Role of UDP-glucuronosyltransferase isoforms in 13-cis retinoic acid metabolism in humans

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Running title page

Running title: Role of UGT isoforms in 13cisRA metabolism in humans

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Abbreviations: UGT, uridine diphosphoglucuronosyltransferases; 13cisRA, 13-cis retinoic acid; ATRA, all-trans retinoic acid; 9cisRA, 9-cis retinoic acid; CYP, cytochrome P450; HPLC, High performance liquid chromatography; HLM, human liver microsomes; HIM, human intestinal microsomes.
Abstract

13-cis Retinoic acid (13cisRA, isotretinoin) is an important drug in both dermatology and the treatment of high-risk neuroblastoma. 13cisRA is known to undergo cytochrome P450-mediated oxidation, mainly by CYP2C8, but phase II metabolic pathways have not been characterized. In the present study, the glucuronidation activities of human liver and intestinal microsomes (HLM and HIM), as well as a panel of human UDP-glucuronosyltransferases (UGTs) towards both 13cisRA and the 4-oxo metabolite, 4-oxo 13cisRA, were compared using high-performance liquid chromatography. Both HLM and, to a greater extent, HIM catalysed the glucuronidation 13cisRA and 4-oxo 13cisRA. Based on the structures of 13cisRA and 4-oxo 13cisRA, the glucuronides formed are conjugated at the terminal carboxylic acid. Further analysis revealed UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 were the major isoforms responsible for the glucuronidation of both substrates. For 13cisRA, a pronounced substrate inhibition was observed with individual UGTs and with HIM. UGT1A3 exhibited the highest rate of activity towards both substrates and a high rate of activity towards 13cisRA glucuronidation was also observed with UGT1A7. However, for both substrates, $K_m$ values were above concentrations reported in clinical studies. Therefore, UGT1A9 is likely to be the most important enzyme in the glucuronidation of both substrates as this enzyme had the lowest $K_m$ and is expressed in both the intestine and at high levels in the liver.
Introduction

Retinoids are derivatives of retinol (vitamin A) that regulate numerous biological processes, including embryogenesis, growth, differentiation, vision and reproduction (Evans and Kaye, 1999). Retinoic acid is the main biologically-active derivative of retinol and exists in several stereoisomeric forms, including all-trans retinoic acid (ATRA), 13-cis retinoic acid (13cisRA) and 9-cis retinoic acid (9cisRA). Retinoids exhibit several pharmacological properties useful in dermatology (Peck and DiGiovanna, 1994), as well as in cancer prevention and chemotherapy (Hong and Itri, 1994). The most dramatic clinical benefits with retinoids are observed with ATRA for the treatment of acute promyelocytic leukaemia and 13cisRA in the treatment of high-risk neuroblastoma (Reynolds and Lemons, 2001; Matthay et al., 2009).

The oxidative metabolism of 13cisRA has been extensively examined and involves the cytochrome P450 (CYP) enzyme system, a superfamily of haem-containing monooxygenases that have a central role in the oxidative metabolism of a variety of endogenous and exogenous compounds. As a result of CYP-mediated metabolism, intermediates that may exert toxicity or carcinogenicity are formed. Such intermediates are also targets for phase II drug metabolism which renders them inactive, with the polar products subsequently excreted via the kidneys. Several CYPs can catalyze the oxidation of 13cisRA, including CYPs 3A7, 2C8, 4A11, 1B1, 2B6, 2C9, 2C19 and 3A4 (Chen et al., 2000; Marill et al., 2002). The major metabolites formed following CYP-mediated oxidation, are 4-hydroxy 13cisRA and 4-oxo 13cisRA.

ATRA and its oxidised metabolites undergo glucuronidation catalyzed by uridine diphosphoglucuronosyltransferases (UGTs) in animals and humans. The UGTs catalyse the
transfer of the glucuronic acid moiety from the co-factor UDP-glucuronic acid to the substrate, resulting in a metabolite with greater polarity and water solubility. The human UGTs 1A8 and 2B7 have been identified as responsible for the glucuronidation of ATRA, 4-oxo ATRA, 4-hydroxy ATRA and 5, 6-epoxy ATRA (Cheng et al., 1999; Czernik et al., 2000; Samokyszyn et al., 2000). Analysis of the glucuronidation products revealed that ATRA, 4-oxo ATRA and 5,6-epoxy ATRA formed carboxyl-linked glucuronides, whereas 4-hydroxy ATRA was glucuronidated at its hydroxyl group (Samokyszyn et al., 2000). In view of its strong homology with ATRA, it is probable that 13\textit{cis}RA also undergoes glucuronidation.

To date, 21 functional UGT isoforms have been identified in humans (www.ugtalleles.ulaval.ca), many of which catalyze the glucuronidation of a wide variety of environmental carcinogens, dietary chemopreventatives, and anticancer agents (Nagar and Remmel, 2006). However, no studies examining the glucuronidation of 13\textit{cis}RA have as yet been reported. In the present study the activities and kinetics of human liver and intestinal microsomes as well as a panel of recombinant human UGTs were determined, to identify those likely to be responsible for 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronidation in humans.
13-cis Retinoic acid, all-trans retinoic acid and β-glucuronidase (from *Helix pomatia*) were purchased from Sigma-Aldrich (Poole, UK). 4-Oxo 13-cis retinoic acid (Ro 22-6595) and 4-oxo all-trans retinoic acid (Ro 12-4824) were generously provided by Hoffmann-La Roche (Basel, Switzerland). UGT Reaction Mix Solution A (25 mM Uridine 5′-diphospho-glucuronic acid) and UGT Reaction Mix Solution B (250 mM Tris·HCl, 40 mM MgCl₂, 0.125 mg/ml alamethicin) were purchased from BD Gentest (Oxford, UK). Pooled human liver and intestinal microsomes as well as Supersomes™ expressing the recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17 enzymes were also purchased from BD Gentest. Control microsomes with wild-type baculovirus were also obtained. HPLC grade acetonitrile and glacial acetic acid were obtained from Fisher Scientific (Loughborough, UK).

**Characterization of 13cisRA and 4-oxo 13cisRA glucuronidation**

Glucuronidation activity was determined in pooled human liver microsomes (HLM), human intestinal microsomes (HIM) and Supersomes™ containing different cDNA expressed UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17). Conditions for linearity with respect to time and protein concentration were optimized in preliminary studies. The incubation mixture consisted of enzyme fractions (0.5 mg/ml total protein), 13cisRA or 4-oxo 13cisRA (100 µM), alamethicin (25 µg/ml), MgCl₂ (8 mM), UDPGA (2 mM), methanol (0.25%) made up to a final volume (200 µl) with Tris-HCl buffer (50 mM, pH 7.5). Incubations were carried out in a shaking water bath at 37°C for 60 min. Reactions were initiated by the addition of enzyme and terminated with ice-cold acetonitrile (400 µl).
Incubations conducted with control Supersomes™ served as a negative control. Samples were vortex mixed and centrifuged at 17,900 g for 5 min. Supernatant (200 µl) was removed and evaporated to dryness and the residue reconstituted in 200 µl 0.1% glacial acetic acid pH 5.0, prior to HPLC analysis. All incubations were performed in triplicate.

**Hydrolysis of 13cisRA and 4-oxo 13cisRA glucuronides**

To samples prepared from the incubations as described above, β-glucuronidase (80 units) in potassium phosphate buffer (75 mM, pH 6.8) was added. The mixture was incubated in a shaking water bath at 37°C for 30 min. Reactions were terminated by the addition of ice-cold acetonitrile (400 µl). Control incubations were prepared under the same conditions, but with the addition of β-glucuronidase after the acetonitrile. Samples were vortex mixed and centrifuged at 17,900 g for 5 min. Supernatant (200 µl) was removed and evaporated to dryness. Residue was reconstituted in 200 µl 0.1% glacial acetic acid, pH 5.0, prior to HPLC analysis. All reactions were performed in triplicate.

**HPLC analysis**

Analysis of 13cisRA and 13cisRA glucuronide was carried out using a Waters 2695 Separations Module and a 996 Photodiode Array UV Detector with Empower 2 software for data acquisition and analysis (Waters Corp., Milford, MA, USA). The UV detector was set to monitor 200 to 400 nm, isolating at 374 nm. Separation was performed on a Luna C18 (2) column (50 x 2.0 mm, 3 µm) (Phenomenex, Torrance, CA, USA) by gradient reversed-phase chromatography. The mobile phase consisted of 0.1% glacial acetic acid, pH 5.0 (A) and 100% acetonitrile (B). Analysis was performed at a constant flow rate of 0.2 ml/min and an injection volume of 20 µl and the gradient ran from 60% A to 60% B between 0 and 2 min. It remained at 60% B for 2 min and then switched back to 60% A between 4 and 5 min,
remaining at 60% A for 10 min before switching to 100% B between 15 and 16 min. It remained at 100% B for 10 min and finally switched back to 60% A between 26 and 30 min, remaining at 60% A until 40 min. Analysis of 4-oxo 13cisRA and 4-oxo 13cisRA glucuronide was carried out as described above, but with an alternative gradient, running from 60% A to 60% B between 0 and 2 min and remaining at 60% B for 2 min before switching back to 60% A between 4 and 5 min, where it remained until 20 min. The 13cisRA and 4-oxo 13cisRA glucuronide peaks were identified by treating the metabolites with β-glucuronidase, which hydrolyses the glucuronide back to the aglycone. Analysis of 13cisRA and 4-oxo 13cisRA glucuronides was considered to be semi-quantitative, as authentic standards were not available.

**Determination of kinetic parameters for 13cisRA and 4-oxo 13cisRA glucuronide formation**

Kinetic parameters for 13cisRA and 4-oxo 13cisRA glucuronide formation were determined using HLM and HIM, as well as UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 Supersomes™. All incubations were carried out as described above. 13cisRA concentrations ranged from 0 – 250 µM and 4-oxo 13cisRA concentrations ranged from 0 – 100 µM. There was an upper limit on the 4-oxo 13cisRA due to the concentration of the stock solution in ethanol. Substrate concentration and velocity data for 13cisRA and 4-oxo 13cisRA glucuronidation were fitted by the hyperbolic Michaelis-Menten model (eq. 1), for data exhibiting atypical kinetics, a substrate inhibition model was fitted (eq. 2)

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

\[
(2) \ \nu = \frac{V_{\text{max}}[S]}{(K_m + [S])(1 + [S]/K_i)}
\]
where \( v \) is the rate of reaction; \( V_{\text{max}} \) is the maximum velocity estimate; \( [S] \) is the substrate concentration; \( K_m \) is the Michaelis-Menten constant; and \( K_i \) is the substrate inhibition constant. Units of \( K_m \) and \( K_i \) are given in \( \mu \text{M} \). As authentic standards were unavailable for the 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronides, \( V_{\text{max}} \) units were given as the peak area generated, as determined by HPLC analysis, in \( \mu \text{V} \text{sec} \text{ per sec} = (\mu \text{V} \text{s})/\text{s} \). Non-linear regression was performed with GraphPad Prism software (GraphPad Software Inc., San Diego, CA).
Results

Identification of 13cisRA and 4-oxo 13cisRA glucuronides

Incubation of 13cisRA with HLM, HIM, recombinant UGT isoforms and UDPGA resulted in the appearance of a novel chromatographic peak that was visible using a HPLC UV method previously optimized for the detection of 13cisRA (Figure 1A). The glucuronide peak had a shorter retention time (6.2 min) compared to 13cisRA (23.1 min), and the rate of generation of this peak was dependent on microsomal protein concentration and incubation time.

Following incubation of 4-oxo 13cisRA with HLM, HIM, recombinant UGT isoforms and UDPGA, another novel chromatographic peak was visible using a HPLC UV method which had previously been optimized for the detection of 4-oxo 13cisRA (Figure 1C). An additional substrate peak was also observed. This was identified as 4-oxo ATRA and was formed as a result of minor isomerisation of 4-oxo 13cisRA. The glucuronide peak retention time was 2.4 min, compared to 7.9 min for 4-oxo 13cisRA and 7.2 min for 4-oxo ATRA, again the peak area increased with microsomal protein and incubation time. Treatment of both incubates with β-glucuronidase resulted in complete disappearance of the metabolite peaks, indicating that the metabolites are glucuronide conjugates of 13cisRA and 4-oxo 13cisRA, respectively (Figures 1B and 1D).

Characterization of 13cisRA and 4-oxo 13cisRA glucuronidation

Screening of 13cisRA and 4-oxo 13cisRA glucuronosyltransferase activity was performed using HLM and HIM, as well as 12 recombinant UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17). HLM, HIM, UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 were found to catalyze both 13cisRA and 4-oxo 13cisRA glucuronide formation (Figure 2). UGT1A4, UGT1A6, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17
exhibited no detectable catalytic activity towards either 13\textit{cis}RA or 4-oxo 13\textit{cis}RA under the incubation conditions employed.

**Determination of kinetic parameters for 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronide formation**

Kinetic analyses of 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronide formation were performed using HLM, HIM and recombinant UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 isoforms. Both 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronides were formed in varying amounts and rates across all UGT sources studied. HIM, which comprises a pool of more than one UGT enzyme, had the highest rate of glucuronidation towards both substrates. The formation of the 13\textit{cis}RA glucuronide exhibited atypical kinetics across all UGT sources (Figure 3 and Table 1), except HLM where data were fitted by the hyperbolic Michaelis-Menten model. The $V_{\text{max}}$ and $K_{\text{m}}$ for HLM were 59±17 µV*s/s and 974±342 µM, respectively. For HIM and recombinant UGT isoforms, data were fitted by the substrate inhibition model. Although the model provided a reasonable fit to the data, estimation of the kinetic parameters was possible only for HIM, with $V_{\text{max}}$, $K_{\text{m}}$ and $K_{i}$ values of 1116±804 µV*s/s, 200±98 µM and 18±9 µM, respectively. Estimates of $K_{\text{m}}$ and $K_{i}$ for the individual UGTs were highly correlated and ranged from 14 µM for UGT1A9 to 186 µM for UGT1A7. For reference, reported plasma concentrations of 13\textit{cis}RA in neuroblastoma patients are on average 3-5 µM, and rarely exceed 10 µM (Veal et al., 2007). The range of concentrations of 4-oxo 13\textit{cis}RA that could be used to characterize the kinetics of glucuronide formation was limited by the amount of substrate available and the maximum concentration of 4-oxo 13\textit{cis}RA in the stock solution in ethanol. The available data (up to 100 µM) were fitted well by the hyperbolic Michaelis-Menten model (Figure 4 and Table 1). Values for $V_{\text{max}}$ were 199±70 µV*s/s and 304±30 µV*s/s in HLM and HIM respectively, with corresponding values of $K_{\text{m}}$ being 95±58µM and...
69±14 µM. Upon comparing glucuronidation activities of individual isoforms, UGT1A3 had a higher $V_{\text{max}}$ towards 4-oxo 13cisRA than the other isoforms examined. Estimates of $K_m$ ranged from 18 µM for UGT1A9 to 266 µM for UGT1A3. Reported plasma concentrations for 4-oxo 13cisRA can be up to 10 µM on day 14 of administration, but on average are nearer 6 µM (Veal et al., 2007).
Discussion

An in vitro HPLC-based assay has been developed to characterise the generation of 13cisRA and 4-oxo 13cisRA glucuronides, using HLM, HIM and recombinant UGT isoforms. Positive identification of these metabolites as glucuronides was based on sensitivity to β-glucuronidase treatment, resulting in complete disappearance of the metabolite peaks generated. Both HLM and HIM were found to catalyse the glucuronidation of 13cisRA and 4-oxo 13cisRA, with the highest rate of activity towards both substrates seen for HIM. This could be particularly significant for an orally-administered drug such as 13cisRA. Further investigations revealed that UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 were the major isoforms responsible for the glucuronidation of both substrates.

Enzyme kinetic studies were performed with microsomes from human liver and intestine, and with human UGT isoforms UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 for both substrates. Although only a limited range of concentrations of 4-oxo 13cisRA could be explored, the concentrations far exceeded those reported clinically. For the parent drug, all enzyme sources other than HLM showed evidence of substrate inhibition. Although it was not possible to determine the parameters of this phenomenon precisely, inspection of the kinetic plots and the estimated parameters suggest that for the majority of enzyme sources, atypical kinetics of glucuronidation is not clinically important. The exception to this is UGT1A9, which showed signs of substrate inhibition at 50 μM. Substrate inhibition kinetics have been reported for other UGT substrates, including resveratrol (Iwuchukwu and Nagar). The kinetic profiles for 4-oxo 13cisRA glucuronidation by HLM, HIM and all of the UGT isoforms studied followed Michaelis-Menten kinetics, allowing determination of apparent $K_m$ and $V_{max}$ values. The 4-oxo metabolite appeared to be a better substrate for glucuronidation in HLM, in that the $V_{max}$ value was higher than that for the parent compound. However, it
should be noted that the $K_m$ values for both substrates were higher than the maximum plasma concentrations observed in patients (Veal et al., 2007). In addition, glucuronidation in the intestine may be more relevant as the rate of reaction was highest for HIM, particularly at concentrations typically achieved in plasma. The intestine may be exposed to higher concentrations during drug absorption, but the degree of substrate inhibition was not marked at concentrations below 100 μM in enzyme sources other than UGT1A9.

Caution in the interpretation of the estimated parameters is necessary since, unlike P450s, UGTs do not have a measurable chromophore representative of active protein; therefore the amount of active UGT in any given recombinant preparation is unknown and activities are normalized to mg total protein rather than pmol UGT. The inability to quantify UGT expression levels in recombinant systems makes it difficult to verify the relative importance of individual isoforms in a particular glucuronidation pathway where multiple UGTs may be involved. One approach to overcome this problem is through implementation of the relative activity factor (RAF) method. This method was first used to investigate P450-dependent activity (Crespi and Miller, 1999), and measures the activities of an enzyme-selective probe substrate in recombinant UGT enzymes and pooled human liver microsomes (HLMs). However, due to broad overlapping substrate specificity between the UGTs, one caveat with the RAF method for UGT reaction phenotyping is that probe substrates have not been identified for all the major UGT isoforms.

Examination of the structures of 13\textit{cis}RA and 4-oxo 13\textit{cis}RA (Figure 5), and comparison with the glucuronidation of ATRA, allows for a tentative assignment of the position of glucuronidation. Since glucuronidation requires either a –COOH, –OH, –NH$_2$ or –SH functional group for conjugation with α-D-glucuronic acid, it is likely that the glucuronides
formed in this study are conjugated at the terminal carboxylic acid. This prediction is in line with the previous finding by Samokyszyn et al., that the UGT2B7-mediated glucuronidation of ATRA and 4-oxo ATRA is directed towards the carboxyl function, whereas the 4-hydroxylated metabolite is glucuronidated almost exclusively at the hydroxyl function (Samokyszyn et al., 2000). It is also likely that 4-hydroxy 13cisRA undergoes glucuronidation. This would most likely occur at the hydroxyl function, as has been demonstrated for 4-hydroxy ATRA (Samokyszyn et al., 2000). However, since this metabolite is not readily available, investigations into the potential glucuronidation of 4-hydroxy 13cisRA could not be carried out.

Despite being unable to determine the exact contribution of each of the recombinant UGT isoforms examined in this study, our data suggest that UGT1A3 has the highest rate of activity towards both substrates, as assessed by the $V_{\text{max}}$ estimates. UGT1A3 is one of the main isoforms responsible for the glucuronidation of the majority of carboxylic acid compounds (Sakaguchi et al., 2004), supporting the hypothesis that the carboxyl-function is the site of 13cisRA and 4-oxo 13cisRA glucuronidation. However, whilst UGT1A3 is mainly expressed in the liver, the level of expression is significantly lower than that of UGT1A1 or UGT1A9 (Ohno and Nakajin, 2009). Furthermore, as the plasma concentrations of 13cisRA and 4-oxo 13cisRA are reported to be below the $K_m$ concentrations for the enzymes tested in this study (Veal et al., 2007), it is probable that UGT1A9 is the most important isoform in the glucuronidation of both 13cisRA and 4-oxo 13cisRA, as this enzyme has the lowest $K_m$ value for each substrate, is expressed at high levels in the liver, and is also expressed in the intestine (Ohno 2009). The importance of glucuronidation in the intestines is emphasized by the very high degree of glucuronidation observed with human intestinal microsomes and the high level of expression of the relevant isoforms in this tissue (Ohno 2009). Identification of
UGT1A9 as an important isoform involved in the glucuronidation of both 13cisRA and 4-oxo 13cisRA provides a rationale for investigating the impact of UGT1A9 genetic polymorphisms on the reported variability associated with 13cisRA pharmacokinetics and metabolism in children with neuroblastoma (Veal et al., 2007).
Acknowledgements

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Footnotes

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Legends for figures

Figure 1. Representative HPLC chromatograms illustrating the *in vitro* glucuronidation of 13*cis*RA and 4-oxo 13*cis*RA by UGT1A3. Absorbance units are measured on the y-axis and time in minutes on the x-axis. (A) 13*cis*RA glucuronidation. 13*cis*RA (i) eluted at 23.1 min and 13*cis*RA glucuronide (ii) at 6.2 min. (B) HPLC chromatogram of the same incubation shown in A, following treatment with β-glucuronidase. (C) 4-oxo 13*cis*RA glucuronidation. 4-oxo 13*cis*RA (i) eluted at 7.9 min, 4-oxo ATRA (ii) at 7.2 min and 4-oxo 13*cis*RA glucuronide (iii) at 2.4 min. (D) HPLC chromatogram of the same incubation shown in C, following treatment with β-glucuronidase.

Figure 2. 13*cis*RA and 4-oxo 13*cis*RA glucuronidation.

(A) 13*cis*RA glucuronidation. (B) 4-oxo 13*cis*RA glucuronidation. Glucuronidation activity was determined in pooled human liver and intestinal microsomes and Supersomes™ containing different cDNA expressed UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15 and UGT2B17). Data are shown as the mean ± SEM, *n*=3. Incubation conditions were 60 minutes at 37°C with 0.5 mg/ml protein and assays were performed in triplicate.

Figure 3. Atypical kinetic profiles observed for 13*cis*RA glucuronide formation.

Kinetic analysis was performed using pooled human liver and intestinal microsomes, as well as recombinant UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 isoforms. Data are shown as the mean ± SEM, *n*=3. Incubations were performed in triplicate and carried out across a substrate concentration range of 0 – 250 μM.

Figure 4. Kinetic profiles observed for 4-oxo 13*cis*RA glucuronide formation.
Kinetic analysis was performed using pooled human liver and intestinal microsomes, as well as recombinant UGT1A1, UGT1A3, UGT1A7, UGT1A8 and UGT1A9 isoforms. Data are shown as the mean ± SEM, $n=3$. Incubations were performed in triplicate and carried out across a substrate concentration range of 0 – 100 µM.

**Figure 5. Structures of 13cisRA and 4-oxo 13cisRA.** Arrows indicate the predicted site of glucuronidation.
Tables

Table 1. Kinetic parameter estimates for the formation of 13\textit{cis}RA and 4-oxo 13\textit{cis}RA glucuronides in human liver and intestinal microsomes and recombinant UGT isoforms

<table>
<thead>
<tr>
<th>UGT protein source</th>
<th>(V_{\text{max}})</th>
<th>(K_m)</th>
<th>Goodness of fit (r^2)</th>
<th>(V_{\text{max}})</th>
<th>(K_m)</th>
<th>(K_i)</th>
<th>Goodness of fit (r^2)</th>
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<tr>
<td>HLM</td>
<td>199±70</td>
<td>95±58</td>
<td>0.88</td>
<td>59±17</td>
<td>974±342</td>
<td>n.a.</td>
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<td>HIM</td>
<td>304±30</td>
<td>69±14</td>
<td>0.98</td>
<td>1120±800</td>
<td>200±98</td>
<td>18±9</td>
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<td>175±27</td>
<td>0.99</td>
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<td>*78</td>
<td>*77</td>
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<tr>
<td>UGT1A3</td>
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<td>266±76</td>
<td>0.99</td>
<td>*134</td>
<td>*161</td>
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<tr>
<td>UGT1A7</td>
<td>19±3</td>
<td>51±17</td>
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<td>*186</td>
<td>*182</td>
<td>0.73</td>
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<td>36±7</td>
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<td>UGT1A9</td>
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<td>0.88</td>
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Data are expressed as mean ± SEM, \(n=3\).

Units are given as follows: \(V_{\text{max}} = \mu V*s/s\); \(K_m\) and \(K_i = \mu M\).

n.a. , not applicable

*Precise estimates of the parameters could not be determined.
13-cis retinoic acid

4-oxo 13-cis retinoic acid