Farnesol is an isoprenoid found in many aromatic plants and is also produced in humans, where it acts on numerous nuclear receptors and has received considerable attention due to its apparent anticancer properties. Although farnesol has been studied for over 30 years, its metabolism has not been well characterized. Recently, farnesol was shown to be metabolized by cytochromes P450 in rabbit; however, neither farnesol hydroxylation nor glucuronidation in humans have been reported to date. In the present paper, we show for the first time that farnesol is metabolized to farnesyl glucuronide, hydroxyfarnesol and hydroxyfarnesyl glucuronide by human tissue microsomes, and we identify the specific human UGTs (uridine diphosphoglucuronosyltransferases) involved. Farnesol metabolism was examined by a sensitive LC (liquid chromatography)–MS/MS method. Results indicate that farnesol is a good substrate for glucuronidation in human liver, kidney and intestine microsomes (values in nmol/min per mg).

**INTRODUCTION**

Farnesol (trans,trans-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) is a sesquiterpene/isoprenoid originally discovered and isolated from plants, although it is now produced synthetically. FPP (farnesyl pyrophosphate, doubly phosphorylated farnesol) is a key synthetic intermediate in the mevalonate pathway (Scheme 1) [1]. It is the last common intermediate for the synthesis of cholesterol, coenzyme Q and dolichol, and it is also used as the substrate for protein isoprenylation [2].

In recent years, interest in farnesol has focused on its anticancer properties, and farnesol has been shown to be an effective chemopreventive agent [3]. It has been tested on cancer cell lines [4–6], and, in addition to tests in vitro, dietary supplementation has been shown to be effective against pancreatic cancer growth [7]. It is postulated that these actions are mediated via a variety of signalling pathways, including links with PKC (protein kinase C)-dependent signal transduction in farnesol-induced apoptotic cell death [8]; farnesol acts on nuclear receptors PPAR (peroxisome-proliferator-activated receptor) γ and PPARε [9]; farnesol is also a substrate for the bile acid receptor (farnesoid X receptor) [10], and can activate CAR (constitutive androstane receptor) [11].

Apart from its potential use as an anticancer agent, farnesol has also generated interest because of its key role in the mevalonate/cholesterol synthesis pathway. Statins inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, the enzyme that synthesizes mevalonate (Scheme 1). Farnesol, in the form of FPP, is a product of this pathway, downstream of HMG-CoA, therefore endogenous FPP (and consequently farnesol) levels are also reduced by statins. In fact, some of the effects of statins can be reversed if farnesol is given [12], which indicates that farnesol is converted into FPP in vitro. In addition to this, farnesol is believed to be a non-sterol regulator for HMG-CoA reductase levels [13], although its effects might be indirect [14], and this means that farnesol could play an important role in regulating cholesterol levels.

Given these important physiological roles, a thorough understanding of farnesol metabolism is essential; however, to date, it has not been well characterized. Apart from the conversion of farnesol into FPP, the only known metabolic pathway in mammals is via CYP (cytochrome P450). Studies have indicated that, in rabbit, farnesol is both a substrate and an inhibitor for CYP [15]. In humans, it has been shown that farnesol can inhibit the metabolism of retinoic acid by CYP [16]. Neither the product(s) from this reaction nor the individual CYP isoforms that metabolize farnesol have been identified, nor have any other direct metabolic pathways.

To date no conjugative (Phase 2) metabolism of farnesol has been identified in any system. In the present paper, we report the kinetics of farnesol glucuronidation in human liver microsomes and identify the human UGTs (uridine diphosphoglucuronosyltransferases) that are responsible for its metabolism in vitro. In addition, we show that farnesol can be hydroxylated and that this hydroxyfarnesol metabolite can also be glucuronidated.
Acetyl-CoA → HMG-CoA → Farnesyl-CoA → Farnesol → Cholesterol

**Scheme 1** The mevalonate synthesis pathway showing the role of farnesol

Farnesol originates from three sources: (i) synthesis of FPP via the mevalonate pathway, followed by action by FPP phosphatase, (ii) degradation of prenylated proteins (although this has not been proved experimentally), or (iii) external sources, such as dietary or pharmaceutical. The broken arrow indicates the feedback role that farnesol has in controlling HMG-CoA reductase levels. Farnesol can be metabolized to farnesyl phosphate and then to FPP, to hydroxyfarnesol, farnesol or to the farnesyl glucuronide. F, farnesol; FP, farnesyl phosphate; P-450, unknown CYP; ADH, alcohol dehydrogenase.

**EXPERIMENTAL**

**Chemicals and reagents**

2-Aminobiphenyl, DMSO, hydoxoycholic acid, 4-methyl umbelliferone, morphine, 3-β-D-glucuronide, 1-naphthol, octylgallate, propofol, UDPGA (β-D-uridine diphosphoglucuronic acid) and farnesol were purchased from Sigma-Aldrich (Gillingham, Dorset, U.K.). [Glucuronyl,14C(U)]-UDPGA was purchased from PerkinElmer (Beaconsfield, Bucks., U.K.). Methanoic (formic) acid and acetonitrile (HPLC grade) and all other chemicals (highest grade available) were purchased from Merck Eurolab (Poole, Dorset, U.K.).

**Sample preparation**

Microsomes were prepared from frozen human tissue, obtained as anonymized surgical or donor surplus, by standard methods [17]. Detailed information on donors was not available. Ethical approval for use of human tissue was obtained from the Tayside Committee on Medical Research Ethics. Cell lines expressing other human UGTs have been created previously in this laboratory [18–21], except UGT1A4 [kindly donated by Dr Robert Tukey (La Jolla, CA, U.S.A.)]. Frozen cell pellets (originally from two anonymized surgical or donor surplus, by standard methods [17]) were thawed and centrifuged at 14,000 g for 5 min. The supernatant was removed and stored at −20 °C until analysis for the presence of farnesyl glucuronide; farnesyl glucuronide was stable at −20 °C (results not shown).

**Sample analysis by LC (liquid chromatography)–MS/MS**

Samples were diluted 10-fold in 50% (v/v) acetonitrile/water before analysis by LC–MS/MS using a HP1100 LC system (Agilent Technologies, Stockport, U.K.), connected to a Micromass LC Quattro (Micromass, Manchester, U.K.) with a 10 µl injection per run. The LC separation used a mobile phase of 0.1% methanoic acid (buffer A), and acetonitrile containing 0.1% methanoic acid (buffer B). LC separation and elution were achieved using a 1 min isocratic portion at 80% buffer A followed by a short step to 50% buffer A and a linear gradient of 50–95% buffer B over 2 min. This was followed by a 4 min wash at 95% buffer B and a 3 min re-equilibration step at 80% buffer A. Chromatography was performed on a Waters Spherisorb (OADS2) 2 µm, 2.1 mm × 150 mm column at a flow rate of 0.3 ml/min with a 2 cm Hypersil (OADS2) guard column.

Mass spectral analysis was performed with direct infusion into the electrospray source, with column diversion during the first 3 min to protect the source from excessive salt. The glucuronide peak from the LC column was analysed using an MRM (multiple reaction monitoring) method in negative-ion mode. Optimized MRM conditions used the transition from 397.3 → 112.8 m/z at a cone voltage of 30 eV and capillary voltage of 3.0 eV. Collision energy was 20 eV and the collision gas was at 3 mbar (300 Pa). The nebulizing gas was set at 110 l/h, and the desolvation gas at 700 l/h.

To quantitate the glucuronide produced, two identical farnesyl glucuronide standards, one radio-labelled and one non-radio-labelled, were generated by parallel incubation of farnesol with human liver microsomes as described above (performed in triplicate). The amount of farnesyl glucuronide produced was calculated by analysis of the [14C]farnesyl glucuronide using a HPLC radiochemical detection assay (see below). As the non-radio-labelled samples were generated in parallel, they contained the same amount of farnesyl glucuronide as the calculated value for the radio-labelled samples. The non-radioactive standard was then used in conjunction with all analyses to quantify the amount of farnesyl glucuronide present.

**[14C]UDPGA assay**

The method used was based on that described by Ethell et al. [23]. Incubated samples were prepared as before, except the UDPGA stock solution contained 0.2 µCi of [14C]UDPGA. Sample (150 µl) was injected onto a 150 mm × 4.6 mm Techsphere ODS2 column (HPLC Technology, Welwyn Garden City, U.K.) connected to a Gilson binary injection pump with mixer. Elution from the column was achieved with a gradient from 95% buffer A (10 mM ammonium acetate, pH 6.5) to 95% buffer B (acetonitrile) over 15 min. Detection of the [14C]UDPGA was achieved using a Reeve 9710 radioactivity monitor (Reeve Analytical) fitted with a 200 µl heterogeneous cerium-activated lithium glass flow cell. Elution of UDPGA was at 2.4 min, and the farnesyl glucuronide was eluted at 8.5 min.
Kinetic parameters for farnesyl glucuronide formation were determined in duplicate using the standard assay above, with variations in the concentration of farnesol (final concentrations were 0, 5, 10, 20, 50, 100, 200, 350, 500, 750, 1000, 5000 and 10000 µM). For human intestine microsomes and cell lines expressing UGT2B7, additional kinetic data were generated as the \( K_m \) was below 5 µM. Assays were performed as above and with an additional set of low farnesol concentrations (0, 0.1, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 5 and 10 µM). Owing to substrate inhibition, standard Michaelis–Menten curves could not be used to calculate all the kinetic parameters. In these cases, \( K_m \) and \( V_{\text{max}} \) were determined using a modified Michaelis–Menten equation by nonlinear least-squares regression (Kaleidagraph, Synergy Software, Reading, PA, U.S.A.) [24]:

\[
\text{Rate} = \frac{V_{\text{max}}}{1 + (K_m/S) + (S/K_i)}
\]

where \( K_i \) is the self-inhibition constant. When the \( K_m \) was >10 mM, the standard Michaelis–Menten equation was used to calculate \( K_m \) and \( V_{\text{max}} \).

To confirm the nature of the kinetics in the human tissue microsomes, where multiple enzymes may be operating, Eadie–Hofstee plots were generated and the multiple \( K_m \) values were calculated.

### Inhibition of farnesol glucuronidation by UGT probe substrates

To investigate further the role of certain UGT isoforms in the glucuronidation of farnesol, a competitive inhibition assay was performed with known human UGT probe substrates [25]. Octylglucate was used for UGT1A1, 2-aminohiphenol for UGTs 1A3 and 1A4, 1-naphthol for UGT1A6, propofol for UGT1A9, and morphine for UGT2B7 and 2B4. The farnesol concentration was fixed at 50 µM, and the other compounds used were at concentrations of 0, 20 µM, 200 µM and 2 mM. Assays were performed for 60 min with human liver microsomes using the same conditions as for the kinetic experiments described above.

To examine the effects of NADPH, farnesol glucuronidation levels were measured as described above, with or without 5 mM UDPGA, and with or without 10 mM NADPH.

### Detection of the novel products formed during farnesol incubation with NADPH and UDPGA

To determine whether human liver microsomes could produce any products of oxidative metabolism from farnesol, the standard assay reaction was carried out with NADPH alone or with NADPH and UDPGA together in the same reaction mixture, and the resulting incubation mixtures were analysed using LC–MS. The MS was set to scan in negative-ion mode over a mass range of 200–600 m/z and a scan speed of 2 s/spectrum. The spectrum was generated with a cone voltage of 40 eV and a capillary voltage of 3.0 eV. The desolvation and source block temperatures were 250 °C and 80 °C respectively. The nebulizing gas was applied at a flow rate of 100 l/h, the desolvation gas at 500 l/h. The LC trace was then scanned for any likely metabolites. A daughter ion scan was then performed on these peaks at a mass of 237.3 for monohydroxylated farnesol products and at 413.4 for glucuronide(s) of hydroxyfarnesol. The scan was performed in negative-ion mode over a mass range from 100 to the mass of the main ion, at a speed of 2 s/spectrum. The spectrum was generated with a cone voltage of 40 eV, a capillary voltage of 3.0 eV and with varying collision energy (optimized to give the biggest peak). The collision gas was at 3 mbar, and the desolvation and source block temperatures were 250 °C and 80 °C respectively. Control reaction mixtures were also analysed in the same way.

### RESULTS

#### LC–MS assay

A very effective LC–MS assay was developed to detect farnesyl glucuronide produced from UDP-expressing cell lines or human tissue microsomes (Figure 1). The limit of detection of this method was calculated as 30 fmol, which translates to a minimum detectable UGT reaction rate of 4 fmol/min per mg (for a standard 1 h assay, with 250 µg of protein). This is 2000 times more sensitive than the best radioactive LC assay used routinely to detect glucuronides [23].

#### Confirmation of farnesyl glucuronide

The structure of farnesol and the predicted structure of the glucuronide are shown in Figure 2. Theoretically, farnesol can form only one glucuronide, through the farnesol alcohol moiety. The mass spectrum of the farnesyl glucuronide is shown in Figure 3(a). The fragmentation peaks are consistent with the breakdown of a glucuronide with characteristic peaks at m/z 113, 157 and 175, which is common for glucuronide fragments in negative-ion mode electrospray [26,27]. No farnesol aglycone ion peak was observed.
in the fragmentation (which is expected in negative-ion mode), but one of the peaks can be attributed to a farnesyl glucuronide fragment (m/z 279). No peak was observed when control experiments were performed without UDPGA, microsomes or farnesol. Farnesyl glucuronide was stable at 4 °C (for 24 h) and at −20 °C (for 1 month).

Table 1 Table of kinetic data parameters of farnesol glucuronidation

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Glucuronidation of farnesol by human microsomes and expressed human UGTs

Table 1 shows the formation of farnesyl glucuronide by different human UGT isoforms and human tissue microsomes. It is clear from these data that UGT2B7 and UGT2B4 have the highest rate,

Figure 3 Mass spectra of farnesyl β-D-glucuronide and hydroxyfarnesyl β-D-glucuronide

(A) Mass spectrum of farnesyl β-D-glucuronide was run from 100 to 400 m/z in negative-ion mode; the spectrum was derived from a daughter ion scan at the farnesyl glucuronide mass (m/z 397), from an incubation of farnesol with human liver microsomes and UDPGA. The inset shows the proposed fragmentation of farnesyl β-D-glucuronide with main fragment masses. Masses at 175, 157 and 113 m/z are common fragments of glucuronide. No peak was observed for a farnesol aglycone fragment (221 m/z). (B) Mass spectrum of hydroxyfarnesyl β-D-glucuronide. Main peaks observed at 193, 175 and 113 m/z are indicative of the glucuronide, and the peak at 237 is the hydroxyfarnesol aglycone. As in the case of the farnesyl β-D-glucuronide fragmentation, no peak was observed for the farnesol moiety.
Glucuronidation of farnesol by human tissues and UGTs

Kinetics of farnesol glucuronidation in human microsomes and expressed human UGT isoforms

Examples of the rate against substrate concentration curves for the glucuronidation of farnesol in each different system are shown in Figures 4(A)–4(E). The kinetic parameters $K_m$ and $V_{max}$ for the three human tissue types and UGT isoforms tested were calculated from these data and are presented in Table 1.

Substrate inhibition

Human kidney and intestine microsomes and recombinant UGTs 2B4 and 2B7 all exhibited substrate inhibition with farnesol. These two tissues and UGTs were analysed by a modified Michaelis–Menten equation as described in the Experimental section. The calculated inhibition constants are shown in Table 1. The $K_i$ indicates that even submillimolar amounts of farnesol are capable of significantly inhibiting UGT2B7. Intestine microsomes exhibited an identical substrate inhibition profile with that of UGT2B7.

Contribution of each isoform to overall glucuronidation

The $K_m$ for farnesol glucuronidation measured with human liver microsomes was significantly different from that of any one individual recombinant UGT isoform, indicating that there is a variety of isoforms in liver with the potential to glucuronidate farnesol. However, the $K_m$ in liver is higher than the $K_m$’s for UGTs

followed by UGTs 1A1 and 1A9. With the exception of UGT2B4, these isoforms are the major xenobiotic-metabolizing UGTs [28].
A. G. Staines and others

Figure 5  Eadie–Hofstee plots of farnesol glucuronidation

Farnesol glucuronidation was carried out by (A) human liver microsomes, (B) human kidney microsomes, (C) human intestine microsomes or (D) human UGT 2B7. The $K_m$ values for the different lines are indicated.

1A9, 2B4 and 2B7, suggesting a significant contribution from UGT1A1, which is the only significant isoform with a higher $K_m$. In the case of kidney microsomes, the $K_m$ for farnesol glucuronidation (very close to the $K_m$ of recombinant UGT1A9) indicates that UGT1A1, which has a significantly higher $K_m$, is unlikely to be involved; there is some slight substrate inhibition, which would suggest a contribution from UGT2B7 or UGT2B4. It has been shown previously that there is no UGT1A1 and high levels of UGT1A9 in human kidney [29,30]. In the case of intestine, the kinetic profile supports a view that UGT2B7 is the major contributor to the glucuronidation of farnesol. The extent of substrate inhibition is important in reaching this conclusion: since UGT2B7 is the only UGT isoform that exhibits significant substrate inhibition, any inhibition in tissue microsomes is likely to indicate a significant role for UGT2B7. This therefore confirms the kinetic data, as intestine exhibits high levels of UGT2B7 (substrate inhibition), kidney exhibits medium levels (slight inhibition) and the liver used in the present study shows no major contribution of UGT2B7, i.e. no substrate inhibition. This is also consistent with previous observations of high UGT2B7 mRNA levels in human intestine [31]. Previous indications that there are high levels of UGT1A1 in intestine are based on the substrate oestradiol [32], which has been shown recently to be a substrate for UGTs 1A8 and 1A10 and 2B7 (BD Bioscience, San Jose, CA, U.S.A.; http://www.bdbiosciences.com/discovery_labware/gentest/), which are also present in intestine [33]. Therefore the data would suggest that, in this case, UGT2B7 is the dominant isoform in intestine and not UGT1A1. Knowledge of the tissue distribution of UGT2B4 is limited, therefore a contribution to glucuronidation in any of these tissues cannot be ruled out.

Eadie–Hofstee plots of data from the kinetic experiments (Figures 5A–5D) also demonstrate that multiple isoforms contribute to farnesol glucuronidation in liver and kidney microsomes. In these samples, at least two distinct activities can be observed, suggesting that at least two UGT isoforms glucuronidate farnesol. In the case of intestine microsomes, the substrate inhibition indicates that a UGT with a low-micromolar $K_m$ for farnesol is the major isoform, and the plot for intestine microsomes is identical with that of UGT2B7.

Inhibition of farnesol metabolism in human liver microsomes

The $K_m$ for farnesol in human liver microsomes is 49.5 $\mu$M, and a concentration of 50 $\mu$M was chosen for this experiment. Each of the probe substrates used is generally accepted to identify a specific UGT isoform, although they do exhibit some cross-reactivity for other isoforms. Only octylgallate was found to inhibit farnesol glucuronidation significantly in liver microsomes (85% inhibition at octylgallate concentration of 2000 $\mu$M). Octylgallate is predominantly metabolized by human UGT1A1, with some activity for human UGT1A9. Propofol is a selective UGT1A9 substrate, and this showed no inhibition of farnesol glucuronidation. Therefore the inhibition by octylgallate is consistent with our finding that UGT1A1 is the dominant isoform for farnesol glucuronidation in liver.

Oxidative metabolism of farnesol

It is known that farnesol can be metabolized to hydroxyfarnesol in rabbit [15], a reaction almost certainly catalysed by a CYP. In the present study, we wished to determine whether farnesol can be
metabolized to hydroxyfarnesol in human tissue and to examine the effect of this reaction on glucuronidation.

Incubation in the presence of NADPH reduced the level of glucuronidation by almost 50%, suggesting that another pathway of farnesol metabolism is operating under these conditions. To confirm that the reduced farnesol glucuronidation in the presence of NADPH was due to the formation of a farnesol CYP metabolite, LC–MS analysis of the products generated following incubation of farnesol with NADPH was carried out. This indicated the formation of a metabolite of the same mass as hydroxylated farnesol, although we could not identify the location of the hydroxy group.

Since farnesol is a good substrate for glucuronidation, it was envisaged that this hydroxylated farnesol might also be a substrate for UGTs. LC–MS analysis of the products from the reaction containing both NADPH and UDPGA incubated with liver microsomes did indeed reveal a metabolite of mass consistent with a hydroxyfarnesyl glucuronide (Figure 3b). This metabolite had similar fragmentation characteristics to farnesyl glucuronide (Figure 3a), with the additional mass corresponding to hydroxyfarnesol. Since this metabolite was found when both UDPGA and NADPH are present, the glucuronidation of hydroxyfarnesol must be able to compete successfully with farnesol glucuronidation itself. Owing to the lack of availability of hydroxylated farnesol, it was not possible to examine the production of the hydroxyfarnesyl glucuronide in more detail.

DISCUSSION

In the present study, we have developed an extremely sensitive assay for farnesol glucuronidation. Detection of the farnesyl glucuronide is 2000-fold more sensitive than that achievable with the next most sensitive technique, use of radiolabelled UDPGA [23]. Although this level of sensitivity is not necessary for the detection of farnesol glucuronidation in liver microsomes, it allowed detection of the lower levels found with some of the minor UGT isoforms and more accurate determination of kinetic data at lower substrate concentrations.

It is important to note that due to significant substrate inhibition with some isoforms, rate measurements do not provide an accurate comparison of the enzyme specificities, therefore complete kinetic analysis must be made. The $K_m$ values for recombinant UGT2B7 and for human intestine microsomes are some of the lowest reported for UGT substrates, especially UGT2B7 (usually between 50 and 500 $\mu$M) [34], with only retinoic acid (1.3 $\mu$M) [35] and androsterone (0.7 $\mu$M) [36] being lower. Without a reliable method for determining UGT protein expression levels in each UGT-expressing cell line, it is impossible to compare the $V_{max}$ values for farnesol glucuronidation accurately between these cell lines. However, comparison with standard substrates would indicate that farnesol is a better substrate for UGT2B7 than morphine, codeine or AZT (azidothymidine), which are often used as probe substrates for this UGT [37], and better for UGT2B4 than androsterone and morphine [37,38]. The UGT1A1 family isoforms all exhibited much lower (10–100-fold) $V_{max}$ values for farnesol than for their prototypical substrates. UGT1A1 (the UGT that metabolizes bilirubin) had the highest overall rate of farnesol glucuronidation for the 1A family, but this was still 10-fold lower than for octylgallate, one of the best UGT1A1 substrates [39].

The low $K_m$ and high $V_{max}$ values obtained for farnesol suggest that farnesol clearance in vivo might be rapid. This has important implications with regard to its effect on nuclear receptors, where rapid detoxification is a potential mechanism for modulation of the effects of farnesol via these pathways. We have demonstrated in the present study that glucuronidation is potentially an important regulator of the levels of farnesol.

The in vitro studies of the present paper have demonstrated three routes of glucuronidation of farnesol. One pathway would be through the liver, presumably largely via UGT1A1. Some metabolism could occur in the kidney via different isoforms, but probably UGT2B7 is the dominant isoform. There is also a significant potential metabolic route through the intestine, where UGT2B7 would most likely be the main isoform. Recent studies on the effects of farnesol on lung carcinoma suggested using farnesol via a direct inhalation method [40]. Lung tissue has low levels of UGTs, and may only express UGT1A7 and UGT2B11 [41], which were not available for testing in the present study, although the majority of orally inhaled drugs enter the gastro-intestinal tract, where metabolism by UGT2B7 could become significant.

Co-administered drugs are unlikely to have a significant influence on farnesol glucuronidation, because farnesol is predicted to be glucuronidated in multiple organs with high activity. Few compounds are metabolized exclusively by UGT1A1 and UGT2B7; however, one exception is buprenorphine, which is mainly metabolized by these two isoforms [42,43]. The $K_m$ values for farnesol glucuronidation by specific UGT isoforms are low, however, especially for UGT2B7, and it is therefore possible that farnesol would competitively inhibit metabolism of drugs that are extensively glucuronidated, especially by UGT2B7, including common drugs, such as morphine, other opiates and some oestrogens [42]. From the kinetic and inhibition data presented, hepatic metabolism of farnesol is likely to involve predominantly UGT1A1, the enzyme responsible for bilirubin glucuronidation. This has potential toxicological implications, particularly since a common UGT1A1 promoter polymorphism is responsible for Gilbert’s syndrome, a mild hyperbilirubinaemia present in approx. 15% of Caucasians. Gilbert’s individuals have impaired UGT1A1 metabolism, which may affect farnesol glucuronidation, and therefore regulation of its function. Unlike UGT1A1, there are currently no known polymorphisms of UGT2B7 that have any significant effect on substrate specificity.

Since no data are available on the in vivo metabolism of farnesol, it was important to confirm that glucuronidation could occur in competition with other metabolic pathways. It has only been reported recently that farnesol can be metabolized by CYP [15]. The evidence presented here indicates for the first time that glucuronidation and hydroxylation of farnesol can occur simultaneously in human liver microsomes.

Farnesol can also be converted into farnesyl phosphate and FPP. It is not clear whether this is a recovery pathway for the disruption of the mevalonate pathway or a compensation for the FPP phosphatase action [44]. Further investigation would be required to see how this enzyme competes with glucuronidation and hydroxylation pathways. However, it should be noted that administration of farnesol does not result in a large increase in mevalonate pathway catabolites. This would suggest that conversion of farnesol into FPP is not a fast process in vivo, which should be compared with the predicted high clearance rate for farnesol glucuronidation.

Farnesol has both structural and functional similarities to retinoic acid. Retinoic acid, like farnesol, is abundant in the diet, and also acts on certain nuclear receptors. Structurally, they both contain a nucleophilic oxygen at the end of a long methylated alkene chain, retinoic acid having a hexene ring at the terminal end of the chain. It has been shown that farnesol can interfere with retinoic acid hydroxylation [45], suggesting that they may both be hydroxylated by the same CYP (CYP26). Retinoic acid and its hydroxy metabolites have been shown to be glucuronidated,
like farnesol, primarily by UGT2B7 [46]; our data support these authors’ hypothesis that UGT2B7 regulates the activity of these cellular signalling molecules.

One additional point of note is the extensive substrate inhibition observed with farnesol. This substrate inhibition would appear to be specific to the 2B family, and human UGT2B7 in particular. This suggests that farnesol could be a potential starting point for the design of specific UGT2B family inhibitors.

In the present study, we have demonstrated that farnesol is a substrate for human UGTs and CYP in vitro, and is metabolized to farnesyl glucuronide and hydroxylfarnesol respectively; glucuronidation can compete with CYP metabolism, and, in addition, the hydroxylfarnesol metabolite from this reaction is also glucuronidated. From kinetic and inhibition data, the glucuronidation reaction is catalysed mainly by UGT1A1 in liver and UGT2B7 in intestine. In the case of UGT2B7, farnesol has a low-micromolar $K_m$, indicating that it is an excellent UGT2B7 substrate. Glucuronidation may therefore represent a novel pathway for modulating the action of this potent signalling molecule.

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