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Application of electrolysis to inactivation of antibacterials in clinical use

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ABSTRACT

Contamination of surface water by antibacterial pharmaceuticals (antibacterials) from clinical settings may affect aquatic organisms, plants growth, and environmental floral bacteria. One of the methods to decrease the contamination is inactivation of antibacterials before being discharged to the sewage system. Recently, we reported the novel method based on electrolysis for detoxifying wastewater containing antineoplastics. In the present study, to clarify whether the electrolysis method is applicable to the inactivation of antibacterials, we electrolyzed solutions of 10 groups of individual antibacterials including amikacin sulfate (AMK) and a mixture (MIX) of some commercial antibacterials commonly prescribed at hospitals, and measured their antibacterial activities. AMK was inactivated in its antibacterial activities and its concentration decreased by electrolysis in a time-dependent manner. Eighty to ninety-nine percent of almost all antibacterials and MIX were inactivated within 6 h of electrolysis. Additionally, cytotoxicity was not detected in any of the electrolyzed solutions of antibacterials and MIX by the Molt-4-based cytotoxicity test.

1. Introduction

The wide clinical use of pharmaceutical and personal care products (PPCPs), e.g., antibacterials, antiphlogistics, antiepileptics, beta-blockers, liquid regulators, vasodilators and sympathomimetics causes nvironmental contamination by these PPCPs through clinical wastewater and human excreta (Jφrgensen and Halling-Sφrensen, 2000; Stuer-Lauridsen et al., 2000; Längea and Dietrichb, 2002; Bound and Voulvoulis, 2004; Jjemba, 2006; Grung et al., 2008) indeed, various PPCPs have been detected in surface water of rivers, lakes, and wastewater treatment plants, and also in garbage plant exudation and wastewater from hospitals (Holm et al., 1995; Terns, 1998; Kümmerer, 2001; Kolpin et al., 2002; Seino, et al., 2004). Antibacterials are released from medical (including both human and veterinary clinics and hospitals), agricultural, stockbreeding, and fishing industrial settings (Heberer, 2002). Antibacterials are relatively stable and highly physicochemically active, and they may affect the ecology (Hirsch et al., 1999; Kümmerer, 2009a, 2009b). Contamination of surface water by antibacterials may affect aquatic floral bacteria (Boxall et al., 2003; Brain et al., 2004). These bacteria acquire drug-resistant genes in a contaminated environment, act as reservoirs of clinically important drug-resistant genes, and transfer the genes to human pathogenic bacteria (Young, 1993). Indeed, antibacterial-resistant bacteria were isolated from river water and lake water (Ash et al., 2002).

A similar assumption can be made in wastewater treatment plants. When such PPCPs flow into an activated sludge plant, bacteria in the sludge may be killed by contaminating PPCPs (Mezrooui and Baleux, 1994; Holm et al., 1995; Kümmerer, 2001; Reinthaler et al., 2003). If antibacterials at low concentrations are loaded to the sludge, antibacterial-resistant bacteria may be induced (Holzel et al.; 2010, Munir et al., 2011; Gao et al., 2012).

Recently, to eliminate antineoplastics in clinical wastewater, which is one of the origins of environmental contamination, an electrolysis treatment method has been developed (Hirose et al., 2005). A conventional apparatus suitable for clinical settings was also fabricated and evaluated to reduce the toxicity of clinical wastewater samples from a cancer chemotherapy center

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(Kobayashi et al., 2008). Moreover, this method is applicable to inactivating antineoplastics in urine (Kobayashi et al., 2012). The method mainly involves the oxidation of antineoplastics by active chlorine generated by anodic oxidation. In the present study, we demonstrate the possibility of applying the electrolysis method to the inactivation of antibacterials from clinical settings by showing the inactivation ability by bench-top scale batch experiment.

2. Materials and Methods

2.1. Antibacterial agents

Commercially available antibacterials commonly used in both human and veterinary clinical settings, mainly those packaged in vials for injection, were used in this study. The ten groups of antibacterials studied were penicillins, cephems, carbapenems, aminoglycosides, fosfomycin, glycopeptides, tetracyclines, chloramphenicols, macrolides and new quinolones. The representative chemicals selected from the ten groups were as follows: benzylpenicillin potassium (PCG, Meiji Seika, Tokyo, Japan) for penicillins, cefazolin sodium hydrate (CEZ; Astellas Pharma Inc., Tokyo, Japan) for cephems, imipenem hydrate and cilastatin sodium (IPM/CS, Banyu Pharmaceutical Co., Tokyo, Japan) for carbapenems, amikacin sulfate (AMK, Banyu Pharmaceutical) for aminoglycosides, fosfomycin sodium (FOM, Meiji Seika) for fosfomycin, vancomycin hydrochloride (VCM, Shionogi Co., Osaka, Japan) for glycopeptides, minocycline hydrochloride (MINO, Wyeth K.K., Tokyo, Japan) for tetracyclines, chloramphenicol sodium succinate, (CP, Daiichi-Sankyo Co., Tokyo, Japan) for chloramphenicols, erythromycin lactobionate (EM, ABBOTT Japan Co., LTD., Tokyo, Japan) for macrolides, and ciprofloxacin (CPFX, Bayer Yakuhin Co., Osaka, Japan) for new quinolones.

Each chemical was reconstituted or diluted with saline (0.9 percent sodium chloride solution) and the resulting solution was electrolyzed. In some experiments, the selected chemicals were mixed and used as experimental clinical wastewater.

2.2. Electrolysis and neutralization

A chemical solution in saline (200 mL) was electrolyzed in a 300 mL tall-form beaker using a pair of platinum-based iridium oxide composite electrodes (50×35 mm, placed 5 mm apart), which are advantageous both in the durability and in the ability of generating active chlorine (Panizza and Cerisola, 2009). The pair of electrodes was inserted into the beaker and the solution was electrolyzed for a designated time at a constant current of 700 mA. The current density was 4 A/dm^2. To eliminate active chlorine generated by the anodic reaction during electrolysis in the bioassays, 500 μL of 1 percent (w/v) sodium thiosulfate was added to 10 mL of an electrolyzed sample. The main reaction is the following: $4HClO + H_2O + Na_2S_2O_3 \rightarrow 2HCl + 2H_2SO_4 + 2NaCl$. The concentration of the added sodium thiosulfate solution was determined so as to quench the residual active chlorine but not to affect the bioassays of antibacterial activity and cytotoxicity.

2.3. Measurement of antibacterial activity

The minimum concentration (μg/mL) of an antimicrobial agent at which bacterial growth is suppressed, that is, the minimum inhibitory concentration (MIC) of a chemical, was measured by the microdilution method as defined by the Japanese Society of Chemotherapy (The Japanese Society of Chemotherapy, 1990). *Staphylococcus aureus* FDA209P (*S. aureus*) and *Escherichia coli* NIHJ JC-2 (*E. coli*) were used in this bioassay by a modified method. In brief, 100 μL of a serially diluted chemical solution in Mueller-Hinton broth (Eiken Chemicals Co., Tokyo, Japan) was mixed with 5 μL of a 10-fold diluted bacterial suspension equivalent to McFarland #0.5 in a U-bottomed microtiter plate. The plate was incubated for 18–24 h at 35 °C. When turbidity or a precipitate was not visible to the naked eye, or when only one precipitate whose diameter was less than 1 mm was observed, the well was considered to be negative for growth. When turbidity or a precipitate of 1 mm diameter or larger was visible to the naked eye, or when there was more than one precipitate whose diameter may be smaller than 1 mm, the well was considered to be positive for growth.

2.4. Measurement of cytotoxicity against human cells

To examine whether toxic substances are generated by electrolysis, the cytotoxicity of an electrolyzed solution was measured as 50 percent cytotoxicity concentration (CC_{50}) using Molt-4 cells, which are human lymphoblastoid cells, with a cell counting kit (WST-8 Cell Counting Kit-8, Dojin, Kumamoto, Japan). Fourfold serially diluted samples were mixed with the same volume of 5×10^5 cells/mL cell suspension and twice the volume of a fresh RPMI-1640 medium supplemented with 10 percent fetal bovine serum (FBS) in a U-bottomed microtiter plate, and cultured at 37 °C for 3 d. After the culture, 100 μL of cell suspension was transferred to a flat-bottomed microtiter plate, and 10 μL of WST-8 solution was added. After incubation at 37 °C for 1 h, optical density (OD) was measured at a wavelength of 450 nm with the reference wavelength at 620 nm.

2.5. Analysis of chemicals and their decomposition

To examine the degradation of AMK, an electrolyzed AMK solution was analyzed by ion chromatography (IC), total nitrogen analysis (TN), and high-performance liquid chromatograpy (HPLC).

The electrolyzed AMK solution was mixed with a solution containing 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ and applied to an IC DX-120 (Dionex Co., Sunnyvale, CA, USA) equipped with a separation column (IonPac AS4A-SC, Dionex) and a guard column (IonPac AG4A-SC, Dionex) at a flow rate of 1.2 mL/min. For HPLC, a 10 μL sample was injected and eluted with 50 mM NH_4PF_6 at a flow rate of 1.0 mL/min at 40 °C in HPLC LC-VP (Shimadzu, Kyoto, Japan) installed with a Wakopac Navi C22-5 column (4.6×250 mm, Shimadzu), and detected at 210 nm wavelength and a retention time of 4.2 min. Organic nitrogen content was calculated by subtracting nitrogen content in NO_3, NO_2, and NH_4 measured by IC from total nitrogen content in the solution measured by TN analysis (TN-30 type; Mitsubishi Chemical, Tokyo, Japan).

The contents of other antibacterials, PCG, CEZ, IPM/CS, VCM, MINO, CP, EM, and CPFX, were measured using a spectrophotometer V-570 (JASCO, Tokyo, Japan) at wavelengths of 322, 270, 300, 280, 352, 275, 210, and 276 nm, respectively. Because FOM does not absorb visible and ultraviolet light, it was colorized by the molybdenum blue method and its content was measured at a wavelength of 740 nm using the same spectrophotometer, in accordance with the Japanese Pharmacopeia, 15th ed. (The Japanese Pharmacopeia, 2006) All quantification determination methods were based on the calibration-curve method.

3. Results

3.1. Electrolyzed AMK solution

To clarify whether antibacterials are inactivated by electrolysis, an electrolyzed AMK solution was examined by MIC assay and HPLC. An unelectrolyzed AMK solution revealed MICs of 0.195 μg/mL and 3.125 μg/mL with *S. aureus* and *E. coli*, respectively, and the MICs increase to 50 μg/mL and >100 μg/mL for *S. aureus* and *E. coli*, respectively within 3 h of electrolysis (Fig. 1). If the decreases were hypothesized to be exponentially, the formula and R^2 (coefficient of determination) were also shown

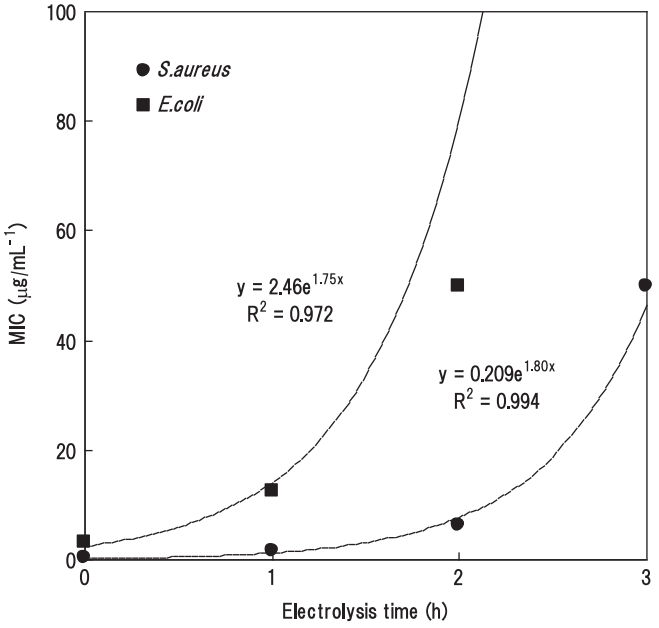

Fig. 1. Relationship between electrolysis time and MIC of AMK. AMK (amikacin sulfate) solution was electrolyzed, and the MIC (minimum inhibitory concentration) for *S. aureus* and *E. coli* was measured. R^2: coefficient of determination.