

Effects of Propofol Analogs on Glucuronidation of Propofol, an Anesthetic Drug, by Human Liver Microsomes

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Abstracts: To prevent rapid forming *O*-glucuronide of propofol (2,6-diisopropylphenol), an intravenous anesthetic, effects of propofol analogs were investigated. Propofol was predominately glucuronidated by human UGT1A9 and intestinal or liver microsomes. 2,5-Diisopropylphenol inhibited the propofol glucuronidation. A possibility of developing orally administrable agents or of reducing propofol dose by coadministration is suggested.

Key Words: Propofol, UGT1A9, inhibitor, glucuronidation, human, 2,5-diisopropylphenol.

INTRODUCTION

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation of a broad spectrum of endobiotic and xenobiotic substrates [1,2]. In general, the resulting glucuronides are more hydrophilic, facilitating renal and biliary excretion. In addition to hepatic metabolism, high rates of gastrointestinal glucuronidation in rats and humans have been observed [3].

Propofol (2,6-diisopropylphenol) is administered as a bolus for the induction of anesthesia and as an infusion for maintenance of anesthesia or for sedation [4]. A rapid and complete recovery [5] is a major advantage of this drug, which is attributable to extensive biotransformation of the parent compound. Propofol is suggested to be a substrate for one of the UGT isoforms, UGT1A9 [6,7]. There is a hypothesis that propofol could be orally used with a suitable inhibitor among analogs for preventing its rapid glucuronidation [8].

In order to seek for a suitable inhibitor of propofol biotransformation, we investigated the interactions between propofol and its analogs in the glucuronidation by recombinant human UGT isoforms expressing both in livers and intestines. Here we report that human intestinal microsomes have a high glucuronidation activity toward propofol as well as the liver microsomes and that 2,5-diisopropylphenol could be the suitable inhibitor for propofol among diisopropyl-, butylmethyl- or dimethyl-phenol analogs tested.

MATERIALS AND METHODS

Chemicals

Propofol, 2,5-diisopropylphenol, 2-*tert*-butyl-6-methylphenol, 2-*tert*-butyl-5-methylphenol, 2,6-dimethylphenol, and 2,5-dimethylphenol were obtained from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals and reagents used were obtained in the highest grade available commercially.

Enzyme Preparations

Rat liver microsomes were prepared as described previously [8,9]. Rat intestinal microsomes and human liver and

intestinal microsomes were obtained from Tissue Transformation Technologies (Edison, NJ, USA). Recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 expressed in microsomes of insect cells (Supersomes) were obtained from BD Gentest (Woburn, MA, USA).

Glucuronidation Assays

The glucuronidation activities of propofol and its analogs were determined according to the methods [8] with slight modifications. Briefly, a typical incubation mixture (100 μ l of total volume) contained 50 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl₂, 3 mM UDP-GA, 0.05 mg alamethicin/ml [10], intestinal or liver microsomes (1.0 mg protein/ml) or recombinant UGT forms (0.5 mg/ml), and substrate dissolved in dimethyl sulfoxide. Blank incubations were performed without UDP-GA. When the suppression of the propofol glucuronidation was investigated, propofol analogs dissolved in dimethyl sulfoxide were simultaneously added to the reaction mixtures. After the incubation at 37°C for 10 min, the reaction was terminated by the addition of 0.1 ml ice-cold methanol. The linearity of product formation for the incubation time and protein concentrations was confirmed for 20 min and to 1.0 mg/ml. After removal of the protein by centrifugation at 10,000 g for 10 min, a 50 μ L portion of the sample was subjected to HPLC system equipped with a C₁₈ 5- μ m analytical column (150 x 4.6 mm i.d). The mobile phase was 40 % acetonitrile containing 0.1% of acetic acid at 1.0 ml/min. The glucuronides formed were determined by UV absorbance at 220 nm.

Statistical Analysis

The kinetic analyses of glucuronidation activities were performed by fitting the Michaelis-Menten equation using a nonlinear regression analysis program (Kaleida Graph, Synergy Software, Reading, PA, USA).

RESULTS AND DISCUSSION

The glucuronosyltransferase activities toward propofol in liver and intestinal microsomes were investigated (Fig. (1)). The transferase activities toward propofol of intestinal microsomes from humans were similar to those from human livers (Fig. (1)A). However, the activities of rat intestinal microsomes toward propofol were one-tenth of those from

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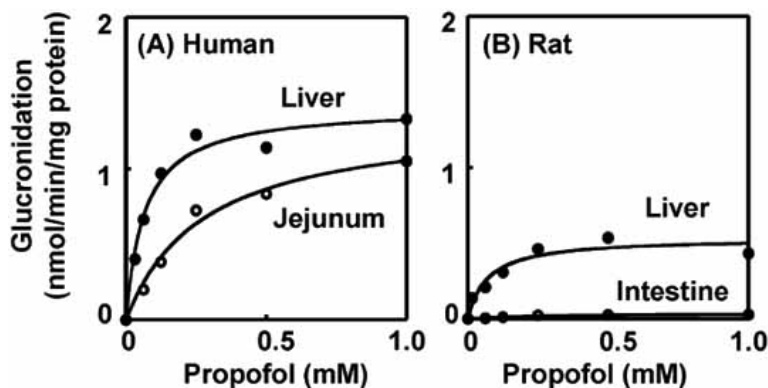


Fig. (1). Glucuronidation of propofol by liver or intestine microsomes from humans (A) and rats (B). Intestinal or liver microsomes (1.0 mg protein/ml) were incubated with propofol for 10 min at 37°C in the presence of 3 mM UDP-GA.

rat livers (Fig. (1)B). Apparent K_m and V_{max} values of propofol glucuronidation by human liver and intestinal microsomes were $64 \pm 9 \mu\text{M}$ (mean and SE) and $1.40 \pm 0.05 \text{ nmol/min/mg protein}$ and $283 \pm 87 \mu\text{M}$ and $1.37 \pm 0.19 \text{ nmol/min/mg protein}$, respectively. The K_m value for human liver microsomes was corresponded to the clinical ranges of plasma propofol concentrations ($\sim 50 \mu\text{M}$) in patients [11]. A marked species difference was seen in the intestinal propofol glucuronidation. These results suggested that first pass effects might be seen if propofol would be orally administrated to humans.

Roles of human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 in glucuronidation of propofol and its analogs were investigated (Fig. (2)). Propofol was pre-

dominantly glucuronidated by UGT1A9. The activity of recombinant UGT1A9 at the substrate concentration of $50 \mu\text{M}$ of propofol seemed to be almost saturated (Fig. (2)A). In contrast, glucuronidation of 2,5-diisopropylphenol, 2-*tert*-butyl-5-methylphenol or 2-*tert*-butyl-6-methylphenol catalyzed by these UGT forms were low or undetectable levels (Figs. (2)B-(2)D). 2,6-Dimethylphenol and 2,5-dimethylphenol were glucuronidated by UGT1A6, UGT1A9 and/or UGT2B7 (Figs. (2)E, (2)F).

Effects of propofol analogs on the propofol glucuronidation ($10 \mu\text{M}$) catalyzed by UGT1A9 were investigated (Fig. (3)). 2,6-Substituted compounds, namely 2,6-dimethylphenol and 2-*tert*-butyl-6-methylphenol, did not inhibit the reaction. On the other hand, 2,5-substituted compounds inhibited the

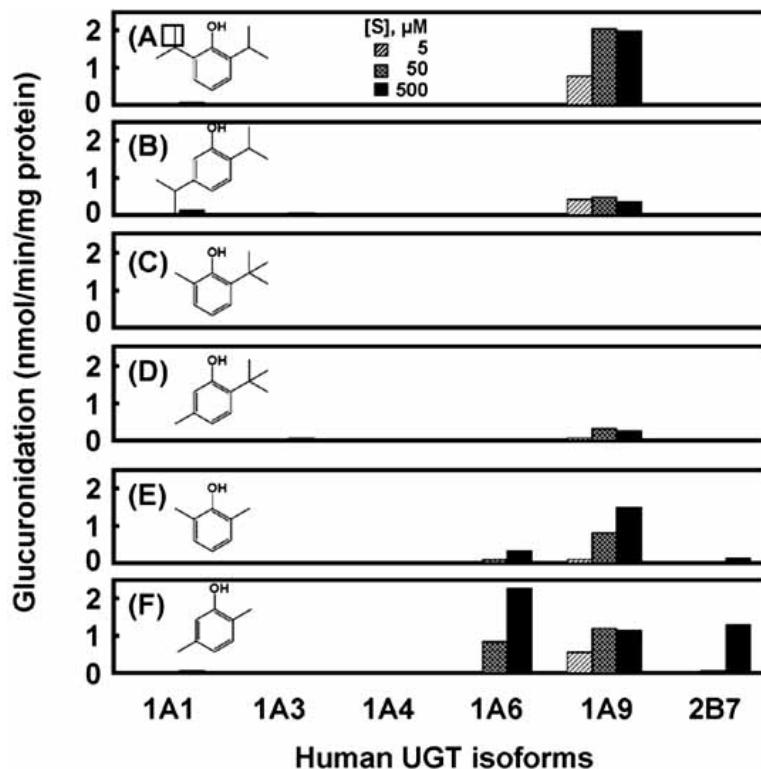


Fig. (2). Glucuronidation of propofol and its analogs by recombinant human UGT isoforms. The transferase activities of recombinant UGT forms toward propofol and its analogs were determined at three substrate concentrations of 5-500 μM as indicated.

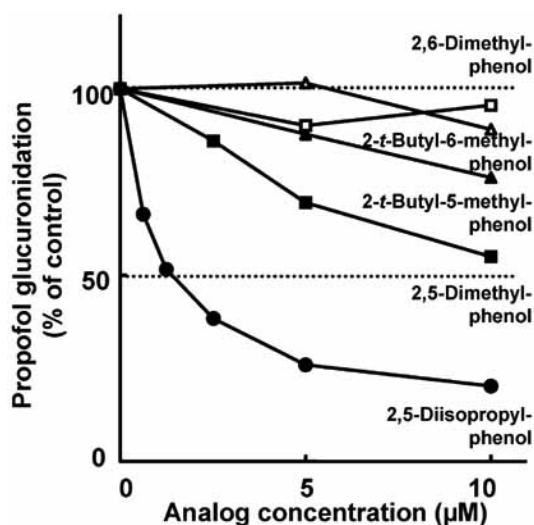


Fig. (3). Effects of propofol analogs on glucuronidation of propofol by UGT1A9. Recombinant UGT1A9 (0.5 mg microsomal protein/ml) was incubated with propofol (10 µM) for 10 min at 37°C in the presence of 3 mM UDPGA and the propofol analogs.

reaction. The potency of inhibition was in the order of 2,5-diisopropyl-, dimethyl-, and 2-*tert*-butyl-5-methyl-derivatives. Apparent 50% inhibition concentration (IC_{50}) of 2,5-diisopropylphenol to propofol glucuronidation by UGT1A9 was 1.0 µM. An Eadie-Hofstee plot for propofol glucuronidation by human liver microsomes in the absence or presence of 2,5-diisopropylphenol revealed that 2,5-diisopropylphenol showed inhibition in a non-competitive manner with a K_i value of ~16 µM (not shown).

Regarding propofol glucuronidation, it has been proposed contribution of UGT1A9 [6] which is commonly expressed in human livers and intestines [1,2]. The present results suggest the possibility of reducing dose of propofol by coadministration with 2,5-diisopropylphenol and of developing orally administrable agents including propofol and its analog. To investigate intestinal glucuronidation of small compounds with phenol group such as propofol, species difference should be paid an attention in terms of clinical and/or toxicological consequences.

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