3.4 Discussion

The drug-drug interaction between enoxacin (ENX) and theophylline is severely underpredicted when reversible inhibition by the parent drug is the only considered mechanism (Obach et al., 2006). The interaction of ENX with caffeine, a more sensitive *in vivo* probe of CYP1A2 activity based upon fraction metabolized, is accordingly greater. While there has been a long-standing consensus that the increase in exposure to theophylline and caffeine is due to inhibition of CYP1A2 by ENX *in vivo*, no plausible mechanism which can account for the magnitude of the interaction has been identified.

The interaction between ENX and theophylline was first reported and studied by Wijnands et al (1984). At a therapeutic dose, ENX raised the plasma concentration of theophylline 3.5-fold without affecting renal clearance or plasma protein binding. We note here that the full increase in theophylline exposure developed gradually over five days. The plasma concentration of theophylline was also measured on day three of this study, at which point theophylline concentration had only increased 2.0-fold. A similar time-dependence for inhibition of theophylline clearance was once again captured in another study two years later (Wijnands et al., 1986). The time-scale for reaching the full inhibitory effect on theophylline metabolic clearance is consistent with, albeit not exclusive to, a mechanism whereby metabolites of ENX with greater inhibitory potential than the parent drug must be generated. Furthermore, since ENX is a substrate of CYP1A2, an investigation into whether a metabolite of ENX may serve as a mechanism-based inhibitor was warranted.

The possibility that inhibition of CYP1A2 by ENX involves mechanism-based inactivation has been raised in the literature (Spaldin et al., 1994). However, a detailed mechanism for this process has never been described. To fill this knowledge gap, the current study sought to expose any linkage between the metabolism of ENX and its inhibitory effect upon CYP1A2. Previously, work performed in this laboratory uncovered a potential role for the secondary

hydroxylamine metabolite, N-OH-ENX, in mediating inactivation of CYP1A2 (Smith, 2007). Specifically, the formation of a metabolic-intermediate complex (MI complex) due to a ring-opened nitroso metabolite of ENX coordinating to the ferrous heme-iron was suspected. However, formation of the MI complex could only be demonstrated with N-OH-ENX as the substrate and with poor reproducibility while evidence for MI complex formation from the parent drug, ENX, was lacking. Further assessment of these initial findings was needed to confirm whether or not MI complex formation is relevant to inhibition of CYP1A2 by ENX.

In this chapter, time-dependent inhibition of CYP1A2 by ENX was more thoroughly examined. Evidence was provided to support the hypothesis that metabolism of ENX enhances inhibition of CYP1A2. Ultimately, mechanism-based inactivation of CYP1A2 by a metabolite of ENX was suspected. MI complex formation was observed from both the parent drug and the N-hydroxylated metabolite, N-OH-ENX. The inactivation of CYP1A2 was highly efficient suggesting that a therapeutic dose regimen of ENX could substantially impact hepatic levels of CYP1A2. Interestingly, we observed no lags when monitoring MI complex formation or CYP1A2 activity loss. Mechanistically, three oxidations and a ring-opening are required to convert ENX to a nitroso metabolite. As such, it appears that MI complex formation occurs both rapidly and efficiently in a process whereby intermediate species are not readily released from the CYP1A2 active site.

In a larger context, this work seeks to clarify the DDI between ENX and CYP1A2-substrates while also supporting the area of research dedicated to rationalizing metabolite-mediated DDI. It is important to note that two distinct scenarios are possible when assessing a metabolite-mediated enzyme inactivation. In one scenario, intermediate metabolites which are on-path to enzyme inactivation may be released from the active site. Once these intermediates reach sufficient levels, they will compete with their precursor and proceed towards enzyme inactivation. The kinetics of inactivation in these situations will be highly complex and heavily

influenced by incubation variables, such as enzyme concentration. The opposite scenario is one in which intermediates are rarely released from the enzyme active site in the steps leading to enzyme inactivation. As this appears to be the case in the present study, it is appropriate to treat the multiple enzymatic steps involved in the inactivation of CYP1A2 by ENX as a single step. A major finding of this study is the ability to predict a metabolite-mediated DDI using inactivation parameters obtained from the parent drug.