be safe and effective in a special population of OATP1B1 variant patients?  2C. How would your drug’s ADME be affected by changes in route of administration (IV vs PO)?  2D. How would your drug’s ADME be affected by changes in food coadministration (fasted vs fed)? |
| 3 | Ibuprofen | 1B. Is your drug expected to be safe and effective in a special population of pediatric patients?  1E. Is your drug expected to be safe and effective in a special population of CYP2C9 variant patients?  2A. How would your drug’s ADME be affected by changes in formulation type (tablet vs solution)?  2D. How would your drug’s ADME be affected by changes in food coadministration (fasted vs fed)? |
| 4 | Amoxicillin | 1A. Is your drug expected to be safe and effective in a special population of elderly patients?  1B. Is your drug expected to be safe and effective in a special population of pediatric patients?  1E. Is your drug expected to be safe and effective in a special population of PepT1 variant patients?  2A. How would your drug’s ADME be affected by changes in formulation type (tablet vs suspension)? |
| 5 | Digoxin | 1A. Is your drug expected to be safe and effective in a special population of elderly patients?  1E. Is your drug expected to be safe and effective in a special population of P-glycoprotein variant patients?  2A. How would your drug’s ADME be affected by changes in formulation type (tablet vs capsule vs suspension)?  2E. How would your drug’s ADME be affected by coadministration of a P-glycoprotein inducer? |

Supplementary Table 4: Rubric for grading of the pre- and post-assessments. Each question has a total possible score of 3 points.

|  |  |  |  |
| --- | --- | --- | --- |
| Question | Identify (1 point) | Describe (1 point) | Explain (1 point) |
| 1 Explain the molecular basis for midazolam having a logP of 2.7? | | | |
|  | Aromatic rings, halides are hydrophobic (increases logP) | Small number of hydrogen bonding groups (decreases logP) | Skews lipophilic since more hydrophobic than hydrophilic moieties |
| 2 Is midazolam ionizable? If so, is it an acid or a base? Justify your reasoning | | | |
|  | Yes, it is ionizable | Base | One of the nitrogens can be protonated and/or more soluble at low pH |
| 3 Where in the Gastrointestinal tract does midazolam have the highest solubility and why? | | | |
|  | Stomach |  | Solubility higher at lower pH |
| 4 What is the most likely mechanism of absorption for midazolam in the duodenum, and what is the charge of the species that crosses the gut wall? | | | |
|  | Passive | Neutral | Moderately high logP, sufficiently neutral in duodenum (pKa) |
| 5 What relationship do solubility and permeability have with the bioavailability of midazolam? | | | |
|  | Midazolam has reasonable solubility, especially early in GI tract, determines amount accesible for absorption | Midazolam has good permeability/moderately high LogP, should absorb 'fast' | Would expect to have good bioavailability |
| 6 What is the effect of food on the pharmacokinetics of midazolam tablets, and what is a likely explanation? | | | |
|  | Decreases Cmax and extends Tmax, increases AUC | Slower transit times and higher pH with food, decreased initial dissolution | Slower absorption blunts Cp-Time profiles, but meal lipids/slower transit time increase total absorption |
| 7 Why is the Fup 4.4% What is the effect of Fup on volume of distribution, tissue partitioning, and clearance? | | | |
|  | Hydrophobic and primarily unionized in blood | Vd goes down | High amount of protein binding means small amount of drug accesible for distribution/clearance |
| 8 How do pKa, LogP, and RBP affect the distribution of midazolam into individual tissues, and what tissues would you expect it to distribute into? | | | |
|  | pKa indicates it will be mostly neutral at pH 7.4, neutral species distribution determined mostly by logP, which is hydrophobic | Fraction that is still charged will be affected by RBP, since it is a cationic compound | Neutral charge at physiologic pH and lipophilic compound result in high adipose/fatty tissue partitioning, generally will partition more out of plasma (also ok to say most stays in plamsa due to low Fup) |
| 9 Do you expect midazolam to be cleared primarily by the liver, the kidney, or both? Would you expect it be cleared as the parent compound or a metabolite? | | | |
|  | It is a CYP3A4 substrate, which indicates metabolism will occur in liver | Low solubility at pH 7.4 and highly bound, so not likely to be renal clearance | CYP3A4 metabolism indicates it would be cleared as metabolite (ok to say metabolite cleared by kidney) |
| 10 What relationship do AUC and Cmax have with dose, and why? | | | |
|  | AUC increases by a greater factor than dose dose does, effect more pronounced at higher doses | Cmax increases by a greater factor than dose dose does, effect more pronounced at higher doses | This is indicative of a saturable mechanism of absorption and/or clearance |
| 11 What are the differences in observed plasma-concentration time profiles between dosage forms, and what are possible reasons for any differences? | | | |
|  | Identifies correct data to compare same doses (15mg PO sol vs 15mg PO tab) | Solution generally has a earlier Tmax than tablet, and has a higher Cmax and exposure (AUC), than tablet. | Solution starts dissolved and/or enters small intestine faster (as liquid) so can start absorbing earlier (tmax & Cmax); more dissolved allows more total absorption and/or faster absorption saturates CL/FPE (AUC) |
| 12 What is the effect of grapefruit juice on midazolam exposure, what is the most likely reason for this effect, and where does this effect most likely occur? | | | |
|  | Increases expsoure/AUC/Cmax | Inhibits FPE and/or CL, and/or a process that prevents absorption | Based on study 1 (IV vs Oral), midazolam has incomplete absorption and/or significant FPE. Most likely explanation is inhibition of first-pass metabolism, gut efflux also acceptable hypothesis. Location of GFJ effect on FPE is likely gut, since no impact on systemic CL, meaning likely not liver. |