A Human Physiologically-Based Model for Glycyrrhizic Acid, A Compound Subject to Presystemic Metabolism and Enterohepatic Cycling

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Purpose. To analyze the role of the kinetics of glycyrrhizic acid (GD) in its toxicity. A physiologically-based pharmacokinetic (PBPK) model that has been developed for humans.

Methods. The kinetics of GD, which is absorbed as glycyrrhetic acid (GA), were described by a human PBPK model, which is based on a rat model. After rat to human extrapolation, the model was validated on plasma concentration data after ingestion of GA and GD solutions or licorice confectionery, and an additional data derived from the literature. Observed interindividual variability in kinetics was quantified by deriving an optimal set of parameters for each individual.

Results. The a-priori defined model successfully forecasted GA kinetics in humans, which is characterized by a second absorption peak in the terminal elimination phase. This peak is subscribed to enterohepatic cycling of GA metabolites. The optimized model explained most of the interindividual variance, observed in the clinical study, and adequately described data from the literature.

Conclusions. Preclinical information on GD kinetics could be incorporated in the human PBPK model. Model simulations demonstrate that especially in subjects with prolonged gastrointestinal residence times, GA may accumulate after repeated licorice consumption, thus increasing the health risk of this specific subgroup of individuals.

KEY WORDS: glycyrrhizic acid; modeling; enterohepatic cycling; PBPK; pharmacokinetics.

INTRODUCTION

Glycyrrhizinic acid (GD) is of interest for the treatment of chronic hepatitis, since long-term administration of this compound reduces the development of hepatocellular carcinomas in this disease (1). Due to its sweet taste, approximately 50 times sweeter than sucrose, GD is also applied as a sweetener in a diversity of food products and in chewing tobacco (2).

When considerable doses of GD are consumed habitually, mineral corticoid excess-like side effects may occur due to the inhibition of the enzyme 11-β-hydroxysteroid dehydrogenase (11-β-HSD) by its main metabolite, glycyrrhetic acid (GA) (3). In case of overconsumption, this may lead to hypertension, edema, and electrolyte disturbances. The differences in susceptibility for licorice-induced adverse effects between subjects might be due either to interindividual differences in the pharmacokinetics or in the pharmacodynamics of GD, or a combination of both (2). For the identification of the subgroups that are at higher risk for licorice-induced adverse effects, a detailed insight into these relationships is essential.

Over the past 15 years, physiologically-based pharmacokinetic (PBPK) models have been applied for the risk assessment of several xenobiotics (4,5). Due to a realistic anatomical and physiological representation of the organism, data from in vitro, in vivo, and in situ studies can be used to describe the kinetics of a compound in vivo. In addition, PBPK models can be readily scaled from one species to another. Moreover, the biological fate of a chemical can be predicted under a variety of exposure situations. Hence, PBPK models are useful for risk assessment purposes, since the actual exposure is often different from the exposure in the experimental setting. In combination with a quantitative relationship between the biologically active dose and its toxic effect(s), an adequately validated model may elucidate why and under which circumstances some subgroups of the population are more at risk than others (4,6).

The aim of the present study is to develop and validate a human PBPK model for GD, based on a previously developed rat model (7). In study in healthy volunteers on the pharmacokinetics of GD after consumption of a suspension of GA, a solution of GD or two different types of licorice confectionery was performed to calibrate this model. Moreover, information on the interindividual distribution of the model parameters that have the most influence on the model forecast was obtained from this study. The model was validated by comparison of its forecast with the results of previously published clinical studies. Finally, the impact of the model parameters on the pharmacokinetics of GD is discussed and used to identify subgroups of subjects at risk for GD-induced adverse effects.

METHODS

Model Description

It is assumed that the structural PBPK model, which has been developed previously to describe the pharmacokinetics of GD and its main metabolite glycyrrhetic acid (GA) in rats (7), can also be applied to describe the kinetics of both compounds in humans (Fig. 1). GD is absorbed as its aglycon GA after enzymatic hydrolysis by commensal bacteria (Eqs. 21–23; see Appendix). In addition to the observation that GA is 200–1000 times more potent an inhibitor of 11-β-HSD in vitro, the kinetics of GA are of interest after oral GD treatment. GA distribution into body tissues other than the liver is minimal (Eq. 1–6). Following hepatic uptake by capacity limited carriers, GA is metabolized to mainly glucuronide metabolites. The observed rapid excretion of GA metabolites
(GM) can only be explained if is assumed that GA is metabolized instantaneously, and that its metabolites are subsequently excreted into the bile (7). This suggests involvement of binding proteins, like 3α-hydroxysteroid dehydrogenase, which prevent the reflux of the conjugates to plasma. The biliary excretion of GM is facilitated by the canalicular multispecific organic anion transporter (cMOAT). In the rat model, the hepatobiliary clearance of GA was described adequately on the assumption that the rate of hepatic uptake of GA and biliary excretion of GA metabolites are equal (Eq. 16, 17). Biliary excreted GA metabolites (GM) are, once excreted into the duodenum, reconverted into GA by bacteria in the gastrointestinal tract (Eq. 24) and subsequently reabsorbed into the systemic circulation (Eq. 6).

To overcome the lack of reliable data on human gastrointestinal lumen volumes, the four physiological compartments (stomach, small intestine, cecum, and colon) of the gastrointestinal tract model for rats were lumped into three compartments, representing the stomach and the small and large intestines (Fig. 1B). In humans, the gastric emptying and the transit time in the intestines has been measured by gamma scintigraphy and is generally defined as the time in which 50% of the contents pass the specific gastrointestinal compartment (8–11). It is assumed that the contents of the gastrointestinal tract pass with a constant (zero order) rate (Eq. 7–15). In the human model, biliary excreted GA metabolites are stored in the gall bladder and are excreted instantaneously into the gut after the ingestion of a meal containing fat (Eq. 18–20) (12).

**Clinical Study**

Human data on the pharmacokinetics of GA after oral administration of glycyrrhizic acid were obtained by performing a clinical study in conformity with the current rules for Good Clinical Practice (GCP). All subjects (8 males and 8 females) refrained from consuming food products containing GD within a 72 hour period foregoing product administration and during the entire study. In a 4-way random crossover design, subjects received four treatments (with a two week wash-out period): I) an aqueous suspension of 130 mg GA (equivalent to 225 mg GD) in 250 ml water-propyleneglycol (80-20% v/v); II) an aqueous solution (250 ml) of 225 mg GD; III) 150 g sweet (unsalted) licorice confectionery containing 225 mg GD and 5% w/w NH₄Cl. GA and the monoammonium salt of GD were purchased from Acros Chimica (Geel, Belgium), and Red Band Venco BV (Rozendaal, The Netherlands) kindly supplied the sweet and salted licorice. Food consumption was recorded during the first 24 hours. It took place at 4, 6, and 9 hours post-dosage. Blood was sampled at −1 (baseline), 2, 2.5, 4, 5.5, 7, 8.5, 10, 11.5, 13, 14.5, 18, 22, 32, 48, and 56 hr after the product administration. The blood samples were centrifuged immediately after sampling and the plasma was stored at −20°C until analysis. The GA plasma concentrations were determined by high performance liquid chromatography (HPLC) analysis. Briefly, GA was extracted from plasma by solid-phase extraction, using a C18 column. For the HPLC analysis of GA, a reversed phase C18 column and a gradient system of 78%–83% v/v methanol/water acidified with 0.3% acetic acid was used. GA was detected at 250 nm. All chemicals used in the HPLC analysis were of analytical grade and were purchased from Merck (Darmstadt, Germany).

**Model Calibration**

The values of the anatomical and physiological parameters were obtained from literature (Table I). In the literature, the gastric emptying time of non-solid material ranged from 0.12 to 0.62 hr (mean 0.38 hr) (9,11), whereas the gastric emptying time of a solid formulation ranged from 0.13–3.51 hr (mean 1.83 hr) (8–11). There is no difference in the small intestinal transit time between liquid or solid formulations (9,10), and the transit time in the small and large intestines is