

Comparison of the inhibition capability of oleanolic acid and betulinic acid towards drug-metabolizing enzymes

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Abstract

Background: Human UDP-glucuronosyltransferases (UGTs) are important membrane proteins located in endoplasmic reticulum, and play important roles in metabolism of a variety of endogenous and exogenous compounds.

Aims: To determine the influence of subtle difference in the structure of oleanolic acid and betulinic acid towards the inhibition towards the activity of UGT isoforms.

Methods: In vitro glucuronidation of 4-methylumbelliferone (4-MU) reaction was employed as the probe reaction to determine the inhibition of these two compounds towards UGTs' activity.

Results: The inhibition of capability of oleanolic acid towards UGT1A6 and UGT1A8 were higher than betulinic acid. However, no significant difference was observed for the inhibition of oleanolic acid and betulinic acid towards UGT1A7. Furthermore, concentration-dependent behaviour was determined for the inhibition of oleanolic acid and betulinic acid towards UGT1A6 and UGT1A8. At various concentrations of oleanolic acid and betulinic acid, the inhibition of oleanolic acid towards UGT1A6 and UGT1A8 was higher than betulinic acid.

Conclusion: Given that UGT1A6 and UGT1A8 play key role in the the inhibition of oleanolic acid towards UGT1A6 and UGT1A8 will induce drug-drug interaction and the risk of diseases.

Keywords: UDP-glucuronosyltransferases(UGTs), drug-drug interaction, oleanolic acid, betulinic acid

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Introduction

Human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS) is defined as a disease spectrum of the human immune system caused by infection with human immunodeficiency virus (HIV)^{1,2}. Searching the efficient drugs to treat HIV/AIDS patients is very important and necessary.

Betulinic acid is a naturally occurring pentacyclic triterpenoid, and has been demonstrated to exhibit antiretroviral, antimalarial, and anti-inflammatory biochemical properties. Betulinic acid has been frequently reported to exert anti-tumor activities towards multiple

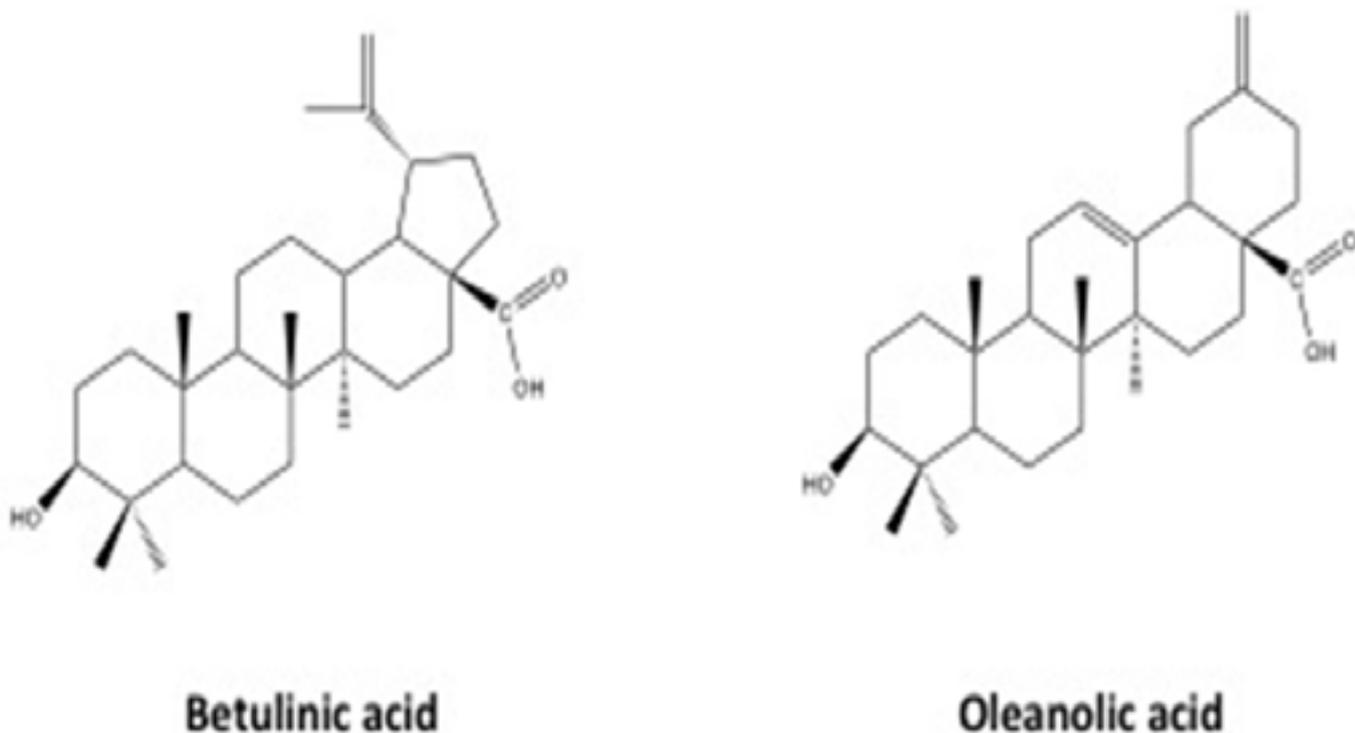
tumor types, including melanoma and breast cancer^{3,4}. Oleanolic acid is a naturally occurring triterpenoid isolated from many food and medicinal plants, including *Olea europaea* (leaves, fruit), *Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant), *Mirabilis jalapa* and *Ternstroemia gymnanthera* (aerial part)⁵. Oleanolic acid has very similar structure with betulinic acid (Figure 1). Betulinic acid and oleanolic acid are also the potential drug candidates for the treatment of HIV/AIDS patients⁶.

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Figure 1 The structures of betulinic acid and oleanolic acid.

Figure 1



The present study aims to investigate the inhibition capability of these two compounds towards the activity of drug-metabolizing enzymes UDP-glucuronosyltransferases (UGTs) 1A6, 1A7, and 1A8.

Materials and methods

Chemicals and reagents

4-Methylumbelliferone (4-MU), 4-methylumbelliferone-b-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin, and uridine-diphospho-glucuronic acid trisodium salt (UDPGA) were purchased from Sigma-Aldrich. Recombinant UDP-glucuronosyltransferase (UGT) isoforms were obtained from BD Gentest Corp. (Woburn, MA). All other reagents were of HPLC grade or of the highest grade commercially available.

Investigation of the inhibition of UGTs' activity by oleanolic acid and betulinic acid

For UGT supersomes-catalyzed in 4-MU glucuronida-

tion probe reactions, the incubation system (200 μ L) is consisted of 0.05 mg/ml UGT supersomes, 5 mM UDPGA co-factor, 5 mM of $MgCl_2$, 50 mM Tris-HCl (pH=7.4), and 4-MU in the absence or presence of different concentration of oleanolic acid and betulinic acid. The concentration of used protein and the incubation time can ensure the linear reaction. The concentration of 4-MU was equal to the K_m or S_{50} values for each UGT isoform. The analytical conditions have been described in the previous literatures^{7,8}.

Statistical method

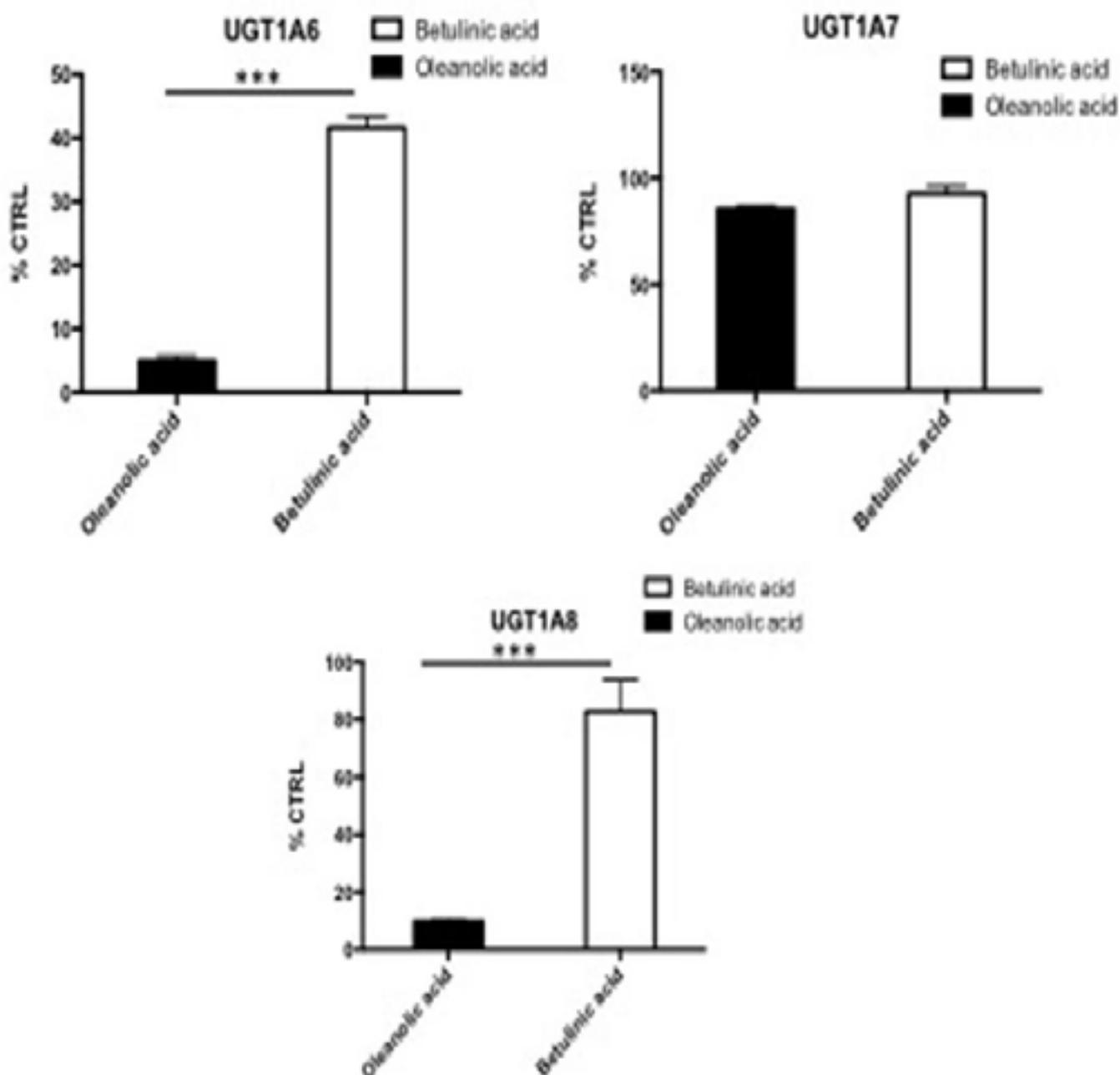
The statistical difference was performed using two-tailed student t-test.

Results

The inhibition screening of oleanolic acid and betulinic acid towards three important UGT isoforms UGT1A6, UGT1A7, and UGT1A8 was firstly investigated, and the results were given in Figure 2.

Figure 2 Initial screening the inhibition capability of oleanolic acid and betulinic acid towards the activity of UGT1A6, UGT1A7, and UGT1A8. 100 μ M of oleanolic acid and betulinic acid were used, and the statistical difference was given as*** $p < 0.001$.

Figure 2



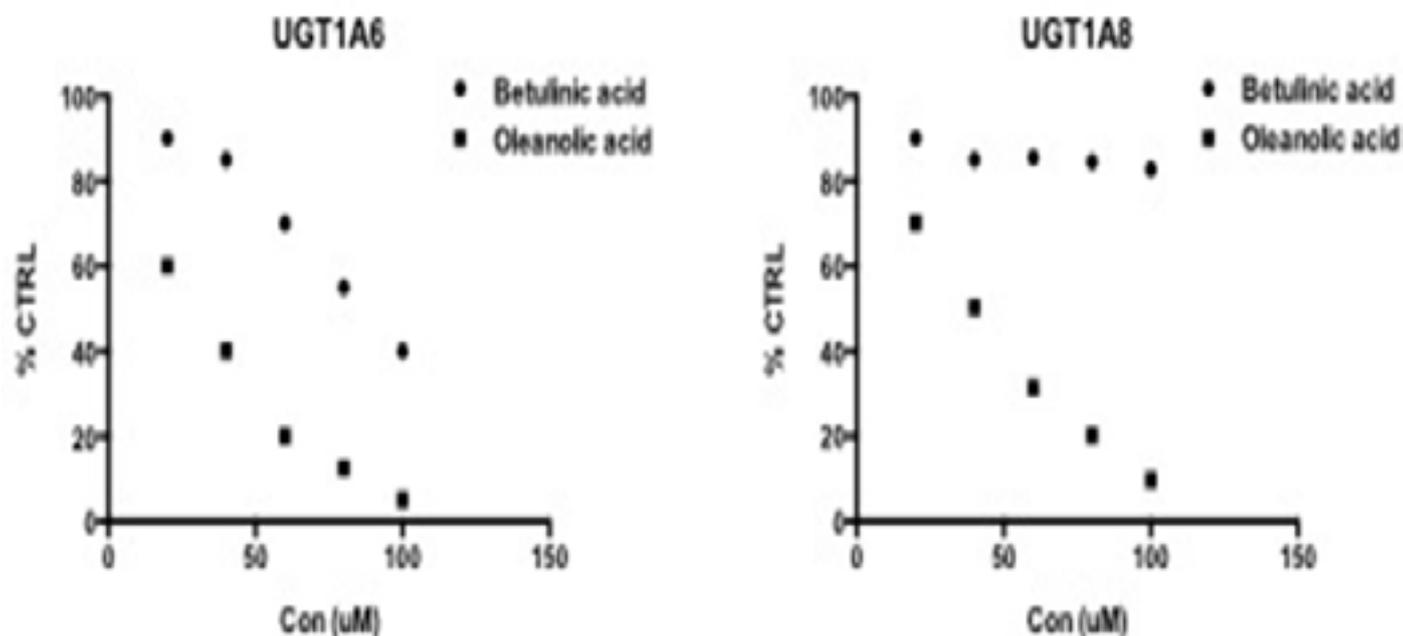
The inhibition of capability of oleanolic acid towards UGT1A6 and UGT1A8 were higher than betulinic acid. However, no significant difference was observed for the inhibition of oleanolic acid and betulinic acid towards UGT1A8. Furthermore, concentration-dependent behaviour was determined for the inhibition

of oleanolic acid and betulinic acid towards UGT1A6 and UGT1A8 (Figure 3).

At various concentrations of oleanolic acid and betulinic acid, the inhibition of oleanolic acid towards UGT1A6 and UGT1A8 was higher than betulinic acid.

Figure 3 Concentration-dependent inhibition of betulinic acid and oleanolic acid towards towards the activity of UGT1A6 and UGT1A8.

Figure 3



Discussion

Human UDP-glucuronosyltransferases (UGTs) are important membrane proteins located in endoplasmic reticulum, and play important roles in metabolism of a variety of endogenous and exogenous compounds⁹. Many UGTs-inhibition based adverse effects cases have been reported. For example, indinavir, an HIV therapeutic drug, can significantly induce the elevation of unconjugated bilirubin in serum through inhibition of UGT1A1-mediated bilirubin glucuronidation¹⁰.

Many compounds have been reported to exert inhibition potential towards UGT isoforms, such as warfarin¹¹ and arbidol¹². The present study showed that subtle difference for the structure of oleanolic acid and betulinic acid will result in the significant difference of these two compounds' inhibition towards UGT1A6 and UGT1A8. Besides the high contribution of these two UGT isoforms towards the metabolism of xenobiotics, high correlation between the activity of these two UGT isoforms and cancers was reported. For example,

UGT1A6₁₉_GG genotype has been reported to be a breast cancer risk factor¹³. The lower activity of UGT1A8 will increase the risk of esophageal cancer risk¹⁴. Therefore, the inhibition of oleanolic acid towards UGT1A6 and UGT1A8 will induce drug-drug interaction and the risk of diseases.

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