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Short Communication

Characterization of the binding affinities of peramivir and oseltamivir carboxylate to the neuraminidase enzyme

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ABSTRACT

With the continued threat of morbidity and mortality from influenza and the development of resistance to influenza antiviral drugs, there is increasing interest in new treatments, such as the investigational intravenous drug peramivir, and in combination treatments. In this study, we determined the impact of oseltamivir carboxylate on the binding affinity of peramivir/neuraminidase (NA) enzyme complex and vice versa. Influenza NA was incubated with peramivir and oseltamivir carboxylate alone and in combination. Dissociation rates of the enzyme–inhibitor complex measured in the presence of NA substrate for peramivir alone and the combination were similar, suggesting that peramivir competitively inhibits the neuraminidase enzyme and that oseltamivir carboxylate when added to peramivir does not impact the binding affinity of peramivir to the NA enzyme.

Combinations of antiviral drugs may be administered purposely or inadvertently to treat influenza virus infections, especially in seriously ill and hospitalized patients or patients found to be infected with drug-resistant viruses. During the 2009 H1N1 pandemic, the investigational intravenous antiviral drug, peramivir, was made available to severe hospitalized patients with influenza through clinician request under FDA Emergency IND regulations and later through Emergency Use Authorization, and many of the peramivir-treated patients concomitantly received oral oseltamivir (Hernandez et al., 2011; Sorbello et al., 2010), a marketed antiviral effective in both prophylaxis and treatment of influenza A and B viruses (Hayden et al., 1999; Kaiser et al., 2003). Peramivir has been approved for the treatment of influenza in Japan and South Korea and is in Phase 3 clinical trials elsewhere, making its use in combination with oseltamivir a real possibility.

Both oseltamivir and peramivir are neuraminidase (NA) inhibitors that bind to the NA site and inhibit the release of virions from infected cells and reduce further infection by causing aggregation of released virion (Mendel et al., 1998), but peramivir appears to have a tighter binding affinity than oseltamivir carboxylate (Bantia et al., 2006) and demonstrates in vitro activity that is comparable or better than oseltamivir carboxylate, the active metabolite (Babu et al., 2000; Bantia et al., 2001; Govorkova et al., 2001).

In vitro cell culture studies and in vivo animal studies have not shown any antagonism between peramivir and oseltamivir (Smee et al., 2010). Instead, additive to synergistic responses were observed (Smee et al., 2010). The purpose of the current investigation was to determine the impact of oseltamivir carboxylate on the formation of the peramivir/NA enzyme complex and the impact of peramivir on the formation of the oseltamivir carboxylate/NA enzyme complex.

The influenza A virus used in this study, A/NWS/33 (H1N1), was obtained from American Type Culture Collection (ATCC), Manassas, VA. Peramivir and oseltamivir carboxylate were synthesized by BioCryst Pharmaceuticals, Inc. (Birmingham, AL). The substrate, 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid (MuNANA), and 2-morpholinoethanesulfonic acid (MES) for the assay buffer were purchased from Sigma–Aldrich (St. Louis, MO). Bio-Spin P-6 columns were purchased from Bio-Rad.

Serial dilutions (5–100 fold) of H1N1 A/NWS/33 influenza virus were prepared in assay buffer (32.5 mM MES and 4 mM $CaCl_2$ in diH_2O, pH 6.25) and incubated with MuNANA substrate for 30 min at RT. A standard fluorometric assay was used to measure NA enzyme activity as fluorescence (excitation: 360 nm and emission: 450 nm). The lowest virus dilution (concentration) required to yield a signal to background ratio (S/B) >5 was selected for use in the assay. Influenza NA (H1N1 virus) was incubated at 37 °C for 1 h with one of the following: buffer alone (control), peramivir alone at a concentration of 100 nM, oseltamivir carboxylate alone at a concentration of 100 nM, or a combination of peramivir and oseltamivir carboxylate (100 nM each), to form an enzyme–inhibitor (EI) complex. The IC_{50}s of peramivir and oseltamivir were 0.11 and 0.69 nM, respectively, and the IC_{90}s were 0.72 and 9.14 nM, respectively, against this enzyme (Bantia et al., 2001). A final

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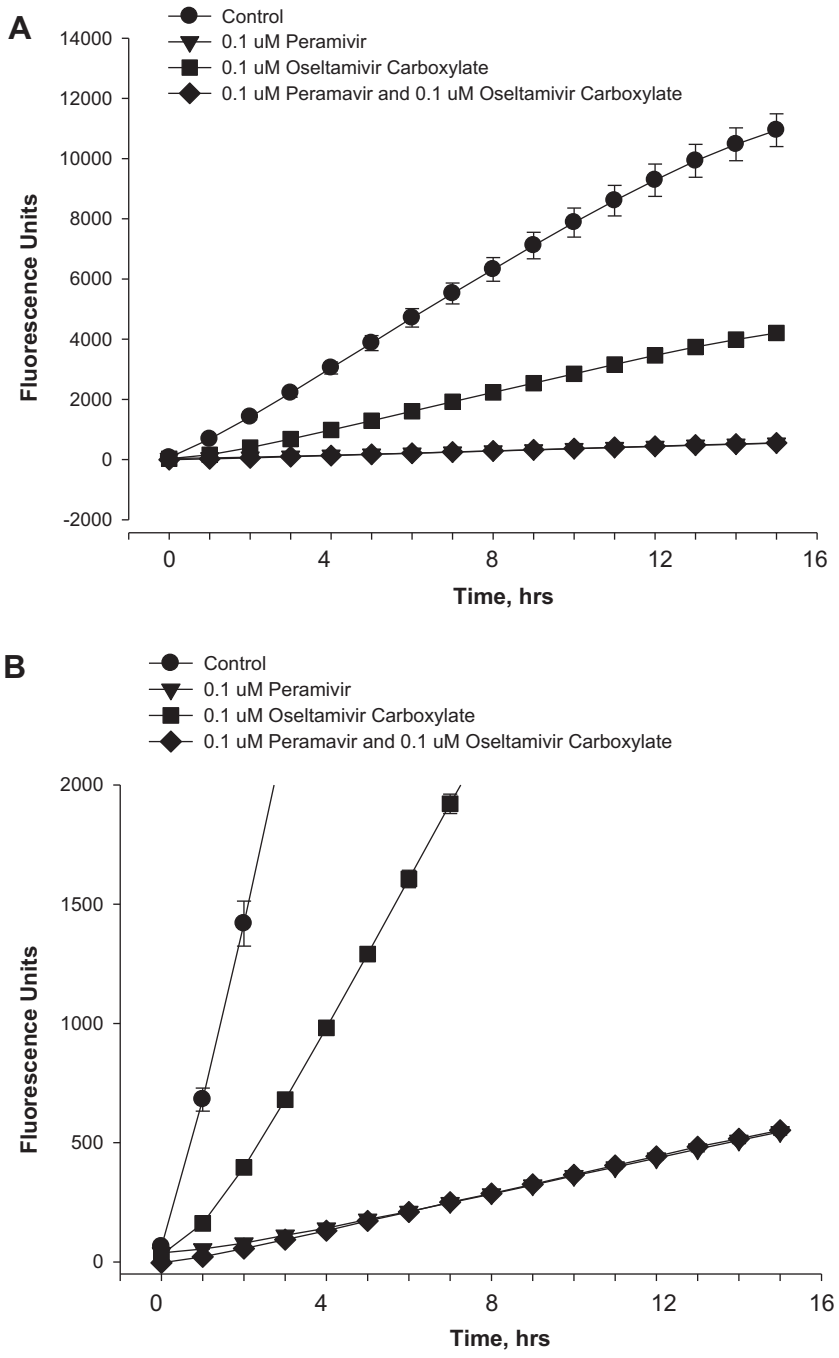

Fig. 1. Dissociation rates of the EI complex of peramivir and oseltamivir carboxylate. (B) is derived from (A), i.e., *Y* scale is expanded to show the difference in the initial rates.

concentration of 100 nM, which is at least 100-fold greater than the IC_{50} and 10-fold greater than the IC_{90} of these compounds, was chosen in an attempt to achieve almost complete saturation of the enzyme with the inhibitor.

Free compound was removed from the complex by passing through a Bio-Spin P-6 column, and the on-site dissociation rates of the EI complex were measured by mixing the complex with substrate (75 μM MuNANA). The substrate MuNANA was cleaved by the dissociated free enzyme to yield a product that could be quantified by fluorometry (excitation: 360 nm and emission: 450 nm). Results were based on an average of three measurements. The data were analyzed by Sigma Plot (Windows Version 10.0, SPSS, Chicago, IL) and Sigma Stat (Windows Version 3.5, Jandel Corporation, San Rafael, CA) software.

The rate of product formation in the control group (buffer alone) was 716 fluorescence units (FU)/h. As shown in Fig. 1, peramivir was tightly bound and had a slow off-rate from the EI complex resulting in a lower rate of product formation (37 FU/h) compared with oseltamivir carboxylate (294 FU/h). The rate of product formation of the EI complex derived from an equimolar combination of peramivir and oseltamivir carboxylate (37 FU/h) was similar to the rate derived from peramivir alone.

Peramivir and oseltamivir carboxylate are potent inhibitors of A/NWS/33 neuraminidase enzyme with IC_{50} and IC_{90} values in the picomolar to nanomolar range (Bantia et al., 2001). The results of this study suggest that peramivir competitively inhibits the neuraminidase enzyme and that oseltamivir carboxylate when added to peramivir does not impact the binding affinity of perami-

vir to the NA enzyme, although these are in vitro observations only and may not necessarily be replicated in vivo. These results also confirmed previous studies (Bantia et al., 2006) demonstrating that peramivir binds to the influenza NA with much tighter binding affinity than oseltamivir carboxylate. NA enzyme from the influenza strains resistant to peramivir and/or oseltamivir was not assessed in this study.

Previous in vitro and in vivo animal studies with a combination of peramivir and oseltamivir were undertaken with the assumption that additive or antagonistic effects are possible since both drugs had the same mechanism of action and different binding affinities (Smee et al., 2010). Smee et al. (2010) found that the combination did not lead to antagonism but rather produced additive to synergistic responses. When influenza-infected mice were treated concomitantly with oral oseltamivir and intramuscular peramivir, consistent and significant improvements in weight loss and mortality were seen compared with single drug treatments.

There remains significant morbidity and mortality worldwide from the influenza virus despite the development of effective antivirals, and the recent 2009 H1N1 pandemic virus was particularly virulent (WHO 2010). Clearly, new strategies for treating influenza are needed. While the adamantanes, including rimantadine and amantadine, are effective against influenza A (Jefferson et al., 2006) and in preclinical studies demonstrate synergistic efficacy when used in combination with oseltamivir (Govorkova et al., 2004; Galabov et al., 2006) and peramivir (Bantia et al., 2010), the prevalence of resistant viruses has diminished their usefulness (Deyde et al., 2007; Bright et al., 2006; Nelson et al., 2009). Likewise, virus variants resistant to oseltamivir and zanamivir, the other marketed NA inhibitor, have spread (Moscona, 2009; Hurt et al., 2009). Combination treatments may not only be synergistic and, thereby, more efficacious than single treatments, but they may delay the emergence of drug-resistant viruses (Ilyushina et al., 2006).However, one report suggests antagonism between NAIs in vitro (Nguyen et al., 2010), and clinical antagonism between oseltamivir and zanamivir was suggested in another report (Duval et al., 2010). Recently, Chavas et al. (2010) reported apparent in vitro inhibition of human sialidases by peramivir and zanamivir and recommended monitoring for potential related adverse reactions.

In the many cases of human treatment with concomitant peramivir and oseltamivir during the 2009 H1N1 pandemic, there were no apparent safety and tolerability problems linked to this combination treatment (Hernandez et al., 2011; Sorbello et al., 2010). However, efficacy of the combination could not be established in that uncontrolled environment, and careful evaluation in clinical trials is warranted. Peramivir and oseltamivir when administered alone in Phases 2 and 3 clinical trials demonstrate similar clinical efficacy and tolerability in outpatients (Kohno et al., 2009) and hospitalized adults with seasonal influenza (Ison et al., 2009).

The results of the current study demonstrating that oseltamivir carboxylate, the active metabolite of oseltamivir, does not impact peramivir binding provide further indication that it may be possible to administer peramivir and oseltamivir in combination to patients with influenza.

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