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Acitretin exhibits inhibitory effects towards UDP-glucuronosyltransferase (UGT)1A9-mediated 4-methylumbelliferone (4-MU) and propofol glucuronidation reaction

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The present study aimed to evaluate the potential risk of drug-drug interactions associated with acitretin which is a drug for therapy of psoriasis approved by the Food and Drug Administration (FDA). The initial screening of acitretin's inhibition towards 4-methylumbelliferone (4-MU) glucuronidation catalyzed by important UDP-glucuronosyltransferase (UGT) isoforms in the liver showed that UGT1A9 activity was strongly inhibited by acitretin with other UGT isoforms negligibly influenced. The inhibition type is best fit to competitive inhibition, and the inhibition kinetic parameter (K_i) was determined to be 3.5 μM. The inhibition behaviour of acitretin towards UGT1A9 activity did not exhibit probe substrate-dependent behaviour when selecting human liver microsomes (HLMs)-catalyzed propofol-O-glucuronidation as probe reaction of UGT1A9. The same inhibition type and similar inhibition parameters (K_i = 3.2 μM) were obtained. Using the maximum plasma exposure dose of acitretin (C_{max}), the C_{max}/K_i values were calculated to be 0.23 and 0.25 when selecting 4-MU and propofol as probe substrates, respectively. All these results indicate a potential clinical drug-drug interaction between acitretin and 4-MU or propofol.

1. Introduction

Psoriasis is an autoimmune disease affecting the skin, and severely affects health-related quality of life to an extent similar to the effects of other chronic diseases, such as depression, hypertension, congestive heart failure or type 2 diabetes (Sampogna et al. 2006). Acitretin (Soriatane) is a drug for therapy of psoriasis approved by the Food and Drug Administration (FDA) in 1997 as a replacement for etretinate, which is another important drug to treat psoriasis (Ellis and Voorhees 1987). Despite the similar chemical structure of acitretin and etretinate, physicochemical properties of both compounds are rather different. The extremely lipophilic properties of etretinate make it easily binding strongly to plasma proteins, particularly lipoproteins and albumin, which results in long terminal elimination half-life (approximately 120 days) (Lucek and Colburn 1985). In the contratry, acitretin is less lipophilic than etretinate, making it eliminated from the body more rapidly (2 days).

Human UDP-glucuronosyltransferases (UGTs) are the most important phase II drug metabolizing enzymes (DMEs) involved in the metabolic elimination of many clinical drugs and multiple endogenous substances such as bilirubin, steroid hormones, thyroid hormones, bile acids and fat-soluble vitamins (Xing and Che 2012). Drug-drug interaction due to the inhibition of UDP-glucuronosyltransferases (UGTs) both as perpetrator and victim has been regarded as an important reason for adverse effects of clinical drugs (Zhang et al. 2010). Many pharmaceutical companies are regarding inhibition screening of UGT isoforms as an important task in drug research and development.

The aim of the present study was to evaluate the inhibitory effect of acitretin towards four important UGT isoforms in human liver, including UGT1A1, UGT1A6, UGT1A9, and UGT2B7. The detailed inhibition kinetic information was furtherly determined, including inhibition kinetic type and parameters (K_i). To avoid the probe substrate-dependent inhibition behaviour of acitretin towards UGT1A9, the probe reaction propofol-*O*-glucuronidation was also used.

2. Investigations and results

Acitretin (100 μM) was used to initially screen the inhibition potential towards the important UGT isoforms in the liver, including UGT1A1, UGT1A6, UGT1A9, and UGT2B7. The results (Fig. 1) showed that the activity was inhibited by 17.1%, 21.6%, 63.1%, and –10.1% for UGT1A1, 1A6, 1A9, and 2B7, respectively. The UGT isoform with more than 50% inhibition extent underwent further study to determine the inhibition kinetic type and parameter (K_i). The intersection was located in the second quadrant in Dixon plot, and in the vertical axis in Lineweaver-Burk plot, which suggested that the inhibition of recombinant UGT1A9-catalyzed 4-MU glucuronidation by acitretin best fit to the competitive inhibition type. The K_i value was calculated to be 3.5 μM. To avoid that the inhibition

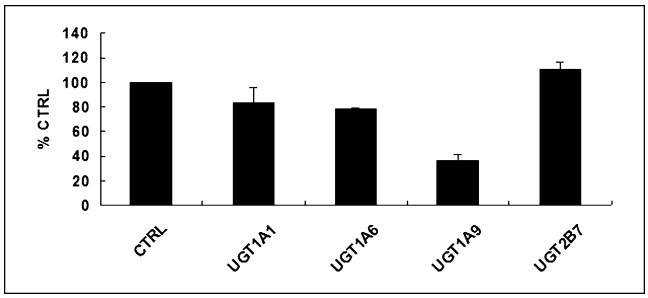


Fig. 1: Initial screening of acitretin's inhibition potential towards UDP-glucuronosyltransferase (UGT) 1A1, 1A6, 1A9 and 2B7. Recombinant UGT isoforms-catalyzed 4-methylumbelliferone (4-MU) glucuronidation was used as probe reaction

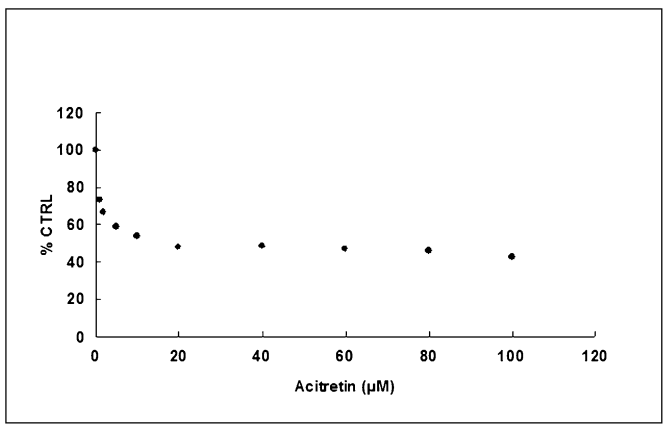
behaviour was influenced by the selection of probe substrate and enzyme source human liver microsomes (HLMs)-catalyzed propofol glucuronidation was also selected to evaluate the inhibition potential of acitretin. The same inhibition type and similar inhibition kinetic parameters ($K_i = 3.2$ μM) were obtained.

3. Discussion

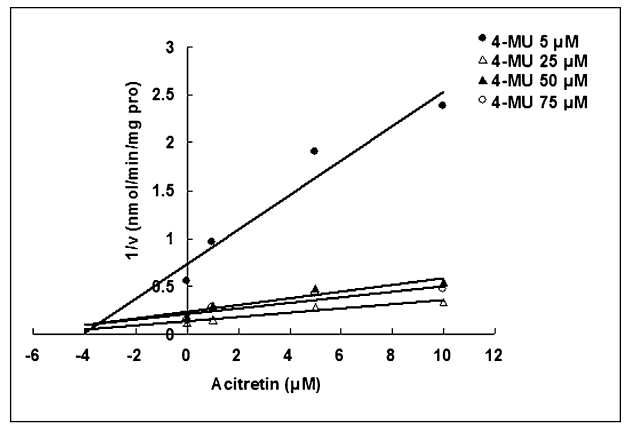
Within the UGT1A subfamily, UGT1A9 has been demonstrated to exhibit high expression in a variety of tissues, such as liver, intestines and kidney (Strassburg et al. 1999). UGT1A9 is involved in the metabolic elimination of many xenobiotics and endogenous substrates, such as planar phenols, bile acids, fatty acids, hormones, and tobacco pro-carcinogens (Tsoutsikos et al. 2004). UGT1A9 is sole elimination pathway for propofol, and the predominant route for mycophenolate (Rowland et al. 2008; Mackenzie 2000). UGT1A9 activity is closely associated with the adverse effects of clinical drugs. For example, the altered activity of UGT1A9 resulting from single nucleotide polymorphisms (SNPs) has a strong relationship with serious toxicity induced by the inhibitors of reversible catechol-O-methyltransferase (COMT) (Ferrari et al. 2012).

In the present study, 4-MU was firstly selected to screen the inhibitory effect of acitretin towards several important UGT isoforms in the liver, including UGT1A1, UGT1A6, UGT1A9, and UGT2B7. The UGT1A9-catalyzed 4-MU glucuronidation activity was competitively inhibited with a K_i value of 3.5 μM. However, negligible influence of acitretin was found towards the other UGT isoforms tested. Probe substrate-dependent inhibition behaviour has been widely detected in evaluation of xenobiotics' inhibition towards drug-metabolizing enzymes, including cytochrome P450 (CYPs) and UGTs. For example, ginsenosides exhibited different inhibition behaviour when different probe substrates of CYP3A4 were selected (Hao et al. 2008). Carvacrol exhibited competitive inhibition towards 4-MU glucuronidation catalyzed by recombinant UGT1A9. However, the noncompetitive inhibition was obtained when propofol was selected as probe substrate and human liver microsomes were used as enzyme source. In the present study, when propofol was employed for evaluation of acitretin's inhibition, the same inhibition type was obtained, and the similar inhibition kinetic parameter (K_i) was calculated, indicating the absence probe substrate-dependent inhibition for acitretin.

Besides the *in vitro* kinetic parameters, *in vivo* exposure concentration of drug is an important determinant for drug-drug interactions. The maximum plasma exposure dose of acitretin



(A)



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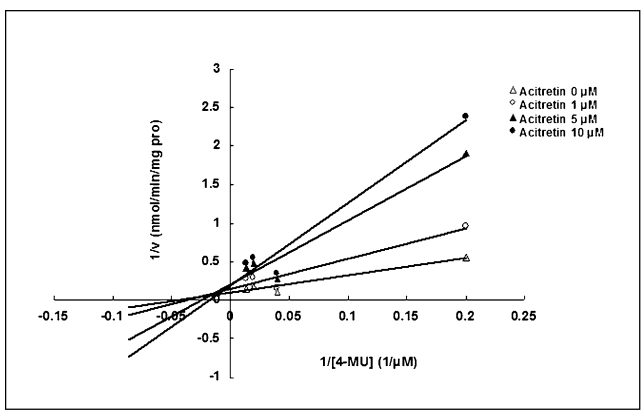

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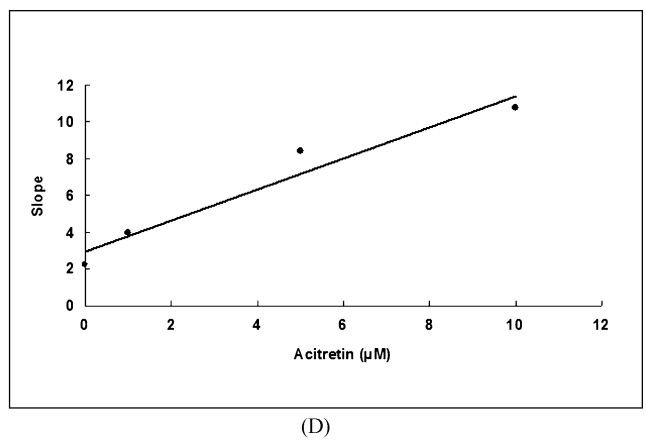

(D)

Fig. 2: Inhibition of acitretin towards recombinant UGT1A9-catalyzed 4-MU glucuronidation. (A) The concentration-dependent inhibition of acitretin towards UGT1A9-catalyzed 4-MU glucuronidation. (B) Dixon plot of acitretin's inhibition towards UGT1A9-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of acitretin's inhibition towards UGT1A9-catalyzed 4-MU glucuronidation. (D) Second plot using slopes obtained from Lineweaver-Burk plot versus acitretin concentrations

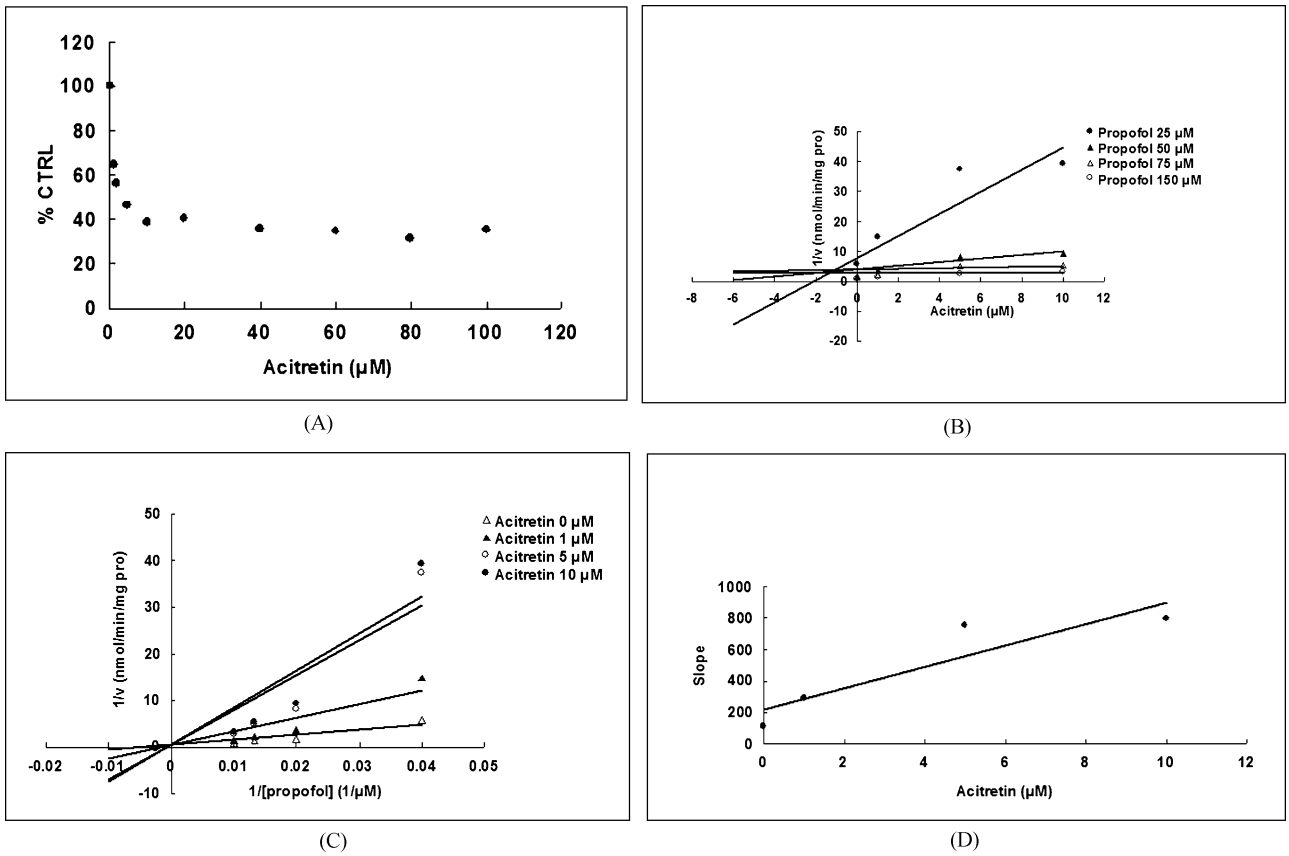

Fig. 3: Inhibition of acitretin towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation. (A) The concentration-dependent inhibition of acitretin towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation. (B) Dixon plot of acitretin's inhibition towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation. (C) Lineweaver-Burk plot of acitretin's inhibition towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation. (D) Second plot using slopes obtained from Lineweaver-Burk plot versus acitretin concentrations

(C_{max}) in patients was reported to be 248 μg/L (0.8 μM) (Stuck et al. 1989). The C_{max}/K_i was calculated to be 0.23 and 0.25 when selecting 4-MU and propofol as probe substrate, respectively. The C_{max}/K_i values have been widely utilized as the judgement for the possibility of drug-drug interaction. The critical values were as followed: $C_{max}/K_i < 0.1$, not possible; $0.1 < C_{max}/K_i < 1$, possible; $C_{max}/K_i > 1$, very posssible.

In conclusion, the higher C_{max}/K_i values of acitretin's inhibition towards UGT1A9-catalyzed 4-MU and propofol glucuronidation indicates a high risk of drug-drug interactions between acitretin and 4-MU or propofol.

4. Experimental

4.1. Chemicals

Acitretin (HPLC purity ≥ 98%), propofol, 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine 5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). Propofol glucuronide (PG) was obtained from Toronto Research Chemicals (North York, Ontario, Canada). Recombinant human UGT supersomes (UGT1A1, UGT1A6, UGT1A9, and UGT2B7) expressed in baculovirus-infected insect cells were obtained form BD Gentest Corp. (Woburn, MA, USA). Pooled human liver microsomes (HLMs, 20 mg/ml protein) were obtained from BD Biosciences (San Jose, CA). All other reagents were of HPLC grade or of the highest grade commercially available.

4.2. Determination of acitretin's inhibition towards 4-MU glucuronidation reaction catalyzed by various UGT isoforms

A modified determination method described previously by Gong et al. (2012) was used to analyze the 4-MU glucuronidation reaction. The corresponding experiment was established to ensure the linear reaction within the range of the used incubation time and protein concentration. The incubation media (total volume = 200 μL) contained recombinant UGT isoforms (0.25, 0.025, 0.05 and 0.05 mg/ml for UGT1A1, 1A6, 1A9 and 2B7), 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH = 7.4), and 4-MU in the presence and absence of different concentrations of acitretin. The used concentrations of 4-MU were 110 μM, 110 μM, 30 μM, and 350 μM for UGT1A1, 1A6, 1A9 and 2B7, respectively. Acitretin was dissolved in DMSO, and the final concentration was 0.5% (v/v). After 3-min pre-incubation at 37 °C, the reaction was initiated through adding UDPGA, and the incubation time was 120 min for UGT1A1 and UGT2B7, 30 min for UGT1A6 and UGT1A9. The analysis method was performed as previously reported (Gong et al. 2012).

4.3. Inhibitory effect of acitretin towards human liver microsomes (HLMs)-catalyzed propofol glucuronidation

To evaluate the inhibition of acitretin towards HLMs-catalyzed glucuronidation of propofol, acitretin was co-incubated with the typical probe substrate of UGT1A9 propofol in the human liver microsomes (HLMs) system containing UDPGA as cofactor. The incubation and analysis conditions were the same as previously described (Vree et al. 1999).

4.4. Data analysis

Metabolic rate was expressed as nanomoles per minute per milligram of protein. The Dixon plot and Lineweaver-Burk plot were frequently used for both identification of the likely mechanism of enzyme inhibition and for estimation of inhibition kinetic parameter (K_i) (Kakkar et al. 1999). The metabolic velocity was determined using multiple concentrations of probe substrates (4-MU or propofol) and acitretin. The data were fitted using the Dixon and Lineweaver-Burk equations. The inhibition kinetic parameter (K_i) was calculated using the second plot which correlate the slopes obtained from the Lineweaver-Burk plot versus the concentrations of acitretin.

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