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Application of electrolysis for inactivation of an antiviral drug that is one of possible selection pressure to drug-resistant influenza viruses

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A B S T R A C T

The recent development of antiviral drugs has led to concern that the release of the chemicals in surface water due to expanded medical use could induce drug-resistant mutant viruses in zoonosis. Many researchers have noted that the appearance of an oseltamivir (Tamiflu®)-resistant avian influenza mutant virus, which may spread to humans, could be induced by oseltamivir contamination of surface water. Although past studies have reported electrolysis as a possible method for degradation of antineoplastics and antibacterials in water, the validity of the method for treatment of antiviral drugs is unknown. In this study, electrolysis was used to degrade an antiviral prodrug, oseltamivir, and a stable active form, oseltamivir carboxylate, and the degradation process was monitored with HPLC-UV and the neuraminidase inhibitory assay. HPLC-UV-detectable oseltamivir and oseltamivir carboxylate were decomposed by electrolysis within 60 min, and inhibitory activity of neuraminidase decreased below the detection limit of the assay used. Cytotoxic and genotoxic activity were not detected in electrolyzed fluid. These results indicate that electrolysis is a possible treatment for inactivation of the antiviral drug oseltamivir.

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1. Introduction

Oseltamivir (Tamiflu®) is a medicine originally invented as a treatment drug of seasonal influenza (WHO, 2003; Ward et al., 2005). Oseltamivir has been used as a prophylactic drug to prevent the infection to compromised hosts. Future influenza pandemic by a novel virus strain is discussing, and the possible way of emerging pandemic strain is the mutation of avian-origin influenza virus strains (Olsen et al., 2006; Järhult et al., 2011). Oseltamivir was proved to be effective to reduce human-to-human transmission of avian influenza virus (H5N1) (Schünemann et al., 2007). Because of these advantageous properties, oseltamivir is also recommended as a primary preventive antiviral drug in the event of an influenza pandemic (von Itzstein, 2007), and many individual nations and global organizations has stockpiled oseltamivir in the event of influenza pandemic (Harrod et al., 2006).

Orally administered oseltamivir and absorbed from the gastrointestinal tract and is immediately converted to the active metabolite oseltamivir carboxylate, a potent and selective inhibitor of influenza virus neuraminidase (Hill et al., 2002). Biotransformation from oseltamivir to oseltamivir carboxylate is illustrated in Fig. 1. The absolute bioavailability of oseltamivir is approximately 80% when 150 mg of oseltamivir is administered orally (Li et al., 1998; He et al., 1999). Oseltamivir carboxylate is entirely eliminated by renal excretion, and unabsorbed oseltamivir is excreted in feces (Li et al., 1998; He et al., 1999). Several animal species can convert oseltamivir to oseltamivir carboxylate, and excrete oseltamivir carboxylate from the urinary tract (Tamiflu® interviewform, 2009). Oseltamivir carboxylate is stable in the environment, and once the converted chemical enters the surface water, it may be consumed by waterfowl. In this way, when oseltamivir carboxylate reaches an intestinal tract of waterfowls, the virus which exists in the tract replicates and resistant mutants of the virus are induced (Singer et al., 2007). Recently, resistant mutants to oseltamivir carboxylate

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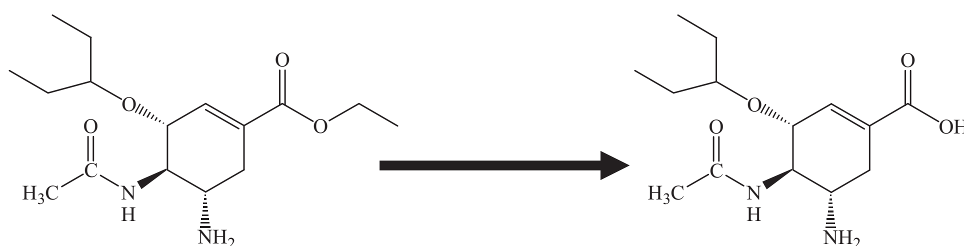

Fig. 1. Biotransformation of oseltamivir carboxylate from oseltamivir. Oseltamivir is catalyzed to the active metabolite, oseltamivir carboxylate by esterase *in vivo*. The reaction formula is shown.

in avian influenza viruses were actually isolated from wild birds (Orozovic et al., 2011).

If an oseltamivir carboxylate-resistant avian influenza virus was to spread as a pandemic in humans, vast oseltamivir phosphate stockpiles would not be helpful for medical treatment and prevention of avian-origin influenza. Therefore, detailed investigations of the persistence and degradability of oseltamivir carboxylate in the aquatic environment have been conducted (Singer et al., 2007; Bartels and von Tümpling, 2008; Saccà et al., 2009; Straub, 2009; Ghosh et al., 2010a,b). Furthermore, several studies have investigated methods for eliminating oseltamivir carboxylate before releasing sewage effluent to the surface water (Saccà et al., 2009; Ghosh et al., 2010a,b; Accinelli et al., 2010a,b; Gonçalves et al., 2011).

The potential for electrolysis methods to detoxify clinical wastewater has been evaluated. By this method, antineoplastics and antibacterials can be effectively inactivated (Hirose et al., 2005; Nakano et al., 2013). A bench-top electrolysis apparatus was shown to detoxify clinical wastewater containing antineoplastics with low energy consumption relative to another method of disposal (Kobayashi et al., 2008). Furthermore, the ability of the electrolysis method to inactivate antineoplastics in urine has been demonstrated (Kobayashi et al., 2012).

Oseltamivir and oseltamivir carboxylate solutions were electrolyzed on a small scale and whether our electrolysis method could decompose the chemicals was examined.

2. Materials and methods

2.1. Chemicals and reagents

Oseltamivir phosphate (Ro 64-0796) and D-tartrate of oseltamivir carboxylate (Ro 64-0802) were kindly provided by F. Hoffmann-La Roche (Basel, Switzerland). Sotalol hydrochloride was purchased from Tocris Bioscience (Ellisville, Missouri, USA). Ultrapure water was prepared with Yamato Millipore WQ 500 (Yamato Scientific, Tokyo, Japan) and used as the diluent.

2.2. Instruments and analytical condition

Oseltamivir and oseltamivir carboxylate concentrations before and after electrolysis were quantified with HPLC (high performance liquid chromatography)-UV (ultraviolet absorption) detection. The HPLC system (Shimadzu, Kyoto, Japan) consisted of a pump (LC-10ADvp system) and a UV detector SPD-10MAvp set at 220 nm. The analytical column, SUMIPAX ODS L-05-4615 (4.6 mm × 150 mm, 5 μm particle size) (Sumika Chemical Analysis Service, Osaka, Japan) was operated at an ambient temperature of 40 °C. The mobile phase was a 20 min linear gradient of 10% 100 mM phosphate solution to 90% acetonitrile at a flow rate of 1 mL/min. Sotalol hydrochloride was used as an internal standard (Joseph-Charles et al., 2007).

2.3. Stock and working standard solutions

Standard solutions of 320 μg/mL of oseltamivir phosphate and 303.2 μg/mL of D-tartrate of oseltamivir carboxylate were prepared. The 2 times serial dilution of each standard solution was prepared. The calibration curve was plotted with 13 point concentrations ranging from 0 to 320 μg/mL for oseltamivir phosphate, and from 0 to 303.2 μg/mL for D-tartrate of oseltamivir carboxylate, respectively. The internal standard solution (10 μg/mL of sotalol hydrochloride) was prepared. The calibration standards were freshly prepared on each day of analysis. To calculate peak area ratios, the oseltamivir and oseltamivir carboxylate peak areas were divided into the area of the internal standard. The theoretical concentration ratios were calculated as a ratio of oseltamivir or oseltamivir carboxylate concentration to the internal standard concentration. The calibration curve was generated from the peak area and theoretical concentration ratios.

2.4. Electrolysis procedure and neutralization of available chlorine

Solutions of 410.40 μg/mL of oseltamivir phosphate and 386.44 μg/mL of D-tartrate of oseltamivir carboxylate were prepared with 0.9% NaCl. These concentrations are equivalent to 1 mM oseltamivir and oseltamivir carboxylate solutions. A volume of 250 mL of oseltamivir or oseltamivir carboxylate solution was electrolyzed in a 300 mL glass beaker using a pair of electrodes (115 mm × 35 mm, placed 5 mm apart). These platinum-based iridium oxide composite electrode are advantageous for both their durability and their ability to generate available chlorine (Panizza and Cerisola, 2009). In this experiment, 1400–1530 mg/L of available chlorine was generated with 2 h of electrolysis. The electrodes were inserted into the beaker, and the solution was electrolyzed with stirring for 2 h at a constant current of 1 A. The current density was 2.48 A/dm^2. Samples were collected before and during electrolysis. A solution of 20% (w/v) sodium thiosulfate was used to neutralize the available chlorine that was generated by anodic oxidation during electrolysis, and the measurements were verified to ensure that the addition of 20% thiosulfate did not influence the results. The concentration of the samples was determined by analyzing 20 μL with the HPLC-UV system.

2.5. Determination of neuraminidase inhibitory activity

Inactivation of neuraminidase inhibitory activity of oseltamivir or oseltamivir carboxylate by electrolysis was measured using chemiluminescence assay (NA-Star, Influenza Neuraminidase Inhibitor Resistance Detection Kit, Life Technologies Japan, Tokyo, Japan) according to the manufacturer's instructions. For neuraminidase, human influenza virus A/Wyoming/3/2003 (H3N2) provided by Osaka Prefectural Institute of Public Health was used.

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