PROTEIN-PROTEIN ASSOCIATION OF CYTOCHROME P450 AND UDP-GLUCURONOSYLTRANSFERASE: ITS RELEVANCE TO ENZYME FUNCTION
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Drug oxidation and conjugation mediated by cytochrome P450 (P450) and UDP-glucuronosyltransferase (UGT) have long been considered to take place separately. However, in order to minimize toxicity, it would be reasonable to expect that the reactive metabolite produced by P450 is directly transferred to the other enzymes participating in its subsequent metabolism (e.g., the UGTs) via protein–protein interactions. Accumulating evidence provided in our laboratory has suggested interactions between P450 and UGT resulting in a change in UGT function (1-4). Further, we have found recently that UGTs also modulate P450 function. In this symposium, we summarize these interactions, and discuss its relevance to functional alteration.

Protein-protein association of P450 and UGT. We have suggested that CYP3A4 specifically associates with UGT2B7 and alters the regioselectivity of morphine glucuronidation (2). Protein-protein interaction of CYP3A4 and UGT2B7 was confirmed by means of immunoprecipitation, overlay assay and cross-linking. The results obtained suggested that CYP3A4 lacking the N-terminal membrane-binding domain (Tyr25–Ala503) retains the ability to associate with UGT2B7, whereas the Met145–His267 fragment lacks this function. One of the anti-CYP3A4 antibodies, which has an ability to interfere with co-immunoprecipitating UGT2B7, was incapable of recognizing CYP3A4 when once CYP3A4-UGT2B7 complex was formed. The epitope of the antibody was mapped to a region located in the J-helix of CYP3A4. It is, therefore, suggested that the CYP3A4 J-helix contributes to the interaction with UGT2B7 (3, 4).

Modulation of P450 function by UGTs. The understanding whether UGT can modulate CYP activity is superficial, while we have learned that P450s modulate UGT function. Thus, we examined whether major human UGT isoforms, UGT2B7, 1A1, 1A6 and 1A9 modulate CYP3A4 function. Microsomes from human lymphoblast cells expressing both CYP3A4 and NADPH-P450 reductase (fp) and microsomes carrying a human UGT isoform were mixed and the activity of CYP3A4 was determined using Luciferin derivatives as substrates. The amount of UGT used was unified by adding control microsomes. CYP3A4 activity was enhanced by all UGTs in an added amount-dependent fashion. The activation by UGT2B7 was enhanced along with an increase in fp/CYP3A4 ratio. The enhancement was confirmed with both low and high affinity substrates. Similarly, UGT2B7 also enhanced the catalytic activities of CYP1A2, 2C8, 2C9, 2D6, 4A11 as well as 3A4. As far as human UGTs we tested, UGT2B7, 1A1, 1A6 and 1A9 are capable of enhancing CYP3A4-catalyzed oxidation. Although the mechanism should be clarified in future studies, UGTs may finely tune to activate the electron transfer from fp to CYP3A4.

References:

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