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Rapid Degradation of Oseltamivir Phosphate in Clinical Samples by Plasma Esterases

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The anti-influenza drug oseltamivir is an ester prodrug activated by hepatic carboxylesterases. Plasma esterases also convert up to 31.8% of the parent compound to the active metabolite after 4 h ex vivo, with wide interindividual variation. This source of error is removed by adding the esterase inhibitor dichlorvos to blood collection tubes.

The threat of pandemic human H5N1 influenza has focused attention on the control and treatment of this potentially devastating disease (8). The only effective orally bioavailable antiviral agent currently licensed for this purpose is the neuraminidase inhibitor oseltamivir (OP) (13), which is being stockpiled for mass use. Although OP has been evaluated in community settings for the prevention and treatment of epidemic seasonal influenza (4), information on the therapeutic response in acute severe disease caused by the H5N1 influenza virus is limited (2).

OP is an ethyl ester prodrug hydrolyzed in vivo, principally at first pass, by high-capacity hepatic carboxyesterases to the active metabolite OP carboxylate (OC) (3, 9). Oral bioavailability is approximately 80%, and OC exposure is reduced during hepatic impairment (11). Plasma concentrations of OP and OC are similar at OP peak concentration (C_{max} 2 h postdosing, while maximum concentrations of OC (at 5 h) are typically fivefold higher than maximum concentrations of OP (3). OC is <3% bound to plasma proteins and is eliminated primarily by renal filtration and active secretion, with <5% of OP excreted unchanged by this route (3). No other metabolites have been identified to date.

Human plasma contains four different esterases, butyrylcholinesterase, paraoxonase, albumin esterase, and acetylcholinesterase (7), although the last binds to the membrane of erythrocytes, which contain additional cholinesterases (10). Butyrylcholinesterase is important for drug activation (bambuterol, isosorbide diaspirinase) and also inactivation (suxamethonium, cocaine, and organophosphosphorus pesticides) (7). Activity is age and sex dependent (5). The genetic locus controlling butyrylcholinesterase expression is highly polymorphic (6, 12), resulting in clinically significant variations in activity (1). We therefore investigated whether the activity of these blood esterases after sample collection might bias the quantification of OC in blood and plasma samples by ex vivo degradation or conversion of OP into OC.

The kinetics of ex vivo hydrolysis under various processing conditions were studied initially in blood obtained from a single healthy volunteer. OP (F. Hoffmann-La Roche Ltd.) dissolved in water was added to heparinized blood to produce a concentration of 10,000 ng/ml and incubated for 30 min at 37.5°C to allow equilibration. Aliquots were then exposed to three differing conditions in a complete two by two by two factorial design: storage as blood or after immediate processing as plasma, storage on ice or at 25°C, and presence or absence of the organophosphate esterase inhibitor dichlorvos (Sigma-Aldrich) at a concentration of 200 μg/ml blood (dichlorvos was diluted in acetonitrile). Triplicate 0.1-ml plasma samples were analyzed at hourly intervals for up to 4 h by liquid chromatography (LC) using solid-phase extraction (SPE) as described previously (14) and UV detection at 220 nm (Fig. 1). Since the extinction coefficients for OP and OC are equivalent at 220 nm (data not shown) and OC is the only product formed by OP, the combined peak area of the two in each sample was used to normalize (i.e., OC acts as an internal standard for OP and vice versa) the amount of remaining OP by using the formula $OP_R = 100 \times A_{OP}/(A_{OP} + A_{OC})$, where OP_R is the percentage of OP remaining, A_{OP} is the peak area of OP, and A_{OC} the peak area of OC.

Significant conversion of the parent compound to the metabolite occurred at room temperature in the absence of dichlorvos, reaching 15.1% by 4 h (paired *t*-test $P < 0.001$). Ex vivo conversion was not arrested completely, even when the samples were placed on ice, reaching 1.6% after 4 h (paired *t*-test $P = 0.006$). In the presence of dichlorvos, no significant change was observed over this period (mean at 4 h irrespective of temperature, 0.4%; paired *t*-test $P = 0.13$). Essentially identical results were obtained for samples allowed to stand as blood or plasma.

Eight healthy volunteers (four male, four female; mean age, 32 years) were then studied to examine the interindividual variability of hydrolysis. Heparinized blood samples were drawn, and plasma was obtained after centrifugation. Plasma (5 ml) was transferred into a tube containing dichlorvos at 200 μg/ml,

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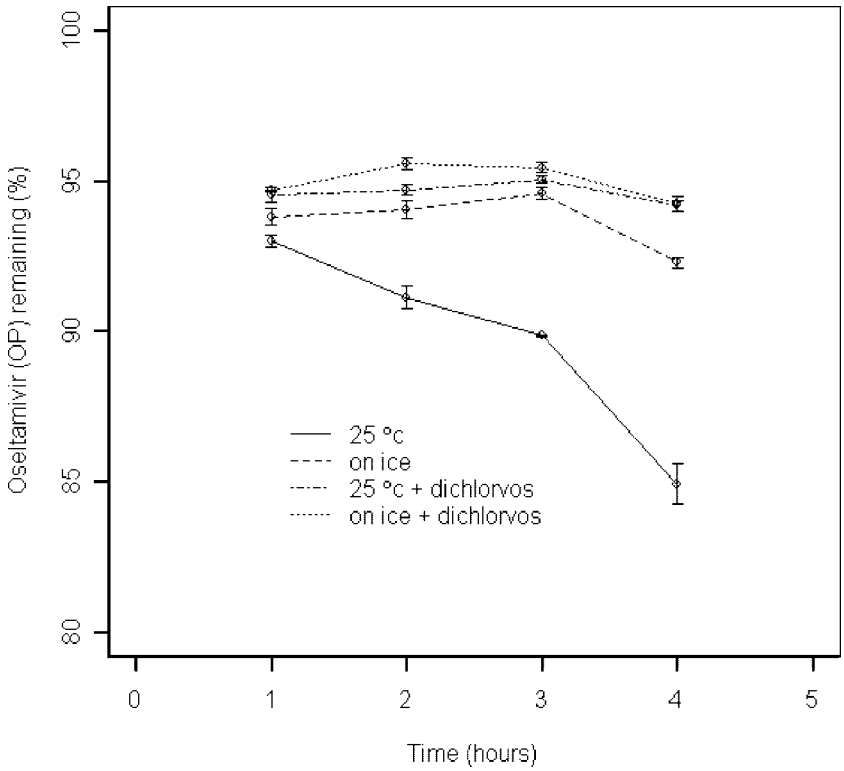

FIG. 1. Degradation of OP in plasma over time as a function of temperature and dichlorvos ($n = 3$, mean with 95% confidence interval shown as error bars). For SPE and LC methodology, see Materials and Methods.

and 5 ml was transferred to a tube without the inhibitor. OP in water (to produce 10,000 ng/ml plasma) was then added, and samples were equilibrated for 30 min at ambient temperature. Triplicate 100-μl plasma samples from each tube were removed at 1, 2, 4, 7, and 24 h, and the normalized OP_R was determined (Fig. 2). There was a minimal trend with time for samples containing dichlorvos with a median change over 24 h of only 1.1% (interquartile range, 0.9 to 1.4; maximum, 1.6%). At all time points, samples without dichlorvos showed a significantly greater reduction in OP levels (paired Wilcoxon test P values of <0.001 for all time points), falling steadily over time to a median of 93.1% at 4 h, 90.5% at 7 h, and 70.6% at 24 h. Importantly, the interindividual variability of hydrolysis was high, with the volunteers with the lowest and highest conversions reaching normalized OP_R levels of 94.9% and 68.2% after 4 h and 81.2 and 13.4% after 24 h, respectively. There was no significant association of the OP conversion rate (using OP concentrations at 24 h) with age or sex (Spearman correlation $P = 0.30$ and Wilcoxon test $P = 0.34$), but the conversion rate increased with the body mass index (Spearman correlation $P = 0.06$).

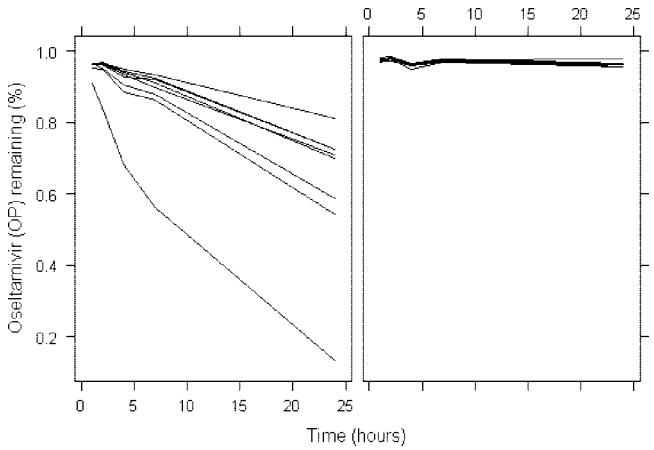

FIG. 2. Degradation of OP in plasma from eight healthy volunteers over time in the absence (left panel) or presence (right panel) of dichlorvos (mean $n = 3$). For SPE and LC methodology, see Materials and Methods.

We conclude that plasma esterase activity can cause significant degradation of OP in blood and plasma samples under conditions likely to be encountered during clinical studies and during assay preparation. This activity may show large variability between individuals and study populations (5, 6, 12). Our results are consistent with a possible systematic overestimation of plasma OC concentrations by as much as 10% during the initial phase of the pharmacokinetic profile. This source of bias could distort all pharmacokinetic parameters, especially parameters related to the absorption phase, such as the maximum concentration and the absorption rate constant. Degradation of OP by plasma esterase activity is not completely arrested, even when samples are kept continuously on ice. Maintenance of the cold chain and rapid processing of samples cannot always be guaranteed under clinical study conditions. We therefore recommend that future studies of the clinical pharmacokinetics of OP

incorporate the use of inhibitors of plasma esterase activity to avoid these potential problems.

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