

Letters to the Editor

Comments on “Anti-Influenza Prodrug Oseltamivir Is Activated by Carboxylesterase Human Carboxylesterase 1, and the Activation Is Inhibited by Antiplatelet Agent Clopidogrel”

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Shi et al. (2006) conclude in their recent article that, because clopidogrel inhibits the hydrolysis and therefore the activation of oseltamivir, the concurrent use of these drugs would render the latter therapeutically inactive. In reaching their conclusions, we believe that the authors have extrapolated their findings beyond the scope of the study and have addressed neither the limitations of their *in vitro* dataset nor the clinical relevance of the drug concentrations evaluated, as illustrated below.

1) The experimental conditions used *in vitro* do not reflect the *in vivo* situation. For oseltamivir, the 50 μM concentration used corresponds to approximately 240 times the maximal plasma concentration obtained with the approved 75-mg twice daily regimen (unpublished observations). For clopidogrel, even the lowest concentration used in the *in vitro* assessments, at which the Shi et al. (2006) reported approximately 30% reduction in hydrolytic activity, was more than 400-fold greater than the plasma C_{max} of clopidogrel using the standard 75-mg once daily regimen (Slugg et al., 2000). Therefore, there is a considerable margin for the liver concentration to be elevated compared with plasma, with no expectation of a significant interaction taking place, especially if unbound clopidogrel concentrations are considered.

The *in vitro* assessments are based on static conditions and do not take into account the decay in the plasma concentrations with time. Therefore, the risk of a sustained interaction that might lead to therapeutic failure of oseltamivir is remote when one considers the small time window over which the highest clopidogrel concentrations are experienced *in vivo*. For example, after a high 600-mg dose of clopidogrel, the C_{max} was 38 ng/ml occurring within 1.4 h after administration, and concentrations rapidly decayed to undetectable levels with a mean \pm S.D. half-life of $1 \pm (0.5)$ h.

Furthermore, the assumption, implicit in the described *in vitro* methodology, is that an increase in oseltamivir prodrug concentrations will equate to a decrease in circulating carboxylate levels by the same percentage. However, this is not relevant to the *in vivo* situation for the following reason. Because metabolism to the carboxylate is the primary route of clearance for oseltamivir [renal clearance accounts for less

than 5% of the dose (He et al, 1999)], a large proportion of the oseltamivir area under the curve will remain to be converted to metabolite once the inhibition by clopidogrel subsides. This supports the view that estimates of the magnitude of increase in exposure to oseltamivir prodrug following concomitant administration with clopidogrel are probably greater than the magnitude of decrease in exposure to the carboxylate.

2) Shi et al. (2006) suggest that the relative concentrations of oseltamivir and clopidogrel are the primary determinant of interaction potential. Although this is partly true, negligible inhibition occurs if concentrations of an inhibitor (clopidogrel) and substrate (oseltamivir) are well below their respective K_i and K_M values, as would be the case using the approved clinical regimens. In fact, the primary driver for an interaction (in this case the magnitude of inhibition) is the relationship between the unbound concentration of the inhibitor (clopidogrel) and the K_i , a routine comparison that Shi et al. (2006) have not made. Citing previous work by Tang et al. (2006), the authors also assert that because clopidogrel is hydrolyzed faster by human carboxylesterase 1 (HCE1) than oseltamivir, clopidogrel is preferentially metabolized by this enzyme. This statement is not relevant to a discussion of the potential for an interaction and confuses the capability of clopidogrel to bind to HCE1 and inhibit oseltamivir hydrolysis (K_i), with the catalytic efficiency of HCE1 for clopidogrel hydrolysis (V_{max}/K_M).

3) Because HCE1 is a liver microsomal enzyme, it would have been reasonable to have used the drug-drug interaction risk assessment procedure recommended by the United States Food & Drug Administration for cytochrome P450 inhibitors (www.fda.gov/cder/guidance/index.htm; United States Food & Drug Administration, 2006), but this was not done. By applying this procedure to the data presented by Shi et al. (2006) and using both the approved 75-mg daily dose and the approved 300-mg loading dose of clopidogrel, the risk of a clinically significant interaction due to clopidogrel inhibition of oseltamivir hydrolysis is judged as remote. Using alternative methods [Ito et al. (1998)] that involve estimation of the unbound inhibitor concentrations at the enzyme level (taking into account the contribution of the systemic circulation and absorption), the same conclusion is reached.

4) Shi et al. (2006) observed reduced viability of transfected

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cells expressing HCE1 that were exposed to high concentrations of oseltamivir (up to 320 μM) but did not comment on how relevant these findings were in relation to the usual clinical exposures of oseltamivir (1500-fold less at 0.21 μM). In long-term toxicological studies in rats and monkeys, doses up to 1000 mg/kg oseltamivir were studied. The maximal plasma concentrations of oseltamivir carboxylate in these animals were approximately 65 $\mu\text{g/ml}$ (229 μM) and 93 $\mu\text{g/ml}$ (327 μM), respectively. Despite these high exposures, no cytotoxicity was found (unpublished observations).

5) Finally, as part of their argument for HCE1 being wholly responsible for the hydrolytic transformation of oseltamivir, Shi et al. (2006) cited pharmacokinetic studies in young children (He et al., 1999; Massarella et al., 2000; Oo et al., 2003; Pope et al., 2005) to support their point that very young children have reduced carboxylesterase capacity and that this affects the pharmacokinetics through delayed hydrolysis. In fact, neither of these statements is supported by the references cited, and it is inaccurate to infer such conclusions from half-life data alone. Indeed, Oo et al. (2003) reported the apparent clearances of oseltamivir (per kilogram body weight) in younger children to be higher than in older children and concluded that carboxylesterase activity in this age group is efficient. Pope et al. (2005) also showed that the range of expression and activity of HCE1 in this age group is similar to that of adults.

In conclusion, whereas this study provides useful information concerning the activation of oseltamivir by HCE1, the data presented indicate that, at the clinical concentrations achieved with oseltamivir and clopidogrel, the likelihood of any significant metabolic interaction between the two compounds is remote.

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Fowler et al. (2007) state that the clopidogrel-oseltamivir interaction (in vivo) is remote because the concentrations used in the in vitro metabolism study were much higher than the maximal plasma concentrations (C_{max}) for both oseltamivir and clopidogrel. To support the argument, they refer to the unbound C_{max} values observed in human pharmacokinetic studies. This argument is invalid because both agents undergo extensive hydrolysis in the liver during the absorption phase, and the observed plasma concentrations of the parent drugs represent only a small fraction of the absorbed dose (escaped from the first pass metabolism) (He et al., 1999). Based on the area under the concentration (AUC) time curve, the exposure to oseltamivir carboxylate was 20-fold higher than the exposure to the prodrug oseltamivir (He et al., 1999; Oo et al., 2002). Likewise, Taubert et al. (2004) reported that the AUC of clopidogrel carboxylate was 1500-fold greater than the AUC of clopidogrel. Such ratios with the predominance of the metabolites suggest that, in the absence of first pass effect, the initial plasma concentrations of the parent drugs would have been much higher than the observed plasma concentrations.

Although it is difficult to predict the exact concentrations in the liver during the first passage, estimates can be made with comfortable confidence according to the volume and flow rate of the portal blood. In healthy adults, the volume of portal blood space is approximately 450 ml, and portal blood flow rate is ~600 ml/min (Hofmann et al., 1983). Therefore, in the first 30 min after oral ingestion of a 75-mg dose of oseltamivir, approximately 18 liters of portal vein blood would pass through the liver. Assuming complete absorption, the concentration reaching the liver can be approximately 4100 $\mu\text{g/l}$ or 13 μM , which is close to the concentrations used in the study reported by Shi et al. (2006). Intuitively, such a small time window may have only limited pharmacokinetic impact. Based on the fact that the AUC value of oseltamivir carboxylate is predominantly greater than that of oseltamivir and that the metabolite rapidly exceeds the parent drug in the blood (He et al., 1999; Abe et al., 2006), the majority of absorbed oseltamivir is hydrolyzed during the first pass of the liver. Apparently, the effective hydrolysis is attributed by the initial high concentrations in the liver. However, when

clopidogrel is coadministered, the initial hydrolysis is inhibited. As a result, the activation of oseltamivir would rely on the subsequent exposure via the systemic circulation. The hydrolysis upon the systemic circulation is less effective, because the plasma concentration is much lower than the initial hepatic concentration. Therefore, only the initial inhibition alone may have profound effect on the overall activation of oseltamivir. The inhibited hydrolysis, on the other hand, would increase the blood concentration of oseltamivir, thus leading to increased elimination in the form of parent drug.

There is a growing interest in developing a strategy to predict clinical drug-drug interactions based on in vitro metabolism data. The widely studied system is the metabolism catalyzed by the cytochrome P450 superfamily proteins. In this system, the ratio of the concentration of an inhibitor (I) over its inhibitory constant (K_i) provides useful predictive information (Wienkers and Heath, 2005; Bachmann, 2006). However, in some cases, the predictability is confounded by certain variables (e.g., the selection of inhibitor concentrations). It remains to be determined whether this method provides similar predictability for carboxylesterase-based metabolism. Nevertheless, Fowler et al. (2007) state that they used this procedure and predicted that the clinical interaction between oseltamivir and clopidogrel is remote. Apparently, they used the plasma but not the liver concentrations for their predication. Strictly speaking, metabolism-based interaction is determined by the relative concentrations at the site of the enzyme involved in the metabolism. In this case, the liver concentrations should be used.

It should be noted that in vitro metabolism studies are usually conducted with relatively high concentrations in order for the assay conditions to be properly controlled. The United States Food and Drug Administration (2006) recommends that inhibition studies be performed with a substrate concentration below its K_m and no more than 10 to 30% depletion of substrate (i.e., oseltamivir) and inhibitor (i.e., clopidogrel) during the assay. In addition, the inhibition of oseltamivir hydrolysis by clopidogrel is competitive in nature (Shi et al., 2006); thus, the inhibition should be dictated by the concentration ratio (clopidogrel over oseltamivir) but not by the absolute concentrations. It is reasonable to believe that similar magnitude of inhibition would have been detected with substantially lower concentrations of oseltamivir and clopidogrel (probably with less enzyme proteins).

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ABBREVIATIONS: AUC, area under the concentration; HCE1, human carboxylesterase.

The cell-based assay provides important mechanistic insight regarding oseltamivir-induced toxicity. Although as high as 320 μM was used for the toxicity study, significant decreases on cell viability were detected at 10 μM (Shi et al., 2006). More importantly, the toxicity was detected in oseltamivir-hydrolytic cells only, suggesting that the intracellular accumulation of the hydrolytic metabolite is responsible for the toxicity. Fowler et al. (2007) state that studies in rats and monkeys detected no toxicity, even though these animals produced high plasma concentrations of the hydrolytic metabolite ($\sim 229\text{--}327 \mu\text{M}$). The precise mechanism on the discrepancy remains to be determined. It is likely that the whole animal studies used toxicological endpoints less sensitive than the cell-based assay. Alternatively, these animals produced the metabolite by serum carboxylesterases, and the negatively charged metabolite stayed in the plasma. Li et al. (2005) reported that there is no serum carboxylesterase in humans. In contrast, animals such as rats express high levels of serum carboxylesterases (Yan et al., 1995), and the serum carboxylesterases presumably hydrolyze oseltamivir as well.

While producing a large amount of hydrolytic metabolite of oseltamivir, hepatocytes may exhibit less toxicity because these cells usually express high levels of transporters. Based on the predominance of the metabolite over the parent drug in the blood (He et al., 1999; Oo et al., 2002), the hepatic effluxing of the metabolite is very effective. On the other hand, decreased hepatic hydrolysis may increase the concentrations of oseltamivir in other types of cells and induce toxicity, particularly in the cells that efficiently hydrolyze oseltamivir but lack the ability of effluxing the metabolite. In humans, there are several mechanisms that may support reduced hepatic hydrolysis of oseltamivir. First, certain drugs concurrently administered may profoundly inhibit the hydrolysis of oseltamivir as suggested with clopidogrel (Shi et al., 2006). Second, people express polymorphic variants of HCE1 with markedly decreased ability to hydrolyze this agent (Shi et al., 2006). And finally, certain cytokines released during influenza infection may down-regulate the expression of HCE1.

In summary, the article by Shi et al. (2006) reports several important findings regarding the metabolism of oseltamivir, cytotoxicity, and potential interaction with clopidogrel. As discussed in the article, although the *in vitro* metabolism

study revealed profound inhibition, *in vivo* interaction between oseltamivir and clopidogrel remains to be established in humans. The cell-based assay provides a molecular explanation on how oseltamivir may induce cytotoxicity, but such explanation may apply to specific types of cells that hydrolyze oseltamivir but lack the ability of effluxing the metabolite.

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