ABSTRACT

Objectives: To investigate potential species-dependence of Oseltamivir hydrolysis in plasma and tissue preparations from human, dog, rat and mouse. Methods: Oseltamivir was incubated with a) intestinal S9 fractions, b) liver S9 fractions, and c) plasma, from different species. The Oseltamivir remaining in the incubate and the Oseltamivir carboxylate formation were determined by LC/MS/MS. Results: In human liver S9 fraction Oseltamivir underwent rapid degradation, with a 31 min half-life; however, it showed little degradation in intestinal S9 fractions and plasma. In rat and mouse plasma, the half-lives of Oseltamivir were, respectively, less than 1 and 10 minutes. In contrast, Oseltamivir underwent minimal degradation in liver S9 fractions from both rat and mouse. Conclusions: The observed differences in Oseltamivir hydrolysis found in the current study highlight the challenge associated with trying to predict in vivo metabolism based on in vitro data and with the interpretation of results from different species.

INTRODUCTION

Oseltamivir (Tamiflu®) is an ester pro-drug, and its carboxylate derivative is the pharmacologically active form. Since the introduction of oseltamivir into the market by Roche (1999 in the US and 2002 in Europe), oseltamivir has been prescribed to approximately 42 million people in 80 countries (1). Some patients who took oseltamivir exhibited abnormal behaviors and committed suicide. Neuropsychiatric disorders reported in 12 children (mainly in Japan) included delirium, behavioral abnormalities, hallucinations, convulsions and confusion. The proposed link between the use of oseltamivir and the occurrence of abnormal central nervous system (CNS) behavior is controversial. A number of studies have attempted to determine whether there is a causal relationship between the administration of oseltamivir and central nervous system disorders. In humans, the conversion of oseltamivir to oseltamivir carboxylate mainly occurs in the gastrointestinal tract and liver. Therefore, in the present study we selected intestinal S9 fractions and liver S9 fractions prepared from species commonly used in pre-clinical studies as test matrices. In addition, because plasma contains high levels of esterases, the stability and conversion profiles of oseltamivir was compared in plasma collected from several species.

MATERIALS AND METHODS

Materials: Oseltamivir phosphate and oseltamivir acid were obtained from Toronto Research Chemicals (Toronto, Canada). Human, dog, mouse, and rat plasma were obtained from Bionacrol (Hicksville, NY). Human, dog, mouse, and rat liver S9 fractions were obtained from Xenotech (Lexena, KS). Human and dog intestinal S9 fractions were prepared by Absorption Systems, LP. Methods: Oseltamivir was added to all tested matrices to a final concentration of 10 μM. All incubation reactions were conducted in a 37°C water bath. All samples were combined with three volumes of ice-cold acetonitrile to terminate the reaction. LC/MS/MS conditions The LC equipment consisted of Perkin Elmer Series 200 autosampler and Agilent 1100 pump. Chromatography was conducted in the reverse phase mode using a Hypersil C18 column (30 x 2.0 mm id 3μm BDS) with a guard column. The mobile phase buffer was 25 mM ammonium formate buffer (pH 3.5); the aqueous reservoir contained 90% deionized water and 10% buffer (v/v); the mobile phase was 25 mM ammonium formate buffer (pH 3.5); the aqueous reservoir contained 90% deionized water and 10% buffer (v/v); the organic reservoir contained 90% acetonitrile and 10% buffer (v/v). The formation rates of oseltamivir in mouse and rat plasma were 350 and 324 pmol/min/mg protein, respectively. The formation rates of oseltamivir carboxylate (Figure 5A) were 1.7 and 1.0 pmol/min/mg protein for Donors 1 and 3, respectively. The initial rate of formation in plasma from Donor 2 was 12.6 pmol/min/mg protein. In dog plasma, the metabolite was undetectable before 6 hours, and after this time the formation rate of oseltamivir carboxylate was 0.83 pmol/min/mg protein.

RESULTS

• In human intestinal S9 fractions, after a 60-minute incubation the average percent remaining oseltamivir was in the range of 80% to 95% (Figure 1A-C). Oseltamivir was similarly stable in dog intestinal S9 fractions (Figure 2). Little or no inter-donor or inter-segment differences were observed in dog intestinal S9 fractions.
• Oseltamivir showed a very rapid degradation in human liver S9 fractions, with a calculated half-life of 31 minutes (Figure 3A). No appreciable degradation of oseltamivir was observed in dog, mouse, or rat liver S9 fractions.
• The degradation of oseltamivir was very rapid in mouse and rat plasma (Figure 4B). The half-life was 0.1 hour in mouse plasma and 0.003 hour in rat plasma. In contrast to the instability in mouse and rat plasma, the compound seemed to be stable in dog plasma, with the percent remaining approximately 90% after 24 hours (Figure 4B). The stability of oseltamivir in human plasma was intermediate between dog and rodent (Figure 4A).

CONCLUSION

The current study demonstrated that oseltamivir hydrolysis exhibits species- and site-dependent differences in in vitro systems. The species specificity reinforces the importance of finding appropriate non-human models to conduct clinically relevant studies with oseltamivir.

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ACKNOWLEDGEMENT
The authors would like to thank Dr. Chris Bode and Colleen O’Neill for technical assistance.