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Occurrence of immunosuppressive drugs and their metabolites in the sewage-impacted Vistula and Utrata Rivers and in tap water from the Warsaw region (Poland)

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HIGHLIGHTS

- Investigation of immunosuppressants and their metabolites in the environment.
- The first time mycophenolic acid in surface water has been detected.
- Occurrence of didesmethyl tacrolimus in surface water cannot be excluded.
- Concentration of mycophenolic acid close to WWTP exceeded PNEC.

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ABSTRACT

Immunosuppresive therapy following organ transplant frequently includes treatment with tacrolimus and mycophenolic acid derivatives. These pharmaceuticals may enter the environment through wastewater treatment plant (WWTP) effluents and may have a potentially harmful effect on aquatic biota. Tacrolimus, mycophenolic acid and their metabolites were measured at specific points of a large Polish river (Vistula), a smaller river (Utrata) and in tap water samples from the Warsaw region. Analysis was performed using liquid chromatography tandem mass spectrometry, after solid phase extraction for water samples, or QuEChERS extraction for sediments. Residues of tacrolimus were below quantitation limits in both water and sediment samples. However, in water samples mycophenolic acid concentrations were measured at up to 180 ng L^{-1} downstream of WWTP outfalls. No immunosuppressive drugs were detected in tap water. Concentrations of mycophenolic acid exceeded the predicted no effect concentration (PNEC) value in some Polish surface water, and risk calculations predicted at least twice higher concentrations in some other countries of the European Union. To the best of the authors' knowledge, this is the first report of these immunosuppressive drug concentrations in the environment.

1. Introduction

Organ transplantation is often the only treatment for the final state of organ failure. The most common transplantation involves the kidney and liver, but also the heart, lung, pancreas and small bowel. The total number of transplants in the European Union is still increasing, reaching 31,165 in 2013, an 18% increase compared to 2004 (Matesanz, 2014). Currently in Poland the transplantation rate is in the middle of the ranking list of transplantations in the European Union. For instance, the rate of kidney transplantation in Poland in 2013 was 30.4 pmp (per million population), whereas in the Netherlands, Spain and the United Kingdom it was over 50 pmp. In Bulgaria the rate was below 10 pmp (Matesanz, 2014).

Abbreviations: ATC, Anatomical Therapeutic Chemical; EC_{50}, half maximal effective concentration; EMEA, European Medicines Agency; EPI, enhanced product ion (mode in the triple quadrupole linear ion trap); ERA, environmental risk assessment; LC_{50}, median lethal concentration; MDL, method detection limit; MEC, measured environmental concentration; MM, mycophenolate mofetil; MPA, mycophenolic acid; MPAG, 7-O-mycophenolic acid glucuronide; MQL, method quantification limit; MRM, multiple reaction monitoring; NFZ, Narodowy Fundusz Zdrowia – National Health Service; NOEC, no observed effect concentration; PEC, predicted environmental concentration; PNEC, predicted no effect concentration; Q-TOF, quadrupole time-of-flight mass spectrometer; RQ, risk quotient; S/N, signal-to-noise ratio; TAC, tacrolimus; WWTP, wastewater treatment plant.

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Transplant recipients should follow complex immunosuppressive therapy to prevent graft rejection. The initial therapy for graft recipients is often the “triple therapy”, including calcineurin inhibitors, antiproliferative drugs and corticosteroids. Calcineurin inhibitors (L04AD in Anatomical Therapeutic Chemical (ATC) classification) are the most important components of immunosuppressive therapy, with tacrolimus (TAC) being the most frequently used (WHOCC, 2015; Kim et al., 2014; Matas et al., 2014). Among antiproliferative drugs, the derivatives of mycophenolic acid (MPA, L04AA in ATC classification) have replaced azathioprine (L04AX in ATC classification) and were used by up to 95% of renal and 80% of liver recipients in the USA in 2012 (Kim et al., 2014; Matas et al., 2014). Thus, in our work we decided to focus on TAC and MPA derivatives.

After oral administration the bioavailability of TAC varies from 20 to 23% (Möller et al., 1999). The absorbed TAC is extensively metabolized by hepatic and intestinal cytochrome P450. Only 0.3 and 0.5% of the unchanged drug are eliminated with urine and faeces. Bile is the principal route of TAC metabolite elimination, as up to 95% of the dose is excreted in faeces (Möller et al., 1999). Fifteen possible metabolites of TAC have been identified in human plasma (Barbarino et al., 2013). The primary metabolites, which account for most of the metabolic clearance of TAC, are O-demethylated metabolites at the 13-, 15- and 31-methoxy group (also known as M–I, M–III and M–II) and 12-monohydroxylated metabolites (also known as M–IV). Didemethylated metabolites include those at the 13- and 15-, 15- and 31-, and 13- and 31-methoxy group (also known as M-VII, M–V and M–VI) (Fig. 1) (Chitnis et al., 2013). Some metabolites exhibited pharmacological activity (Barbarino et al., 2013).

In contrast to TAC, MPA, which is available either as an ester prodrug (mycophenolate mofetil, MM) or as a sodium salt, has high bioavailability (81–94%) following oral administration (Upadhyay et al., 2014). MPA is metabolized in the liver, kidney and intestine to pharmacologically inactive 7-O-mycophenolic acid glucuronide (MPAG). Other minor phase II MPA metabolites are acyl glucuronide and phenolic glucoside (Upadhyay et al., 2014). The conjugates can revert to the parent drug during sewage treatment (Thurman and Ferrer, 2012). The only phase I metabolite is 6-O-desmethyl-MPA (Fig. 1). It undergoes further conjugation to form two glucuronides that constitute very minute fractions of MPA. MPA is primarily excreted in urine as MPAG (87%) and in negligible amounts as free acid (<1%) (Lamba et al., 2014).

The main sources of aquatic environmental contamination by pharmaceuticals and their metabolites are effluents of wastewater treatment plants (WWTPs). Pharmaceuticals and/or their metabolites enter WWTPs via wastewater from the disposal of unused or expired drugs in toilets, as well as human excretion. Human excretion is generally considered to be the primary source of the pharmaceuticals in the environment (Santos et al., 2013). The pharmaceuticals' residues can have harmful or even toxic effects on aquatic biota (Orias and Perrodin, 2013). There are limited data on the ecotoxicity of immunosuppressive drugs. MPA is slightly toxic for planktonic crustaceans and highly toxic for algae (Roche, 2015). Moreover, due to their mechanism of action, immunosuppressive drugs can also cause reproductive toxicity. Cyclosporine, for instance, due to the inhibition of calcineurin expressed in the developing brain and eye, induces structural and functional defects in zebrafish embryos (Clift et al., 2015).

The aim of this study was to determine the presence of the most commonly used immunosuppressive drugs, TAC, MPA and their metabolites, at specific points of two rivers in Poland – the Vistula and the Utrata – as well as in tap water samples in the Warsaw region. The Vistula is the longest river in Poland at 1047 km and a source of water supply for approximately two million people from Warsaw and its surrounding area. The watershed area of the Vistula is 194,424 km^2 of which 168,699 km^2 is located within Poland (Grabowski et al., 2009). In contrast, the Utrata is a small river at

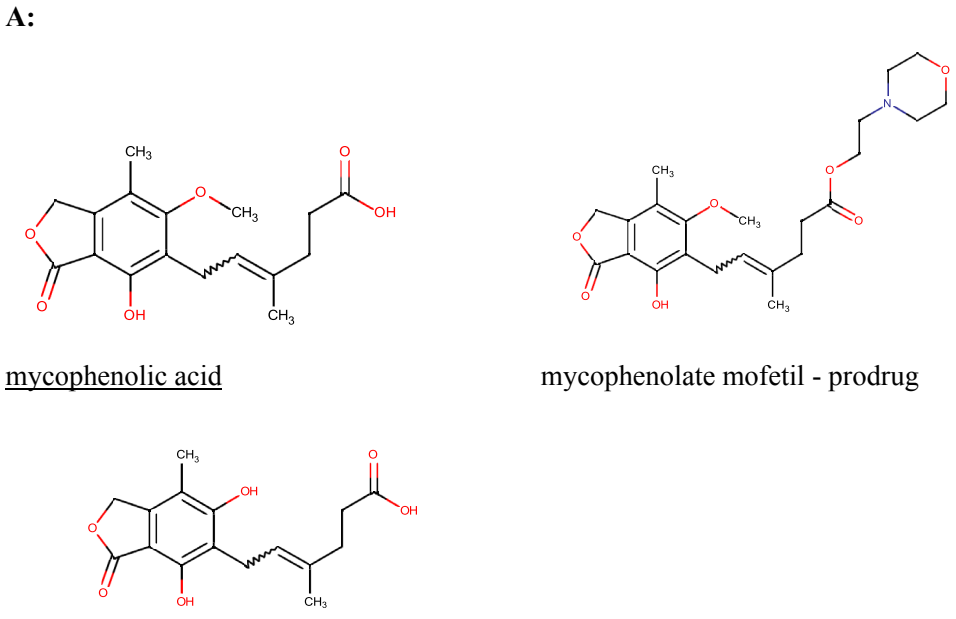

Fig. 1. Chemical structure of mycophenolic acid (A), tacrolimus (B) and their major phase 1 metabolites (according to Iwasaki, 2007).