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An *in vitro* study on efavirenz metabolism and the CYP3A-mediated drug interaction of efavirenz and midazolam

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The benzodiazepine midazolam is widely used in anesthesia and intensive care. Midazolam is extensively metabolized by CYP3A following oral or intravenous application and has become one of the paradigm markers of CYP3A activity *in vitro* and *in vivo*. *In vivo* data suggested a possible acute activation of CYP3A4-catalyzed midazolam metabolism by efavirenz.

The objectives of the study were (1) to develop an *in vitro* method for the quantification of 1´-hydroxymidazolam concentrations by LC/MS/MS in presence and absence of a second substrate, (2) to assess the kinetics of midazolam 1´-hydroxylation in HLM and rCYP3A and to calculate apparent kinetic constants (V_{max}, K_m) in presence of efavirenz and 8-hydroxyefavirenz, (3) to clarify whether activation of CYP3A might be the underlying mechanism, and (4) to assess the kinetics of 8-hydroxyefavirenz formation in HLM and finally (5) to clarify whether the formation of x-efavirenz is catalyzed in a CYP-dependent reaction. A pre-existing analytical method for the quantification of midazolam and 1´-hydroxymidazolam in human plasma was further developed to allow the *in vitro* quantification of the compounds. The internal standard diazepam was exchanged for $^{13}C_3$-1´-hydroxymidazolam and d_5-midazolam and

the labeled standards allowed a more precise quantification, in particular at high midazolam concentrations. The formation of 1′-hydroxymidazolam was studied in HLM, rCYP3A4, and rCYP3A5 in the presence of efavirenz (0.5, 1, and 5 μM). Product formation rates (V_{max}) increased with increasing efavirenz concentrations (~1.5-fold increase at 5 μM efavirenz in HLM and ~1.4-fold in rCYP3A4). The activation in rCYP3A4 was dependent on cytochrome b_5 and the activating effect was also observed in rCYP3A5 supplemented with cytochrome b_5, where V_{max} was ~1.3-fold enhanced. Concomitant inhibition of CYP3A activity with ketoconazole in HLM abolished the increase in the 1′-hydroxymidazolam formation rate, further confirming involvement of CYP3A. The results of this study represent a distinct acute activation of midazolam metabolism and support the *in vivo* observations. Moreover, only efavirenz but not its major metabolite 8-hydroxyefavirenz, was responsible for the activation. The increase in 1′-hydroxymidazolam formation may have been caused by binding of efavirenz to a peripheral site of the enzyme, leading to enhanced midazolam turnover due to changes at the active site.

Efavirenz primary and secondary metabolism is catalyzed by HLM. Incubation of efavirenz with HLM leads to formation of significant amounts of its main metabolite 8-hydroxyefavirenz, although CYP2B6 is the major catalyst for this pathway. However, a new structure, which was identified and isolated in a clinical study, was not formed in a CYP-dependent reaction. Moreover, so-called x-efavirenz may be formed by conversion of 8-hydroxyefavirenz under the influence of organic solvents.