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The effects of the phytoestrogenic isoflavone genistein on the hepatic disposition of preformed and hepatically generated gemfibrozil 1-*O*-acyl glucuronide in the isolated perfused rat liver

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Abstract

Foods and complementary medicines contain phytoestrogenic isoflavones such as genistein, which undergo hepatic glucuronidation and excretion into bile and can potentially interfere with the hepatic elimination of other compounds. To investigate this potential, livers from Sprague–Dawley rats were perfused in single-pass mode with preformed gemfibrozil 1-*O*-acyl glucuronide (GG) (1 μM, n = 12) for 60 min followed by a 30-min washout phase, or with gemfibrozil (1 μM, n = 10) for 120 min. Half of each group of livers were co-perfused with genistein (10 μM) throughout the experiment. Perfusate and bile were analyzed for GG and gemfibrozil by HPLC. Co-perfusion with genistein significantly ($P < 0.05$) decreased the biliary extraction ratio of preformed GG from a mean of 0.82 to 0.65 and the first-order rate constant for transport of GG into bile from 0.054 ± 0.010 to $0.032 \pm 0.008\,min^{-1}$, but increased the first-order rate constant for sinusoidal efflux of GG from 0.128 ± 0.023 to $0.227 \pm 0.078\,min^{-1}$. Co-perfusion with genistein also significantly decreased the biliary extraction ratio of hepatically generated GG from 0.95 ± 0.01 to 0.83 ± 0.05. The findings confirm that genistein increases the potential for hepatic and systemic exposure to hepatically generated glucuronides, which may be important for patients on conventional drugs who consume isoflavones.

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Introduction

Health benefits associated with the consumption of phytoestrogenic isoflavones include reductions in the severity of menopausal symptoms and the incidence of cardiovascular disease, osteoporosis and reproductive system neoplasia (Knight & Eden 1996; Murkies et al 1998). Phytoestrogenic isoflavones can be consumed in the diet (soy, legumes) (Barnes et al 1994; Reinli & Block 1996) or as over-the-counter, complementary medicines for self-medication (Setchell et al 2001) and since many people consuming the phytoestrogenic isoflavones may also be taking medications containing conventional drugs, the potential exists for pharmacokinetic interactions between these compounds. This potential has previously been investigated in the isolated perfused rat liver (IPL), where co-perfusion with mixtures of genistein and biochanin A, or daidzein and formononetin, reduced the hepatic clearance of paracetamol by reducing the formation of paracetamol sulfate, and the co-administration of genistein and biochanin A also enhanced the biliary excretion of paracetamol sulfate, by inhibiting its sinusoidal efflux (Lucas et al 2003). Genistein has also been shown to inhibit the canalicular multispecific organic anion transporter (cMOAT)-mediated transport of the glucuronide conjugates of rhodamine and bilirubin into bile (Jager et al 1997). However, the effects of the phytoestrogenic isoflavones on the sinusoidal uptake or efflux of (preformed) glucuronide conjugates has not been investigated.

Gemfibrozil 1-*O*-acyl glucuronide (GG) has previously been used as a model compound for investigating pharmacokinetic interactions in the IPL (Sallustio et al 1996;

Sabordo et al 1999, 2000). GG is the major hepatically generated metabolite of gemfibrozil and the hepatic disposition of both GG and gemfibrozil in the IPL have previously been described in detail (Sallustio et al 1996; Sabordo et al 1999, 2000). In IPL studies, the liver readily extracts both gemfibrozil and GG, and GG is extensively transported into bile by cMOAT (Sabordo et al 1999).

Genistein is a major phytoestrogenic isoflavone (Setchell et al 2001) and undergoes extensive first-pass hepatic metabolism to sulfate and glucuronide conjugates, which are excreted in bile and urine (Yasuda et al 1994, 1996; Jager et al 1997; Sfakianos et al 1997). The present study examined the effects of genistein on the hepatic disposition of preformed and hepatically generated GG in the IPL and a pharmacokinetic model was used to identify the mechanisms of the observed interaction.

Materials and Methods

Materials

Gemfibrozil, sodium taurocholate and β-glucuronidase from bovine liver (EC 3.2.1.31) were purchased from Sigma-Aldrich (Sydney, Australia). Genistein was purchased from Indofine Chemical Company (Somerville, NJ). The biosynthesis and subsequent extraction of GG from human urine was based on a previously published procedure (Sallustio & Fairchild 1995). The purity of GG was determined by quantification of the molar amount of gemfibrozil produced following hydrolysis of GG with β-glucuronidase. All other chemicals were purchased from Merck (Kilsyth, Australia). Water was purified using a Milli-Q purifier system (Millipore Australia, Australia).

Isolated perfused rat liver preparation

The study was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science, Adelaide, SA, Australia. Male Sprague–Dawley rats (200–350 g) were purchased from the Gilles Plains Animal Resource Centre, Adelaide, SA, Australia.

Livers were perfused in single-pass mode at 37 °C following previously described surgical techniques (Evans & Shanahan 1995), so that samples could be collected from the cannulated bile duct and portal inflow and venous outflow cannulas. The perfusion medium consisted of freshly prepared albumin- and erythrocyte-free Krebs–Henseleit buffer, supplemented with 16.5 mM glucose and 8.33 μM sodium taurocholate. This solution was filtered (0.45 μM), warmed to 37 °C, bubbled with humidified 95% O_2/5% CO_2, adjusted to a pH of 7.4 and pumped through the liver, via the portal vein, at 30 mL min^{-1}.

Liver viability was confirmed throughout each perfusion by measuring hepatic oxygen consumption (>10 μmol min^{-1}, 820 Dissolved Oxygen Meter, Orion, MA), bile flow rate (>5 μL min^{-1}, determined gravimetrically assuming a specific gravity of 1.0), the decrease in pH between inflow and outflow perfusate (>0.05 pH units), and by observing changes in the gross appearance of the liver (Ross 1972).

Loading–washout experiments with GG

Twelve livers were each perfused for 105 min with perfusate containing 0 μM (control, n = 6) or 10 μM (n = 6) genistein. After a 15-min equilibration period, GG was infused into the portal catheter, using a syringe pump, to generate an inflow concentration of 1 μM. Samples of outflow perfusate were collected at 2, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min after starting the GG infusion. The infusion was stopped after 60 min and further samples were collected during the 'washout' phase at 0.167, 0.5, 1, 2, 4, 5, 7, 10, 14, 18, 22, 26 and 30 min. Samples of inflow perfusate (n = 3) were collected from the inflow catheter. Bile was collected in 5-min intervals throughout the experimental period.

The hydrolysis of GG was avoided by adding 15 μL mL^{-1} of 1.5 M orthophosphoric acid to each perfusate sample, and by the dilution of bile 1:5 with 1 M glycine buffer (pH 3.0) (Sallustio et al 1996). Samples were stored at −20 °C until analysed by HPLC.

Steady-state experiments with gemfibrozil

Ten livers were each perfused for 120 min with perfusate containing 1 μM gemfibrozil and either 0 μM (control, n = 5) or 10 μM (n = 5) genistein. After the perfusion with gemfibrozil was commenced, outflow perfusate samples were collected at 5-min intervals and bile samples were collected over 5-min periods throughout the experimental period. At the end of the perfusion, the catheter was removed from the portal vein and samples (n = 3) of inflow perfusate were collected directly from the catheter. Perfusate and bile samples were stabilized and stored as above.

Sample analysis

The HLPC assay for the simultaneous quantification of gemfibrozil and GG in perfusate and bile samples was based on a previously reported method (Sallustio & Fairchild 1995). Perfusate was used undiluted while bile was diluted (1:250) in Milli-Q water. Samples (250 μL) were injected onto an Alltima C_{18} 5 μm pre-column (Alltech, Deerfield, IL) connected to a Waters Symmetry C_{18} column (5 μm, 3.9 × 150 mm, Waters, Milford, MA). The mobile phase consisted of 43% acetonitrile, 5% methanol and 40 μL L^{-1} trifluoroacetic acid and was pumped at a flow rate of 1 mL min^{-1}. The HPLC system consisted of a SIL-10A auto injector, LC-10ADVP pump, SCL-10A system controller, RF-10AXL fluorescence detector (excitation 284 nm, emission 316 nm) and C-R5A chromatopac integrator (Shimadzu, Kyoto, Japan). Typical retention times for GG and gemfibrozil were 8 and 32 min, respectively. Chromatograms of solutions of GG prepared freshly from powder stored at −20 °C contained only one peak with no evidence of rearrangement isomers (Sallustio & Fairchild 1995). There was no

evidence of rearrangement isomers in the chromatograms of perfusate or bile samples from any liver.

Calibration curves for undiluted perfusate and diluted bile were prepared over the range 0.017–4.28 μM for GG and 0.02 to 5 μM for gemfibrozil. Independently prepared quality control samples were 0.085 and 0.85 μM (GG) and 0.1 and 1 μM (gemfibrozil). The limit of quantification was 0.017 μM for GG and 0.02 μM for gemfibrozil. Calibration curves were fitted to $1/y$ weighted data using linear least squares regression analysis (for gemfibrozil and GG, $R^2 > 0.9993$). From repeated analysis of the high and low quality control samples, accuracy was within 12% of nominal values and precision was within 8%.

Data analysis

Pharmacokinetic parameters at steady-state were calculated using data from samples collected between 45 and 60 min (perfusions with GG) or between 105 and 120 min (perfusions with gemfibrozil).

From perfusions with GG, the hepatic clearance of GG was calculated as the product of its hepatic extraction ratio and the perfusate flow rate, where the hepatic extraction ratio was the difference between concentrations of GG in outflow and inflow perfusate divided by the inflowing concentration; the total rate of recovery of GG was calculated as the sum of the rates of recovery of GG in perfusate and bile; the biliary extraction ratio of preformed GG was calculated as the rate of recovery of GG in bile divided by its total rate of recovery; and the mass balance (%) was calculated as the total rate of recovery of GG and gemfibrozil, relative to the inflow rate of GG.

Equations A1, A2 and A3 (see Appendix) were derived from a model for the hepatic disposition of GG (Figure 1, Appendix). These equations were programmed into WinNonlin (Version 3, Pharsight Corporation, Mountain View, CA) with the model designed to estimate the first-order rate constants (k_{eff}, k_{upt}, k_{hyd}, k_{bile}, k_{form} and k_{other}) from simultaneous fitting of the rates of recovery of GG in perfusate and bile (unweighted data), using all samples analysed in each loading–washout experiment. Perfusate flow rate, volume of the vascular space and infusion time were fixed at 30 mL min^{-1}, 2.5 mL and 60 min, respectively. The experimentally determined rate of infusion of GG for each liver was used and after 60 min the infusion rate was set to zero. The user-defined initial parameter estimates were $k_{eff} = 0.15\ min^{-1}$, $k_{upt} = 200\ min^{-1}$, $k_{hyd} = 0.2\ min^{-1}$, $k_{bile} = 0.05\ min^{-1}$, $k_{form} = 10\ min^{-1}$ and $k_{other} = 1\ min^{-1}$.

From perfusions with gemfibrozil, the biliary extraction ratio of hepatically generated GG was calculated as the rate of recovery of GG in bile divided by the total rate of recovery of GG, and mass balance was calculated as the total rate of recovery of gemfibrozil and GG relative to the inflow rate of gemfibrozil.

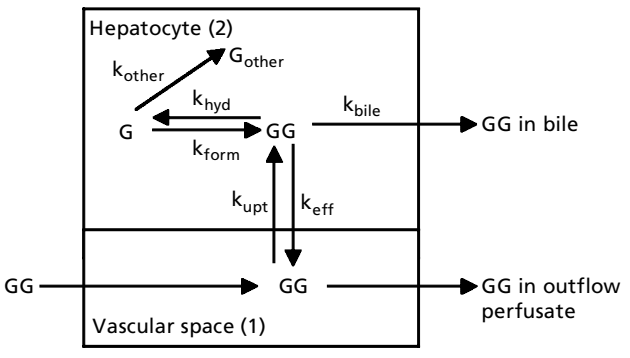

Figure 1 Proposed model for the hepatic disposition of preformed gemfibrozil 1-*O*-acyl glucuronide (GG). GG entering the tissue from the vascular space (k_{upt}) can be transported into bile (k_{bile}), efflux transported back into the vascular space (k_{eff}) or hydrolysed to gemfibrozil (G) (k_{hyd}). Intracellularly formed G can be metabolized back to GG (k_{form}) or to other gemfibrozil metabolites (G_{other}) (k_{other}). k_x is the first-order rate constant for process x.

Statistical methods

Except where indicated, data are presented as arithmetic mean ± s.d. Comparisons between experimental groups were made using unpaired t-tests, with $P < 0.05$ considered statistically significant.

Results

In all cases, the parameters reflecting liver viability remained above minimum acceptance limits over the course of each perfusion experiment. In the loading–washout experiment, the steady-state bile flow rate from livers perfused with 10 μM genistein was significantly higher than that from the control livers (11.30 ± 2.22 vs 8.23 ± 1.58 μL min^{-1}, respectively, $P < 0.05$).

Gemfibrozil could not be quantified in outflow perfusate or bile from any liver perfused with GG. The mean profiles for the concentration of GG in outflow perfusate and rate of recovery of GG in bile over time for each experimental group are presented in Figure 2. At steady-state in the absence of genistein, the hepatic clearance of GG was 25.4 ± 2.9 mL min^{-1}, the biliary excretion rate of GG was 21.8 ± 3.4 nmol min^{-1}, the biliary extraction ratio of GG was 0.82 ± 0.09 and the mass balance was $80 \pm 17\%$. Co-perfusion with genistein had no significant effect on the steady-state hepatic clearance of GG (22.4 ± 3.9 mL min^{-1}, $P = 0.16$) and the overall mass balance ($73 \pm 10\%$, $P = 0.39$), but did significantly decrease the biliary excretion rate of GG to 14.9 ± 4.5 nmol min^{-1} ($P < 0.05$) and the biliary extraction ratio of preformed GG to 0.65 ± 0.15 ($P < 0.05$).

The pharmacokinetic model (Figure 1, Appendix) provided an excellent description of perfusate and biliary data for GG in both the absence and presence of genistein (Figure 3). Genistein co-administration significantly increased k_{eff} and significantly decreased k_{bile}, but had no significant effect on k_{upt}, k_{hyd}, k_{form} or k_{other} (Table 1).

In the experiments with gemfibrozil, the steady-state bile flow rate from livers co-perfused with 10 μM genistein was higher than from the control livers (9.79 ± 1.36 vs 6.43 ± 0.72 μL min^{-1}, respectively, $P < 0.05$). A chromatographic peak at the retention time of gemfibrozil was

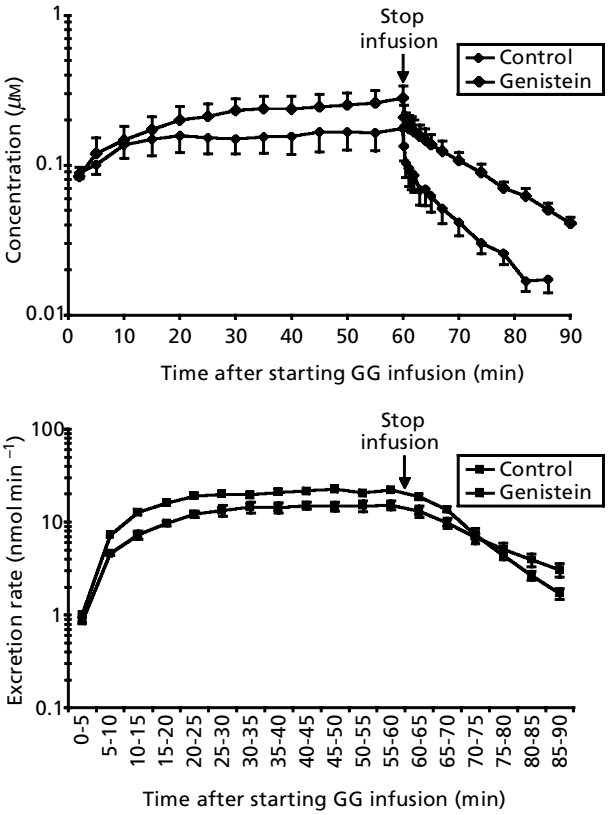

Figure 2 Mean concentration of GG (±standard error of the mean) in outflow perfusate (diamond) and biliary excretion rate of GG (square) from livers perfused in single-pass mode, with 1 μM GG in the absence (open) or presence (solid) of 10 μM genistein.

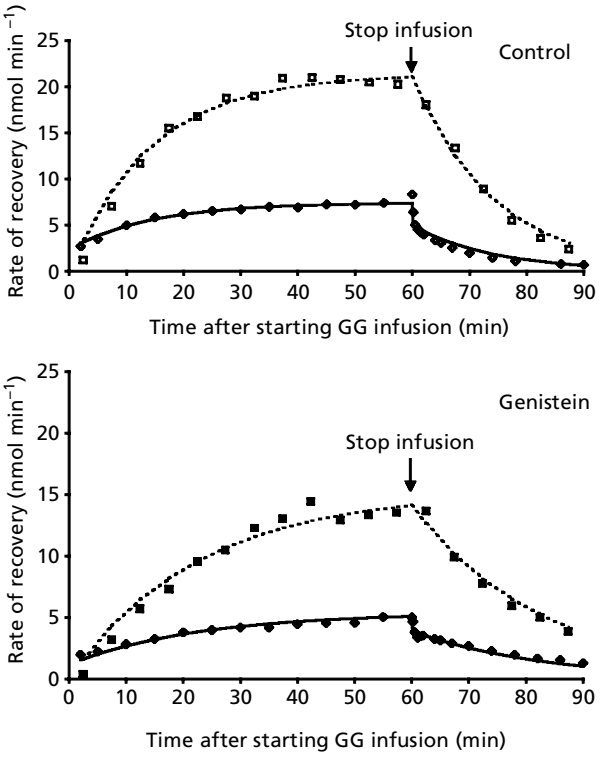

Figure 3 Examples of the rate of recovery of GG in perfusate (diamond) and bile (square) from representative livers, when the liver was perfused in single-pass mode, with 1 μM GG (open) or 1 μM GG plus 10 μM genistein (solid) in albumin- and erythrocyte-free Krebs–Henseleit buffer. The data points are overlaid with the predicted loading–washout curve using the physiologic model described in the Appendix.

Table 1 Mean (± s.d.) pharmacokinetic parameters calculated from livers perfused with 1 μM GG in the absence (control) or presence of 10 μM genistein, in a single-pass, loading–washout design.

Parameter	Control	Genistein
k_{eff} (min^{-1})	0.128 (0.023)	0.227 (0.078)*
k_{upt} (min^{-1})	239 (122)	242 (118)
k_{hyd} (min^{-1})	0.165 (0.081)	0.107 (0.034)
k_{bile} (min^{-1})	0.054 (0.010)	0.032 (0.008)**
k_{form} (min^{-1})	16.5 (14.8)	11.6 (3.9)
k_{other} (min^{-1})	0.958 (0.668)	1.949 (0.860)

k_{eff}, first-order rate constant for the sinusoidal efflux transport of GG; k_{upt}, first-order rate constant for the sinusoidal uptake transport of GG; k_{hyd}, first-order rate constant for the hydrolysis of GG to gemfibrozil; k_{bile}, first-order rate constant for the bile canalicular transport of GG; k_{form}, first-order rate constant for the formation of GG from gemfibrozil; k_{other}, first-order rate constant for the formation of other metabolites of gemfibrozil. *$P < 0.05$, **$P < 0.005$.

observed in outflow perfusate of some livers perfused with gemfibrozil, but in all cases was below the limit of quantification of the assay (0.02 μM). It was therefore assumed that the steady-state hepatic clearance of gemfibrozil approached the perfusate flow rate (30 mL min^{-1}). During perfusions with gemfibrozil, GG was recovered in both outflow perfusate (1.01 ± 0.27 nmol min^{-1}) and bile (18.88 ± 1.25 nmol min^{-1}), giving a biliary extraction

ratio for hepatically generated GG of 0.95 ± 0.01 and a mass balance of $75 \pm 4\%$. Co-perfusion with genistein significantly increased the rate of recovery of GG in outflow perfusate to 3.10 ± 0.79 nmol min^{-1} ($P < 0.05$) and decreased the rate of recovery of GG in bile to 15.71 ± 1.86 nmol min^{-1} ($P < 0.05$), resulting in a significant reduction in the biliary extraction ratio of the metabolite to 0.83 ± 0.05 ($P < 0.05$). Co-perfusion with genistein had no effect on the overall mass balance value ($69 \pm 5\%$, $P = 0.07$).

Discussion

This study was designed to investigate the potential for phytoestrogenic isoflavones to be involved in pharmacokinetic interactions in the liver by examining their effects on the hepatic disposition of preformed and hepatically generated GG. GG was chosen as the marker compound because it is a stable glucuronide conjugate in the IPL (Sallustio et al 1996), and its hepatic disposition is well described (Sallustio et al 1996; Sabordo et al 1999, 2000).

Given that the steady-state plasma concentration of gemfibrozil in humans is between 60 and 100 μM (Todd & Ward 1988) and with 97 to 98.4% binding to albumin (Todd & Ward 1988), the unbound steady-state plasma concentration of gemfibrozil would be in the order of 1 to 2 μM. The target inflow concentrations of gemfibrozil and GG of 1 μM in the IPL experiments described in this paper are therefore within the range of unbound plasma concentrations encountered in humans after gemfibrozil administration.

The inflow concentration of genistein (10 μM) was based on this being within the range of predicted concentration of genistein in portal blood in-vivo after an oral dose of the isoflavone, as the extensive enterohepatic cycling of genistein in humans (Sfakianos et al 1997) means that the concentration of genistein entering the liver is substantially greater than the peripherally measured plasma concentration of 1 to 4 μM (King & Bursill 1998; Setchell et al 2001). In addition, 10 μM was the total isoflavone concentration that has previously been shown to have significant effects on the hepatic disposition of paracetamol in the IPL (Lucas et al 2003).

In both loading–washout and steady-state experiments, the steady-state bile flow rate from livers perfused with 10 μM genistein was higher than that from the control livers. This is consistent with previous reports that genistein causes a dose-dependent increase in bile flow rate in the IPL (Jager et al 1997; Lucas et al 2003). It has been suggested that the increase is an osmotic effect arising from the transport of genistein metabolites into bile (Jager et al 1997).

The hepatic clearance of gemfibrozil in the present study (approximately 30 mL min^{-1}) was considerably greater than that previously reported (2.73 mL min^{-1}) (Sallustio et al 1996; Sabordo et al 1999, 2000). Similarly, the hepatic clearance of GG (25.4 mL min^{-1}) was also greater than that previously reported (9.0 to 19.5 mL min^{-1}) (Sallustio et al 1996; Sabordo et al 1999, 2000). Previous IPL experiments with gemfibrozil and GG utilized albumin in the perfusing medium while the current experiments were performed with a protein-free medium, and the attendant increase in fraction unbound would account for the increased hepatic clearance of gemfibrozil and its metabolite in the present study.

In keeping with previous studies (Sallustio et al 1996; Sabordo et al 1999, 2000), complete mass balance was not achieved in the present study. This probably reflects the contribution of unquantified gemfibrozil metabolites, as at least three (Dix et al 1999) and nine (Thomas et al 1999) metabolites of gemfibrozil have been identified in the bile and urine, respectively, of rats administered gemfibrozil. Co-perfusion with genistein had no effect on the mass balance in either perfusion experiment, suggesting that genistein, or its metabolites, had no overall effect on the hydrolysis or formation of GG or on the formation of other gemfibrozil metabolites. Although there were no changes in the metabolic rate constants (k_{hyd}, k_{form} and k_{other}; Table 1), these three parameters were quite variable, unlike the transport rate parameters. Genistein has previously been shown to inhibit the formation of the glucuronide conjugates of rhodamine 123 and bilirubin in rat liver homogenate (Jager et al 1997); however, the genistein concentration required to produce 50% inhibition in that study (100 μM) was considerably higher than the inflow concentration of genistein in the present study. Even though genistein had no effect on the overall formation of gemfibrozil metabolites in the present study, it is still possible that genistein altered the relative proportions of the non-GG metabolites formed from gemfibrozil. However, it is unclear whether this was the case, as these metabolites were not quantified in the present study.

The biliary extraction ratio of both hepatically generated and preformed GG was high (0.95 ± 0.01 and 0.82 ± 0.09 respectively), indicating that GG within the hepatocyte is efficiently transported into bile. The higher value for hepatically generated GG might reflect the fact that the metabolite generated inside the hepatocytes does not need to transverse the sinusoidal membrane in order to gain access to the canalicular membrane. Co-perfusion with genistein decreased the biliary extraction ratio of GG (both hepatically generated and preformed), indicating that biliary excretion was impaired relative to sinusoidal efflux. This suggests that the isoflavone either decreased the bile canalicular transport and/or increased the net sinusoidal efflux of the glucuronide. This was confirmed by pharmacokinetic modelling of liver washout data, which showed that genistein decreased the bile canalicular transport rate constant of GG and increased the corresponding rate constant for sinusoidal efflux (Table 1).

It has been suggested previously that the bile canalicular transport of GG is mediated by cMOAT, as the biliary excretion of GG in the IPL is inhibited by co-perfusion with the cMOAT substrate dibromosulfophthalein (Sabordo et al 1999). Since co-perfusion with genistein has previously been shown to inhibit the cMOAT mediated transport of the glucuronide conjugates of rhodamine and bilirubin in the IPL (Jager et al 1997), a similar mechanism may be proposed for its effect on the bile canalicular transport of GG.

The ability of genistein to increase the sinusoidal efflux of GG (increase in k_{eff}) might be explained by direct stimulation of the sinusoidal efflux transporter of GG or by counter-transport of GG across the sinusoidal membrane. To the authors' knowledge, direct stimulation of the sinusoidal efflux of glucuronide conjugates from the liver has not previously been established. However, counter-transport of bilirubin diglucuronide with bilirubin has been reported in experiments with sinusoidal membrane vesicles prepared from hepatocytes (Adachi et al 1990). For counter-transport to explain the increase in sinusoidal efflux transport of GG during co-perfusion with genistein, GG within the hepatocyte must be exchanged with a compound that was either absent or at lower concentrations in the perfusate of control livers. Although genistein itself could be exchanged, it is also possible that the major hepatically generated metabolite of genistein, genistein glucuronide, could be exchanged, as this metabolite is also present in the outflow perfusate of livers perfused with genistein (Jager et al 1997). In either case, since the liver efficiently extracts both genistein and genistein glucuronide from portal blood (Sfakianos et al 1997), the potential exists that counter-transport of genistein or genistein glucuronide with intracellular GG could significantly increase the net sinusoidal efflux of GG in-vivo.

Conclusion

In summary, the present study found that genistein decreased the fraction of GG within hepatocytes undergoing transport into bile and increased its sinusoidal efflux into perfusate. The corresponding outcome in-vivo might be an increase in the circulating levels of a hepatically generated metabolite (or a reduction in the biliary excretion of that same metabolite) which, in the case of gemfibrozil, would increase systemic exposure to the potentially toxic metabolite GG (Sallustio & Foster 1995). Since comparable pharmacokinetic interactions could also be predicted for other drugs with similar hepatic disposition, this warrants further investigation.

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Appendix

For the purposes of gaining insight into the mechanism of the interaction between GG and genistein, the data from the loading–washout experiments were analysed using the pharmacokinetic model (Figure 1) described below.

The following assumptions were made:

GG was unbound in perfusate ($f_u = 1$). This assumption is supported by the use of albumin-free perfusate in single-pass mode.

Intracellular GG could be excreted into bile, appear in outflow perfusate, or be hydrolysed to gemfibrozil and did not undergo irreversible sequestration within the hepatocytes. This assumption is supported by previous observations (Sabordo et al 1999).

The vascular space and intracellular spaces of the liver behave as separate well-stirred compartments.

Once transported into the vascular space, GG was available for re-entry into hepatocytes. The assumption that GG can be taken up by hepatocytes is supported by previous IPL studies where the percentage of inflowing GG extracted by the liver was 30 to 65% (Sallustio et al 1996; Sabordo et al 1999, 2000).

Gemfibrozil in the liver could be metabolized to GG and other gemfibrozil metabolites. This assumption is supported by a previous IPL study with gemfibrozil, where even though GG was recovered in outflow perfusate and bile, the mass balance as gemfibrozil and GG was less than 100% (Sallustio et al 1996).

Whether preformed or hepatically generated, GG could be hydrolysed within the liver to gemfibrozil. This assumption is supported by previous IPL studies where gemfibrozil accumulated over time in the perfusate reservoir of livers perfused in recirculating mode with GG, while hydrolysis of GG was not observed in the same apparatus in the absence of a liver, or when GG was incubated with perfusate that had been previously perfused through a liver (Sallustio et al 1996).

Hepatically generated GG is subject to the same dispositional processes within the liver as preformed GG.

Linear pharmacokinetics for GG existed over the concentration range in the study. This assumption is based on the results of preliminary unpublished studies, where similar steady-state pharmacokinetic parameters were determined for livers perfused with 2 μM GG.

Based on these assumptions, a two-compartment venous equilibration model was proposed (Figure 1) and the following differential equations were derived to describe the rate of change in the amount of GG in the vascular space (GG_1) and liver (GG_2), and of gemfibrozil in the liver (G_2), over time:

$$\frac{dGG_1}{dt} = \text{GG infusion rate} + k_{eff} \cdot GG_2 - \left(k_{upt} + \frac{Q}{V_{vasc}}\right) \cdot GG_1 \quad \text{(A1)}$$

$$\frac{dGG_2}{dt} = k_{upt} \cdot GG_1 + k_{form} \cdot G_2 - (k_{hyd} + k_{eff} + k_{bile}) \cdot GG_2 \quad \text{(A2)}$$

$$\frac{dG_2}{dt} = k_{hyd} \cdot GG_2 - (k_{form} + k_{other}) \cdot G_2 \quad \text{(A3)}$$

The volume of the vascular space of the liver (V_{vasc}) has previously been reported to be 0.26 mL g^{-1} liver (Goresky et al 1992) and so the nominal volume of the vascular space was set at 2.5 mL. The perfusate flow rate (Q) was 30 mL min^{-1}.