

## PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS OF THE GLUCOSE-LOWERING EFFECT OF METFORMIN IN DIABETIC RATS REVEALS FIRST-PASS PHARMACODYNAMIC EFFECT

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### ABSTRACT:

Metformin, a commonly used antidiabetic drug, exerts its glucose-lowering effect due to metabolic activities at several sites of action (biophases), including liver, intestine, muscle cells, and adipocytes. The relative contribution of the individual biophases to the overall glucose-lowering effect is not known. Thus, the aims of this investigation were to study the influence of mode of drug administration on the kinetics of glucose-lowering action of metformin in diabetic rats and identify the contribution of different sites of action to the overall response. Streptozotocin diabetic rats received metformin in crossover fashion via intraduodenal, intravenous, and intraportal routes as bolus dose or infusion regimens designed to yield similar pharmacokinetic profiles. Metformin plasma concentrations and blood glucose levels were measured following each mode of administration. Despite the similarity in the concentration-time profiles obtained for different routes of metformin administration,

intraduodenal administration produced larger response than intraportal metformin infusion, and lowest response was observed following intravenous administration. This finding indicates that a significant "first-pass" pharmacodynamic effect, which occurs in the presystemic sites of action (liver and the gastrointestinal wall), contributes to the overall glucose-lowering response of metformin. We applied a combined pharmacokinetic-pharmacodynamic modeling approach to study the nature of the first-pass pharmacodynamic effect. The observed data were successfully described by a novel integrated indirect response pharmacokinetic-pharmacodynamic model that revealed a correlation between the temporal metformin concentrations that transit the portal vein and through the gut wall rather than with drug concentrations that accumulated in the liver and the intestinal wall.

Metformin, a biguanide glucose-lowering agent, is commonly used for management of type 2 diabetes. Despite the wide clinical use of metformin, the mechanism of its action is not fully understood. The glucose-lowering effect of metformin is apparently composed of a combination of several distinct activities in various organs and tissues (Hermann and Melander, 1992; Cusi and DeFronzo, 1998), including: 1) decreased hepatic glucose output due to decreased hepatic gluconeogenesis and increased glycogenesis and lipogenesis (Christiansen and Hellerstein, 1998); 2) reduced rate of intestinal glucose absorption (Wilcock and Bailey, 1991); and 3) increased glucose uptake by muscle cells and adipocytes (Bailey et al., 1996). The multifactorial mechanism of action of metformin and the complex nature of glucose homeostasis in vivo obscures the dose-response relationship of the metabolic effects in individual organs and tissues (Wiernsperger, 1996). As a result, the relative significance of the above-mentioned sites of metformin action (biophases) to produce metabolic effects remains unknown and is still a matter of continuous debate (Bailey et al., 1994; Abbasi et al., 1998; Cusi and DeFronzo, 1998).

In preliminary investigations, we have found that mode of metformin administration affects the kinetics and extent of its pharmaco-

logical action. A bolus peroral administration of the drug produced stronger and longer glucose-lowering response than i.v. administration of an equivalent dose (Stepensky et al., 1998). At the time, the mechanism of this finding was not elucidated. In general, it could be attributed to pharmacokinetic (PK<sup>1</sup>) factors (i.e., changes in the drug concentration versus time profiles at biophases), pharmacodynamic (PD) factors (e.g., nonlinearity of the concentration-effect relationship), or both PK and PD mechanisms (Castaneda-Hernandez et al., 1994; Hoffman, 1998; Hoffman and Stepensky, 1999).

Previously, we reported similar effects of mode of administration on magnitude of response for the lipid-lowering drugs niacin and bezafibrate. Slow input of these drugs to the gut produces a significantly augmented hypolipidemic response compared with administration of the drug by equivalent rate and extent directly to a peripheral vein (Lomnický et al., 1998; Hoffman et al., 1999). These outcomes result from the targeting of the drug to presystemic biophases by continuous administration of the drug to the gastrointestinal (GI) tract and were termed "first-pass pharmacodynamic effect" (Hoffman et al., 1999; Stepensky et al., 2001). Similarly, it has been reported that for an active derivative of simvastatin, continuous mode of drug administration to the GI tract produced stronger cholesterol-reducing

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<sup>1</sup> Abbreviations used are: PK, pharmacokinetic; PD, pharmacodynamic; GI, gastrointestinal; AUC, area under concentration versus time curve; AUEC, area under effect versus time curve; HPLC, high-performance liquid chromatography.

effect than oral bolus administration of this compound to dogs (McClelland et al., 1991).

In the present investigation, we studied the relationship between metformin disposition and glucose-lowering effects. The specific aims were to investigate the influence of the mode of metformin administration on the kinetics of its glucose-lowering action; to distinguish between the pharmacokinetic and pharmacodynamic mechanisms that affect the kinetics of action; and to identify the contribution of the different biophases (i.e., systemic versus GI and liver) to the overall glucose-lowering effect.

For these purposes, we developed a novel PK-PD model, which was used to study the contribution of each of three sites of metformin action to the glucose-lowering effect. To our knowledge, this study applied the indirect response PK-PD model for the first time in a case where the measured response is influenced simultaneously by three different sites of action. This modeling approach was necessary to resolve the contribution of individual sites of action to the overall glucose-lowering effect of metformin.

### Experimental Procedures

**Materials.** Metformin hydrochloride was kindly provided by Teva Pharmaceutical Industries, Ltd. (Netanya, Israel). Phenformin hydrochloride and streptozotocin were purchased from Sigma-Aldrich (Rehovot, Israel). All other reagents used in this study were of analytical or HPLC grade.

**Animals.** Male Sabra rats (200–250 g; Animal Breeding Unit, The Hebrew University of Jerusalem, Israel) were used in this study. This investigation adhered to the principles of laboratory animal care (National Institutes of Health publication 85-23, revised 1985). The animals were housed under standard conditions with a 12-h light/dark cycle with free access to water and food (regular rat chow) with the exception of food deprivation during the period of blood sampling throughout the PK-PD experiments.

An experimentally induced model of type 2 diabetes was produced by streptozotocin injection (50 mg/kg, i.p.). Degree of diabetes was assessed 5 days later by measurements of blood glucose levels using a Glucometer Elite blood glucose meter (Bayer, Brussels, Belgium). Rats with blood glucose below 140 mg/dl following an overnight fast and above 300 mg/dl at fed conditions were selected for the experiment. The baseline time course of blood glucose concentrations was checked on several occasions to ensure that the metabolic status of the rats remained stable throughout the whole experimental period.

**Surgery.** To enable drug administration, cannulas (PE-50 intramedic polyethylene tubing; BD Biosciences, San Jose, CA) were implanted in the duodenum and blood vessels (jugular and portal vein) of the rats. Portal vein cannulation was performed according to the method described by Strubbe et al. (1999) with slight modifications. The surgery was performed under anesthesia (9% ketamine and 1% xylazine solution, i.p.; 1.0 ml/kg) at least 5 days prior to initiation of the experiments. The cannulas were exteriorized at the dorsal part of the neck, which made it possible to carry out the investigation in nonanesthetized and unrestrained rats.

**Experimental Protocols.** The streptozotocin diabetic rats ( $n = 6$ ) received metformin in a crossover experimental design via the following modes: 1) intraduodenal bolus (450 mg/kg); 2) a constant rate intraduodenal infusion (4 h, total dose 450 mg/kg); 3) a constant rate intravenous infusion (4 h, total dose 200 mg/kg); 4) a variable rate intravenous infusion (total dose 200 mg/kg); 5) a variable rate intraportal infusion (total dose 200 mg/kg); and 6) vehicle bolus administration (double-distilled water and saline via intraduodenal and intravenous routes, respectively).

The washout period between the drug administrations was at least 6 days. For parenteral modes of drug administration, metformin was dissolved in saline, and for intraduodenal administration, metformin was dissolved in double-distilled water. Metformin doses were selected on the basis of preliminary experiments to produce similar systemic exposure (measured as area under the concentration-time curve) following gastrointestinal and parenteral modes of drug administration. Variable rate infusions were designed to mimic the plasma drug concentrations versus time profile attained following the intraduodenal infusion mode of administration. For this purpose, the infusion

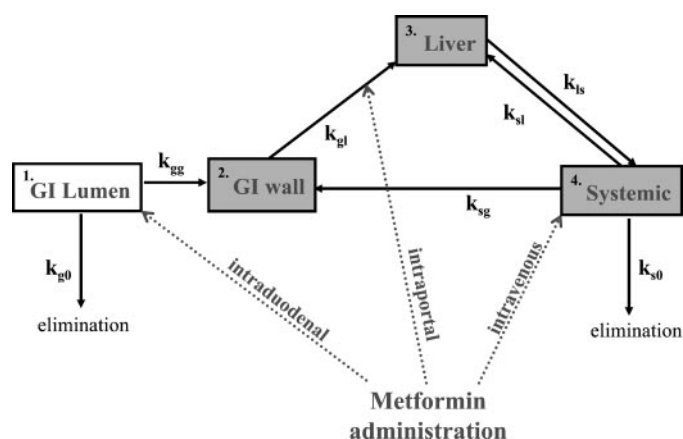


FIG. 1. The pharmacokinetic model of metformin disposition in rat.

The total metformin content in the gastrointestinal tract is composed of two fractions: free drug in the lumen (GI lumen) and the drug within the intestinal wall (GI wall). Additional compartments are attributed to liver and systemic circulation (including blood, different organs, and tissues). Transfer of metformin between different compartments is assumed to occur according to the first-order kinetic processes along the arrows with corresponding rate constants ( $k_{gg}$ ,  $k_{gl}$ , etc.). Elimination occurs from the GI lumen or systemic compartments (with  $k_{g0}$  and  $k_{s0}$  rate constants, respectively).

rate was gradually elevated and then reduced at 1-h steps (infusion rate of 5, 11, 18, 40, 51, 37.5, 27.5, and 10 mg/kg/h for a total dose of 200 mg/kg for both variable rate intravenous and intraportal infusions). Administration of constant and variable rate infusions was performed by means of a microprocessor-controlled syringe pump (Pump 22; Harvard Apparatus, Holliston, MA).

For each mode of metformin administration, blood samples (120- $\mu$ l) were collected from the tail artery at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 10 h (and additional samples at 0.25 and 4.25 h for a constant rate intravenous infusion). Blood glucose levels were immediately measured by a Glucometer Elite blood glucose meter. Plasma samples were obtained from the rest of the blood (by centrifugation at 3500 rpm for 10 min) and stored at  $-20^{\circ}\text{C}$  pending analysis.

**Analytical Procedures.** Plasma metformin concentrations were determined by an HPLC method applying a Kontron HPLC system (Kontron, Zurich, Switzerland) and LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany). The detection was at 234 nm, and phenformin was applied as the internal standard. The mobile phase consisted of 0.01M  $\text{Na}_2\text{HPO}_4$  solution (pH = 6.5), methanol, and acetonitrile (20:3:6, v/v). The quantitation limit was 100 ng/ml. Intraassay and interassay coefficients of variation were 5 and 9%, respectively.

**PK Model.** A multicompartment PK model was used to describe the pharmacokinetics of metformin (see Fig. 1). Transfer of metformin between different compartments was assumed to occur according to a first-order kinetic process along the arrows with corresponding rate constants:

$$dX_1/dt = -X_1(k_{g0} + k_{gg}) \quad (1)$$

$$dX_2/dt = X_1k_{gg} + X_4k_{sg} - X_2k_{gl} \quad (2)$$

$$dX_3/dt = X_2k_{gl} + X_4k_{sl} - X_3k_{is} \quad (3)$$

$$dX_4/dt = X_3k_{is} - X_4(k_{sl} + k_{sg} + k_{s0}) \quad (4)$$

where  $X_1$ ,  $X_2$ ,  $X_3$ , and  $X_4$  are metformin amounts in GI lumen, GI wall, liver, and systemic compartments, respectively. The rate constants are:  $k_{g0}$ , drug elimination with feces;  $k_{gg}$ , drug transfer from the GI lumen to GI wall compartment;  $k_{gl}$ , drug transfer from the GI wall to liver compartment;  $k_{is}$  and  $k_{si}$ , drug transfer from the liver to systemic compartment and in the opposite direction,  $k_{sg}$ , drug transfer from the systemic to GI wall compartment; and  $k_{s0}$ , drug elimination with urine. Metformin is administered into GI lumen, liver, or systemic compartments following intraduodenal, intraportal, and intravenous modes of administration, respectively (see Fig. 1).

The structure of the PK model was based on available data of metformin pharmacokinetics and the physiology of intestinal and portal blood circulation.

Metformin is not metabolized in the body, oral bioavailability reaches 50 to 60%, and the rest of the dose is excreted in the feces (Tucker et al., 1981; Wiernsperger, 1996). Due to the biguanide chemical properties of metformin, the drug is characterized by a unique distribution behavior; following intraduodenal (or oral) administration, part of the metformin is reversibly adsorbed to the luminal surface of the intestinal wall (Hermann and Melander, 1992). Metformin tends also to accumulate in the GI wall following both oral and intravenous administration (Sirtori et al., 1978; Wilcock and Bailey, 1994). This accumulation is not attributed to the GI lumen since no fecal excretion of metformin was observed following i.v. administration (Pentikainen et al., 1979; Tucker et al., 1981). Thus, the "GI wall" compartment introduced in this model receives metformin via arterial blood supply to the intestine (with rate constant  $k_{sg}$ ) and, in addition, comprises the drug amounts that are absorbed from the GI lumen. According to the unidirectional blood flow through the portal system, the PK model allows metformin passage through both the GI wall and liver before reaching the systemic circulation following the intraduodenal route and only through the liver for intraportal administration.

**PK-PD Model.** The overall glucose-lowering effect of metformin was attributed to inhibition of glucose production or stimulation of glucose utilization at the individual biophases according to the sigmoidal  $E_{\max}$  model:

$$I_{\text{liver}} = \frac{I_{\max} L \times (A_L)^{n_L}}{(IA_{50} L)^{n_L} + (A_L)^{n_L}} \quad (5)$$

$$S_{\text{GI}} = \frac{E_{\max} \text{GI} \times (A_{\text{GI}})^{n_{\text{GI}}}}{(EA_{50} \text{GI})^{n_{\text{GI}}} + (A_{\text{GI}})^{n_{\text{GI}}}} \quad (6)$$

$$S_s = \frac{E_{\max} S \times (A_s)^{n_s}}{(EA_{50} S)^{n_s} + (A_s)^{n_s}} \quad (7)$$

where  $I_{\text{liver}}$  is inhibition of glucose production in the liver (L) and  $S_{\text{GI}}$  and  $S_s$  are stimulation of glucose utilization in the GI tract (GI) and by muscle and fat tissues (S), respectively. To reduce the number of estimated parameters, the equations were written in terms of drug amounts and not concentrations, thereby casting off the need for estimating the volumes of the PK compartments and biophases. Therefore, drug effect at certain biophase is a function of metformin amount ( $A_L$ ,  $A_{\text{GI}}$ , and  $A_s$ ), maximum effect ( $I_{\max} L$ ,  $E_{\max} \text{GI}$ , and  $E_{\max} S$ ), metformin amount at the biophase that produces 50% of maximal effect ( $IA_{50} L$ ,  $EA_{50} \text{GI}$ , and  $EA_{50} S$ ), and the shape factor ( $n_L$ ,  $n_{\text{GI}}$ , and  $n_s$ ).

A combination of indirect response models I and IV (Dayneka et al., 1993) was applied to describe the time course of metformin glucose-lowering effect, according to the following equation

$$dR/dt = k_{\text{in}}(I - I_{\text{liver}}) - k_{\text{out}}(1 + S_{\text{GI}} + S_s)R \quad (8)$$

where the response parameter  $R$  is the metformin-related change in glucose blood concentration;  $k_{\text{in}}$  is the zero-order rate of glucose input into the body; and  $k_{\text{out}}$  is the first-order rate of glucose utilization.

**Linked PK-PD Model.** Two approaches were used to model the kinetics of metformin effect(s). Model A assumed that the amounts of metformin accumulated in "liver", GI wall, and "systemic" compartments (see Fig. 1) were responsible for the glucose-lowering effects.

Model B was based on the presumption that only the metformin amount reaching the GI tract and liver at a given time point (i.e., drug flux through intestinal microvessels and the portal vein, respectively), rather than overall accumulated amount, is related to the observed glucose-lowering effect. The drug flux through intestinal microvessels was calculated as amounts of metformin transferred from "GI lumen" to GI wall compartment:  $Q_{\text{gg}} = X_1 \cdot k_{\text{gg}}$  (see Fig. 1). Due to the first-pass effect, metformin concentrations in the portal vein are derived from metformin concentrations in the systemic circulation, infused drug (for intraportal mode of administration), and drug absorption in the GI tract (for intraduodenal bolus and infusion modes of administration). Therefore, metformin flux through the portal vein ( $Q_{\text{gl}}$ ) was calculated as the sum of systemically derived amounts, drug amounts infused in the portal vein (in the case of intraportal infusion), and transfer of metformin from GI wall to liver compartment that was derived from the absorption from GI lumen (for intraduodenal bolus and infusion). Systemically derived drug amounts in the portal vein were calculated as metformin plasma concentrations multiplied by

portal blood flow ( $5.92 \pm 0.97$  ml/min/100 g body weight; Sakaeda et al., 1998).

For the purpose of PK-PD modeling according to model B, estimated values of drug flux through intestinal microvessels and the portal vein ( $Q_{\text{gg}}$  and  $Q_{\text{gl}}$ ) were introduced instead of the amounts at the GI wall and liver ( $A_{\text{GI}}$  and  $A_L$ , respectively) in eqs. 6 and 5. PK-PD linking for systemic compartment (i.e., adipocytes and muscle cells) was not different in models A and B.

**Data Analysis.** To overcome the circadian variations in blood glucose levels and reveal the metformin-driven glucose-lowering effect, the baseline time course of blood glucose levels (following vehicle administration) was subtracted from the observed blood glucose levels following the drug administration. Calculation of area under concentration versus time curve (AUC) and area under effect versus time curve (AUEC) values was performed using the WinNonlin program (version 1.1; Pharsight Corporation, Mountain View, CA) by means of the noncompartmental analysis method.

Analysis of mean pharmacokinetic and pharmacodynamic data following various modes of administration was performed with ADAPT II Pharmacokinetic/Pharmacodynamic Systems Analysis Software (Biomedical Simulations Resource, Los Angeles, CA) applying the mean likelihood objective function (D'Argenio and Shumitzky, 1997). The variance was described by the linear model,

$$\text{Var } R = (a + b \times R)^2 \quad (9)$$

where  $a$  and  $b$  are the variance parameters.

The PK fits were done simultaneously for seven data sets: five sets of mean plasma concentration versus time data for various modes of administration and two estimated data sets representing GI and liver amount versus time data for i.v. bolus administration. The estimated amounts of metformin versus time profiles in the GI tissues and liver were calculated using intestine/plasma and liver/plasma ratios (10 and 5.0, respectively) based on metformin distribution data in streptozotocin diabetic mice (Wilcock and Bailey, 1994). In the second stage, the PK-PD model was fitted to the mean glucose-lowering effect versus time data (simultaneous fit of five modes of administration) using the estimated PK parameters as fixed values.

**Statistical Analysis.** The Kruskal-Wallis analysis of variance with subsequent Newman-Keuls multiple comparisons test were applied for analysis of AUC and AUEC values following different modes of metformin administration. A  $p$  value of less than 0.05 was termed significant.

## Results

**The PK and PD Data.** The mean concentration versus time profiles following different modes of metformin administration are presented in Fig. 2. It can be seen that a constant rate i.v. infusion produced a gradual increase in metformin plasma concentrations and reached a steady state within 2 to 3 h. The PK results for constant rate intravenous infusion served for calculation of clearance and volume of distribution, which were 2.02 l/kg/h and 1.31 l/kg, respectively. These values are comparable (up to 3- to 4-fold difference when normalized by weight) with the results obtained in human studies (Pentikainen et al., 1979, 1986; Tucker et al., 1981; Scheen, 1996).

Following intraduodenal bolus administration, the peak plasma metformin concentrations were obtained 3 h after drug administration and then declined gradually. The concentration-time profile following a constant rate intraduodenal infusion was characterized by slow kinetics of drug absorption to the systemic circulation; the peak plasma concentration was attained 1 h after the termination of the infusion.

The plasma concentration-time profiles of metformin following variable rate i.v. and intraportal infusions were designed to mimic the PK profile obtained following constant rate intraduodenal infusion (see Fig. 2). No significant differences in AUC values were found for the different modes of metformin administration that were studied [see Table 1; the results of intraduodenal bolus and constant rate intraduodenal infusion were published previously (Stepensky et al., 2001)].

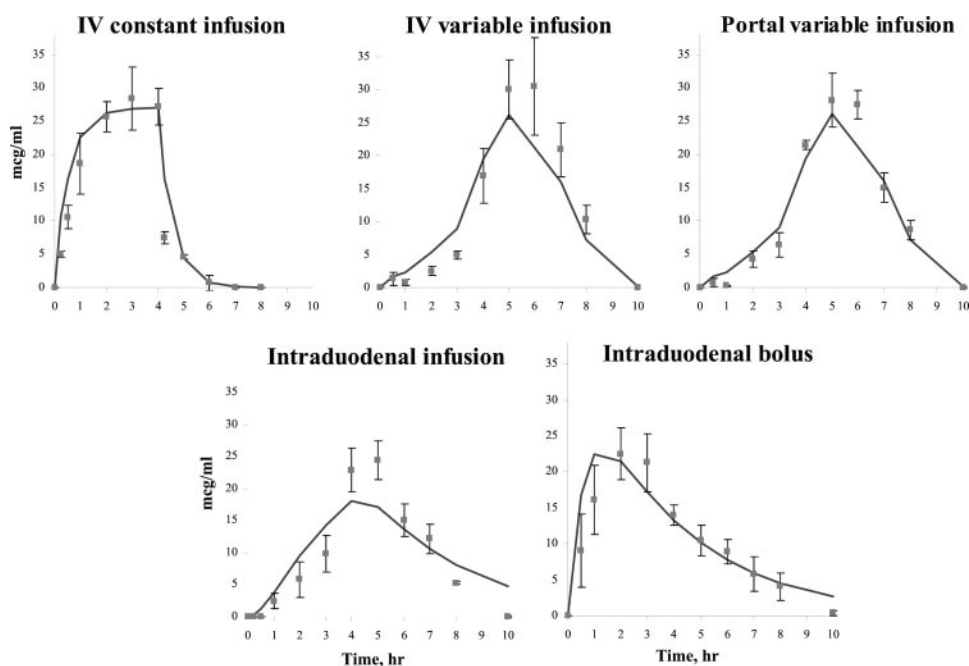


FIG. 2. Plasma metformin concentrations following administration of 450 mg/kg metformin as intraduodenal bolus or intraduodenal infusion or 200 mg/kg metformin as constant rate i.v. infusion, variable rate i.v. infusion, or variable rate intraportal infusion.

Data points are the mean observed pharmacokinetic data, and solid lines are the best fits according to the PK model.

TABLE 1

The observed area under concentration and effect curves following metformin administration (mean  $\pm$  S.D.)

Mode of Administration	AUC	AUEC
	mg/min/ml	mg/100 ml/min ( $10^3$ )
Intraduodenal bolus 450 mg/kg	5.72 $\pm$ 0.72	56.6 $\pm$ 20.6
Constant rate intraduodenal infusion 450 mg/kg	6.35 $\pm$ 1.23	55.3 $\pm$ 22.4
Constant rate intravenous infusion 200 mg/kg	5.92 $\pm$ 0.69	7.68 $\pm$ 4.24 <sup>a,b</sup>
Variable rate intravenous infusion 200 mg/kg	7.34 $\pm$ 1.81	18.2 $\pm$ 10.8 <sup>a,b</sup>
Variable rate intraportal infusion 200 mg/kg	6.98 $\pm$ 1.15	38.3 $\pm$ 19.8 <sup>c</sup>

<sup>a</sup> Significantly different from intraduodenal bolus at  $p < 0.01$ .

<sup>b</sup> Significantly different from constant rate intraduodenal infusion at  $p < 0.01$ .

<sup>c</sup> Significantly different from constant rate intravenous infusion at  $p < 0.05$ .

At the beginning of the experiment, mean plasma glucose levels were approximately 400 mg/100 ml, and following vehicle administration, they declined gradually, reaching approximately 320 mg/100 ml at the end of the data collection period (10 h; graph not shown). The time course of glucose-lowering effects following different modes of metformin administration is shown in Fig. 3. It can be seen that both the kinetics and magnitude of the glucose-lowering effect were highly dependent on the mode of metformin administration. Intraduodenal bolus and infusion produced the highest extent of glucose-lowering effects as evidenced by the augmented AUEC values (see Table 1). Intraportal infusions produced intermediate effects, and the lowest extent of pharmacological effect was observed for constant and variable i.v. infusion modes of metformin administration.

**PK-PD Modeling.** The PK model adequately captured the concentration-time data following all the modes of metformin administration (Fig. 2). The values of estimated PK parameters are presented in Table 2. The values of the Akaike and Schwartz criteria for the results of PK modeling were 209 and 261, respectively.

Estimated exposures of biophases to metformin according to model A are shown in Fig. 4, and those according to model B are presented in Fig. 5. PK-PD analysis according to model A could not describe the observed time course of the drug effects (see *Discussion*). PK-PD analysis according to model B provided adequate description of the

time course of the drug effects (see Fig. 3). The estimated values of the PD parameters are presented in Table 2. To reduce degrees of freedom, the values of  $n_L$ ,  $n_{GI}$ , and  $n_S$  were set to 2, 5, and 5, respectively, based on the preceding PK-PD fitting process. The values of the Akaike and Schwartz criteria for the results of PK-PD modeling were 515 and 549, respectively.

## Discussion

Preliminary work by Stepensky et al. (1998), as well as previous reports of Marchetti et al. (1987), have revealed a lack of direct correlation between the magnitude of glucose-lowering effect and blood metformin concentrations. To clarify the underlying pharmacokinetic and/or pharmacodynamic mechanism(s), we investigated the concentration-effect relationship following metformin infusion by different routes. The major difference between infusion of metformin to the duodenum and the peripheral vein is the targeting of higher drug concentrations to presystemic biophases over a prolonged period of time. This difference may account for the larger magnitude of effect observed for intraduodenal infusion despite the similarity in AUC values (see Table 1). However, this conclusion is questionable because the pattern of the concentration-time profile of the drug in the systemic circulation differed considerably between the intraduodenal and i.v. infusions.

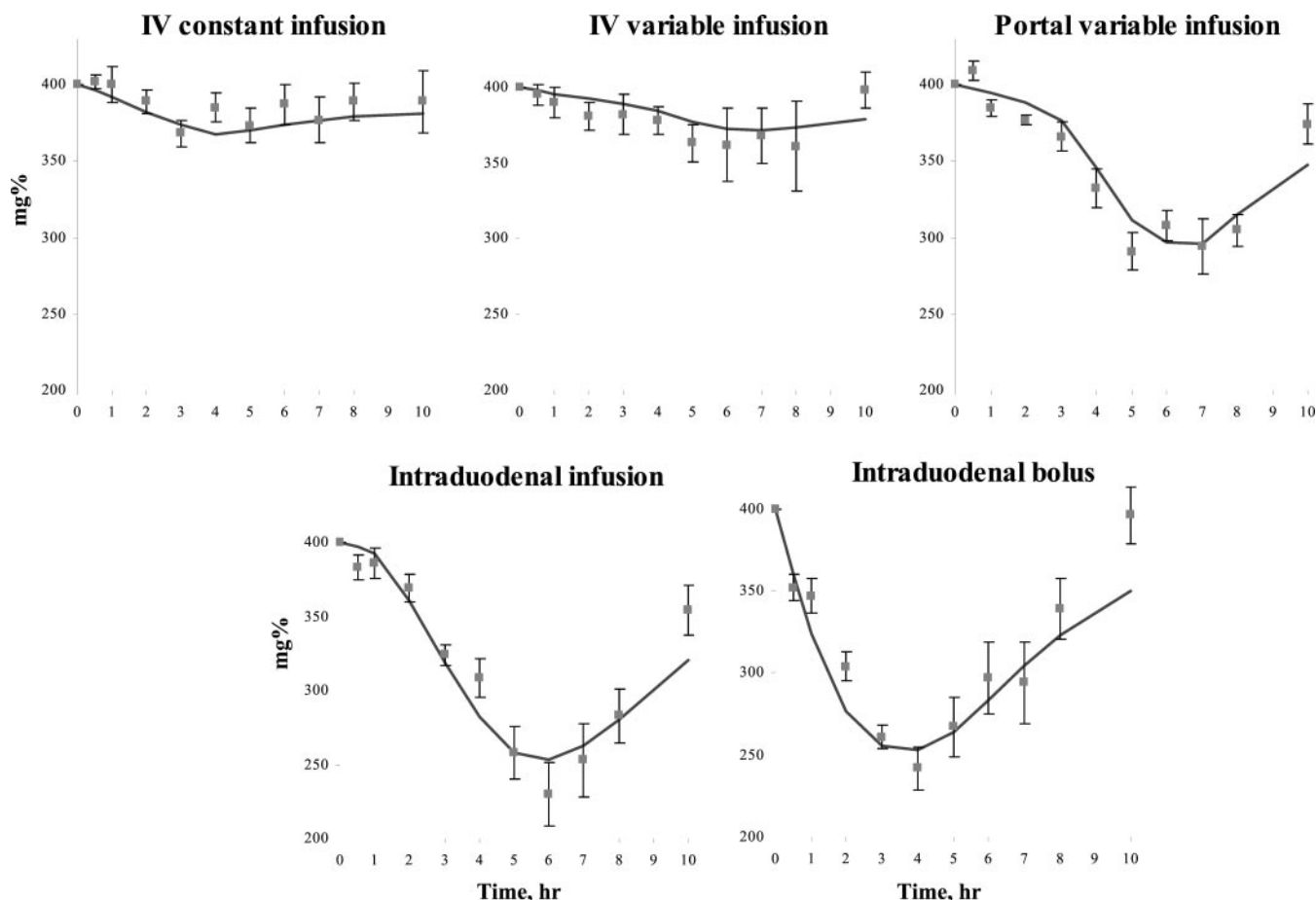


FIG. 3. Glucose-lowering effects following administration of 450 mg/kg metformin as intraduodenal bolus or intraduodenal infusion or 200 mg/kg metformin as constant rate i.v. infusion, variable rate i.v. infusion, or variable rate intraportal infusion.

To reveal the metformin-driven glucose-lowering effect, the baseline time course of blood glucose levels (following vehicle administration) was subtracted from the observed blood glucose levels following the drug administration. Data points are the mean observed glucose-lowering effects, and the solid lines are the best fits according to model B.

TABLE 2

Mean pharmacokinetic and pharmacodynamic (Model B) parameter estimates

PK Parameters			PD Parameters		
Parameter	Estimate	%CV	Parameter	Estimate	%CV
$k_{g0}$ ( $\text{min}^{-1}$ )	1.88e-03	23.3	$k_{in}$ (mg/dl/min)	2.08	34.8
$k_{gg}$ ( $\text{min}^{-1}$ )	1.85e-03	17.3	$k_{out}$ ( $\text{min}^{-1}$ )	5.42e-03	27.8
$k_{g1}$ ( $\text{min}^{-1}$ )	0.458	35.8	$E_{max}GI$	0.486	31.2
$k_{is}$ ( $\text{min}^{-1}$ )	0.910	28.2	$I_{max}L$	0.378	28.3
$k_{sl}$ ( $\text{min}^{-1}$ )	1.01e-02	68.4	$E_{max}S$	0.148	37.3
$k_{sg}$ ( $\text{min}^{-1}$ )	4.13	39.2	$EA_{50}GI$ ( $\mu\text{g}$ )	431	29.0
$k_{s0}$ ( $\text{min}^{-1}$ )	0.509	16.6	$IA_{50}L$ ( $\mu\text{g}$ )	521	33.1
$V_c$ (ml/kg)	70.23	15.0	$EA_{50}S$ ( $\mu\text{g}$ )	1024	37.4
			$n_{GI}^a$	2	
			$n_L^a$	5	
			$n_S^a$	5	

<sup>a</sup> Fixed parameter.

To further investigate this phenomenon, we applied a unique experimental strategy of variable rate i.v. infusion of the drug. The infusion rates were selected to yield the same metformin plasma concentration-time profile as that following infusion of the drug to the duodenum (see Fig. 2). This approach bypassed the PK constraints that evolve due to the slow intestinal absorption rate of metformin (i.e., flip-flop pharmacokinetics) and enabled a clear appreciation of the impact of the administration route on the magnitude of effect. The only difference between the two modes/routes of infusion was that the

first pass of metformin from the GI tract, via the portal vein and the liver, into the systemic circulation was of notable significance to the glucose-lowering effect. This difference in magnitude of response is therefore termed a first-pass PD effect (Lomnický et al., 1998; Hoffman et al., 1999).

To distinguish between the contribution of metformin effects on the GI wall during the first pass and its effects on the liver, the drug was infused directly to the portal vein of unrestrained rats using an input function that produced the same concentration-time profile as the

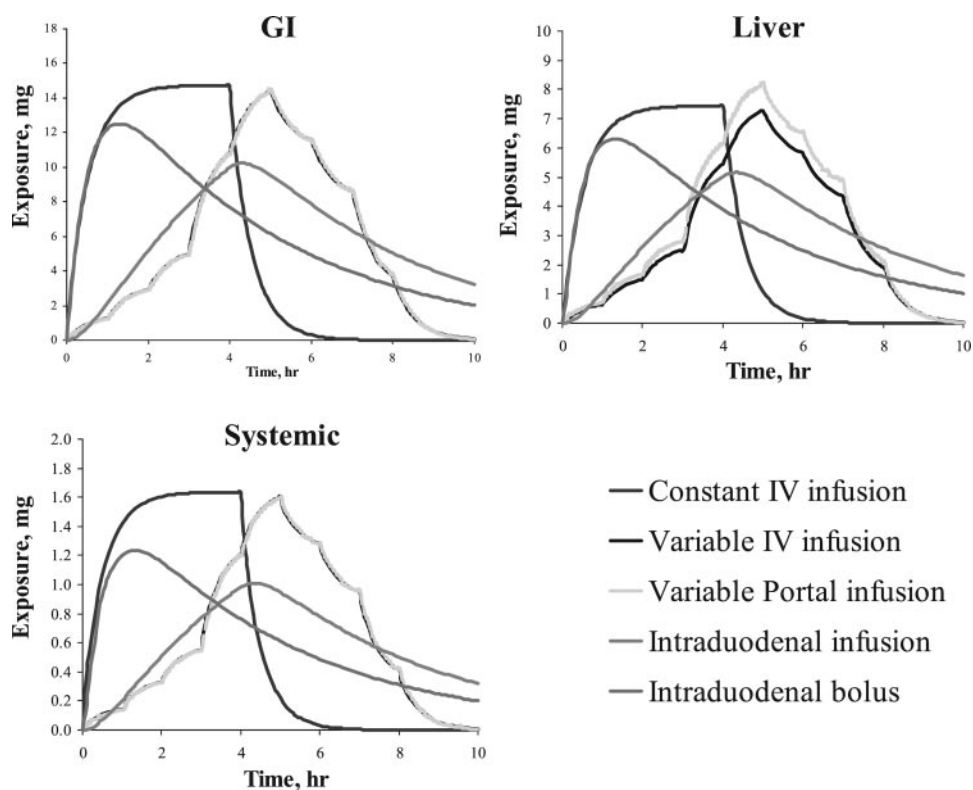


FIG. 4. Model A predicted exposure of biophases to metformin following administration of 450 mg/kg metformin as intraduodenal bolus or intraduodenal infusion or 200 mg/kg metformin as constant rate i.v. infusion, variable rate i.v. infusion, or variable rate intraportal infusion.

Note overlapping curves of the variable rate intraportal and i.v. infusions for the GI and systemic biophases.

intraduodenal (and i.v.) infusion. The results reconfirmed that administration of metformin via the portal-hepatic pathway produces a stronger glucose-lowering response than direct administration to the systemic circulation. The results also clarify the contribution of the first pass of the GI wall to the overall enhanced activity, as the magnitude of effect following direct intraportal infusion yielded a somewhat weaker glucose-lowering response than intraduodenal administration.

**Modeling.** To better understand the mode of administration dependence, we employed a PK-PD modeling approach that enabled an estimation of drug concentration-time data at the biophases, which otherwise are not accessible for sampling. This mathematically oriented approach estimates the major kinetic rate constants and the contribution of individual biophases to the overall drug effect. Due to the complex pharmacokinetic behavior of metformin and the contribution of three different sites of action to the overall glucose-lowering effect, complex models had to be produced. The number of estimated parameters reached 8 for the pharmacokinetic model and 11 for the pharmacodynamic model. To reduce the number of the estimated parameters, we fixed the values of the shape factors in the final run. This fixation was based on extensive modeling efforts applying different initial estimates of the shape factors that revealed that the value of  $n_L$  tends to be 2 and values of  $n_{GI}$  and  $n_S$  tend to be 5. We performed a simultaneous fit of all the experimental results (first PK and then PD) that were collected in a crossover design from the same experimental animals, thus considerably enhancing the power of the parameter estimation and thereby the validity of the derived conclusions.

**PK Model.** The PK model described the distribution of metformin versus time at different sites, including the proposed biophases (see Fig. 1). The simultaneous fit procedure yielded estimated PK param-

eters that adequately described the observed concentration-time data of the five investigated modes of metformin administration (Fig. 2). The fitting produced similar values of the rate constants of absorption and elimination from the GI lumen ( $k_{g0}$  and  $k_{gg}$ , respectively; see Table 2), indicating an overall GI bioavailability of approximately 50%, which is in accordance with the 40–60% bioavailability reported in most studies (Wiernsperger, 1996). The rate constant representing metformin elimination from the systemic circulation ( $k_{s0}$ ) was higher than the absorption rate constant, confirming flip-flop PK behavior of metformin (Scheen, 1996; Cusi and DeFronzo, 1998).

**PK-PD Model.** Since metformin affects blood glucose levels indirectly by altering the rate of glucose production and utilization, the indirect response PK-PD modeling approach (Dayneka et al., 1993) was applied to describe the glucose-lowering effect of metformin. In this investigation, a combined indirect PD model was used for the first time to describe the effect of the drug on the kinetics of the measured response by affecting both the input and output parameters (models I and IV, respectively; Dayneka et al., 1993). For each biophase, metformin concentration versus time profiles estimated from the PK model were linked to the elevation or reduction of blood glucose (eqs. 5–8).

Model A was based on the assumption that concentrations of metformin accumulated in the GI wall, liver, and systemic compartments specified in the PK model were responsible for the glucose-lowering effects. However, exposure of each of these compartments to metformin, according to model A, was similar for all the modes of drug administration (see Fig. 4). For example, intraportal and i.v. infusion exposure to metformin according to model A was similar for liver biophase and identical for the “GI” and systemic biophases, despite the major differences in the observed magnitude of glucose-lowering effect (AUEC) (see Fig. 3 and Table 1). Hence, model A is

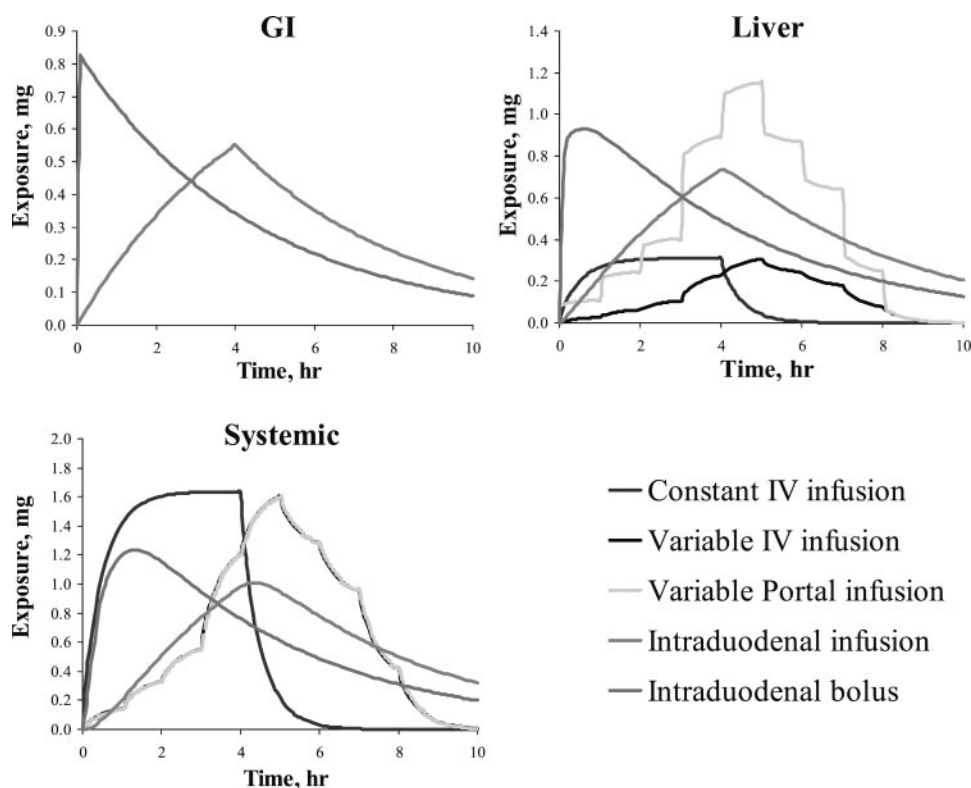


FIG. 5. Model B predicted exposure of biophases to metformin following administration of 450 mg/kg metformin as intraduodenal bolus or intraduodenal infusion or 200 mg/kg metformin as constant rate i.v. infusion, variable rate i.v. infusion, or variable rate intraportal infusion.

Note overlapping curves of the variable rate intraportal and i.v. infusions for the systemic biophase.

not the proper model, and the drug concentrations at these PK compartments do not represent the concentration “seen” by the receptors associated with metformin action.

Model B was based on the working hypothesis that there is a correlation between the temporal metformin concentrations in the portal vein and within the intestinal wall (for liver and GI wall compartments, respectively) and the observed glucose-lowering effect. It differs from model A, which focused on metformin concentrations that accumulated in the above-mentioned compartments. For the liver biophase, this presumption is based on the known heterogeneity in liver anatomical structure, termed “hepatic zonation” (Gebhardt, 1992; Jungermann and Thurman, 1992), and the unique organization of the liver blood supply from both portal vein and hepatic artery. Focusing on the variable rate i.v. and intraportal infusions, model B predicts higher exposure of liver biophase following intraportal infusion (Fig. 5). Appropriate description of the pharmacodynamic data by model B (Fig. 3) indicates that the magnitude of the glucose-lowering effect of metformin in liver is related to the drug concentrations in the portal vein. This finding is in accord with previous knowledge on metformin pharmacologic activity that is mediated through cell membrane events (Klip and Leiter, 1990; Wiernsperger, 1996). An additional possibility is the rapid equilibrium between the metformin concentrations at the portal vein and an intracellular site of action. Thus, the liver/portal biophase could be the subpopulation of hepatocytes whose cellular membrane is exposed to portal blood.

In intraduodenal bolus administration, adsorption of metformin to the intestinal wall and low rate of absorption (i.e., flip-flop PK) lead to prolonged elevation of portal drug concentrations compared with i.v. administration. Even more prolonged elevation of portal metformin concentrations was achieved following intraduodenal infusion.

Therefore, intraduodenal bolus and infusion produced higher portal-vein metformin concentrations and thereby enhanced exposure of the liver/portal biophase to metformin compared with i.v. infusions (see Fig. 5). This conclusion is confirmed by the findings that the portal vein concentrations were consistently higher (by approximately 50%) than drug concentrations in the systemic circulation (inferior vena cava) following oral metformin administration to streptozotocin diabetic mice (Wilcock and Bailey, 1994).

GI administration of metformin, in addition to elevated portal exposure, leads also to higher exposure of the GI biophase to the drug. This is apparent from the comparison between the magnitude of glucose-lowering effect following GI and intraportal modes of metformin administration. model B assumes that the main activity of metformin at the GI wall is contributed from drug that reaches the GI biophase from the GI lumen following intestinal absorption, whereas the impact of the arterial blood input is negligible. The good fit between the response-time profile predicted by model B and the experimental data substantiates the underlying assumption. It means that drug molecules that permeate the intestinal wall account for the first-pass phenomenon, whereas the relatively large amounts of the drug that accumulate there have only a negligible contribution to that effect.

It should be noted that the streptozotocin-induced model of diabetes as applied in this investigation is not a perfect mimic of type 2 diabetes with regard to insulin resistance in peripheral tissues (Weiss et al., 1995). Thus, the contribution of presystemic biophases to the overall glucose-lowering effect may be accentuated in this model. It is, therefore, suggested that the route of administration dependence of metformin in insulin-resistant type 2 diabetes be examined to validate the outcomes and refine the extrapolation of the conclusions of the

current investigation to understanding the PK-PD of the drug in clinical situations.

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