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Aerobic biodegradability of the calcium channel antagonist verapamil and identification of a microbial dead-end transformation product studied by LC–MS/MS

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Abstract

In recent years pharmaceuticals and personal care products have been detected in increasing concentrations in hospital effluents, sewage treatment plants (STP) as well as in different environmental compartments such as surface water, groundwater and soil. Little is known about the elimination of these substances during sewage treatment or about the formation of potential metabolites in the environment caused by bacterial biotransformation. To assess the biodegradability of the popular cardiovascular drug verapamil and the possible formation of potential microbial degradation products, two tests from the OECD series were used in the present study: the widely used *Closed Bottle* test (OECD 301 D) and the modified *Zahn–Wellens* test (OECD 302 B).

In the Closed Bottle test, a screening test that simulates the conditions of an environmental surface water compartment, no biological degradation was observed for verapamil at concentrations of 2.33 mg l^{-1}. In the Zahn–Wellens test, a test for inherent biodegradability which allows evaluation of aerobic degradation at high bacterial density, only a partial biological degradation was found. Analysis of test samples by high performance liquid chromatography coupled to multiple stage mass spectrometry (HPLC–MS^n) revealed 2-(3,4-dimethoxyphenyl)-2-isopropyl-5-(methylamino)pentane nitrile, already known as D617 (Knoll nomenclature), a metabolite of mammalian metabolism, which is the major degradation product and dead-end transformation product of aerobic degradation of verapamil.

Keywords: Closed Bottle test; Zahn–Wellens test; Biodegradation; Bacterial metabolism; Dead-end metabolite; D617

1. Introduction

Since pharmaceuticals were first detected in the aquatic environment in the late 1970s (Hignite and Azarnoff, 1977), a variety of different substances have been found in several environmental compartments (Heberer, 2002). With the increasing sensitivity of modern instrumental chemical analysis, more and more representatives of different pharmaceutical classes can be identified (Ternes, 1998; Trenholm et al., 2006). Most of the pharmaceuticals used in medicine are excreted by patients in an unchanged or only partially metabolized form and end up as such in municipal waste water (Al-Rifai et al., 2007; Gomez et al., 2007). Due to their chemical and physical properties elimination of many pharmaceuticals in the sewage treatment plant (STP) is incomplete so that they end up in the environment, primarily in the water compartment (Kümmerer, 2004).

Verapamil (Fig. 1) is a calcium channel antagonist that is widely prescribed and used for the treatment of supraventricular arrythmias, coronary heart disease, antianginal therapy, mycordial ischemia and arterial hypertension (Singh et al., 1978). In addition, verapamil is a strong inhibitor of P-glycoprotein-mediated transport and has been shown to modify multidrug resistance in cancer

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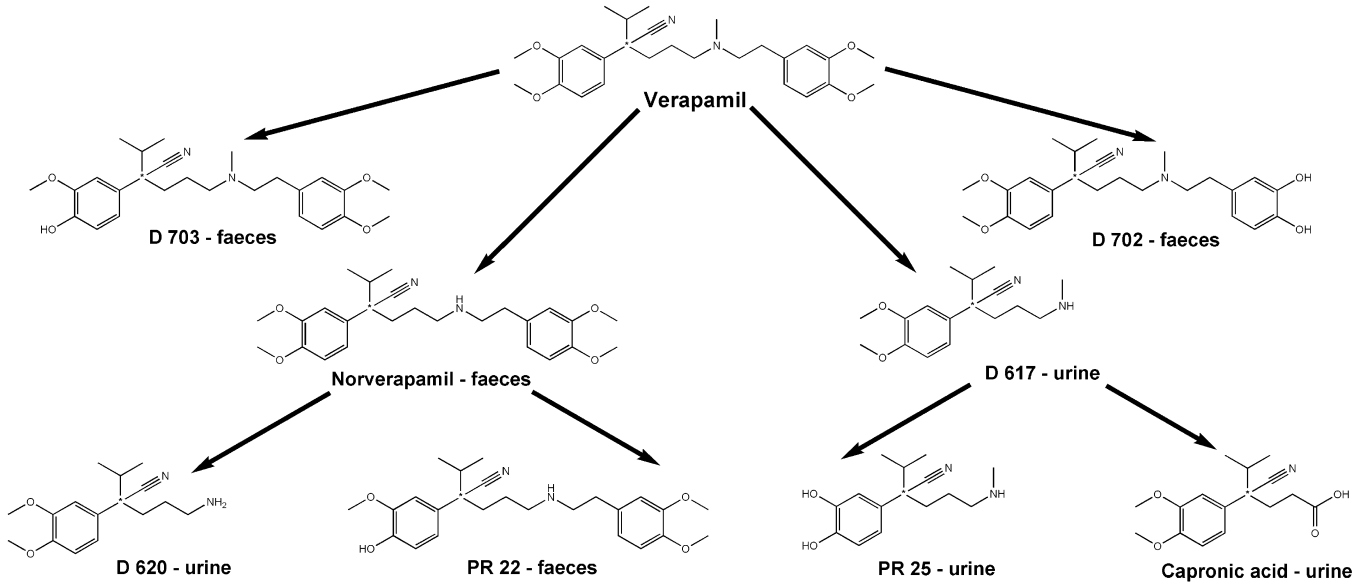

Fig. 1. Main phase I metabolites of verapamil in vivo and their excretion ways, according to Reder-Hilz et al. (2004), modified.

chemotherapy in *in vitro* experiments (Toffoli et al., 1995; Pauli-Mangus et al., 2000). Due to the presence of a tertiary amino group verapamil is a weak base (pK_a 8.9) and predominantly exists in a protonated form in physiological media (Surakitbanharn et al., 1995). It is a lipophilic drug with an octanol–water coefficient $\log p_{ow}$ of 3.79 for the free base (Carmen, 1992). The commercial product is a racemic mixture of (+)-(*R*)-verapamil and (−)-(*S*)-verapamil. The pharmacological potency of these two optical isomers shows considerable difference with (−)-(*S*)-verapamil being 10–20 times more potent than (+)-(*R*)-verapamil.

O- and *N*-demethylation are the two major metabolic pathways for verapamil in humans. *N*-demethylation is carried out by the subfamily CYP3A of the cytochrome P450 enzymes, and leads to the two major metabolites norverapamil (D591) and 2-(3,4-dimethoxyphenyl)-2-isopropyl-5-(methylamino)pentane nitrile (D617) (Busse et al., 1995; Tracy et al., 1999; Wang et al., 2004). These two compounds were also found in cryopreserved hepatocytes of human, rat, mouse and dog as primary metabolites (Reder-Hilz et al., 2004; Fig. 1). The reason for the stereoselectivity of the preferential oxidation of the *S*-enantiomer of verapamil is largely unknown. Interestingly, no stereoselectivity was found in the formation of the metabolites D617 or norverapamil (Kroemer et al., 1993).

Only in recent years have there been attempts to identify conjugation products of verapamil, although it had been speculated earlier that some glucuronides might contribute to the pharmacological and toxicological properties of this drug (Olsen et al., 1992). Walles et al. (2003) investigated verapamil metabolism in rat hepatocytes by LC–MS^n and identified 24 phase I and 14 phase II metabolites. Borlak et al. (2003) studied the metabolism of verapamil in primary human hepatocytes and human urine by LC–MS^n and LC–NMR (nuclear magnetic resonance). They found 21 phase I and 16 phase II metabolites. All of the phase II metabolites (glucuronides) and 11 of the phase I (oxidative) metabolites had been unknown until that point.

Even though the metabolism of verapamil in humans and animals has been well studied, little is known about metabolism in lower organisms. There have been studies about the capability of fungi (Sun et al., 2004) and anaerobic bacteria from rat cecal (appendix) contents (Koch and Palicharla, 1990) to metabolize verapamil, however, nothing is known about the fate of verapamil in the aquatic environment with its high bacterial diversity.

In Germany in the year 2005, 171 million defined daily doses (DDD) of verapamil were prescribed (Schwabe and Paffrath, 2006). Using the DDD conversion factors according to the World Health Organization (WHO ATC/DDD Index, 2007) this is equal to a predicted total amount of 41 tons. Eichelbaum et al. (1979) found 3–4% unchanged verapamil in human urine so it can be assumed that in Germany about 1.5 tons of this drug enter the sewage treatment plants annually via municipal waste water. So far there is very little data about the elimination and biodegradability of coronary pharmaceuticals such as verapamil in the environment. Cardiovascular drugs can cause strong side effects in patients. Verapamil possesses both immunosuppressive (Hailer et al., 1994) and cytotoxic effects (Haussermann et al., 1991). Based on experimental ecotoxicological data from *Daphnia magna* (Villegas-Navarro et al., 2003) and the results of a structure–activity relationship study which both revealed toxic effects at low doses on aquatic organisms (Sanderson et al., 2004), concern has arisen regarding the environmental fate of verapamil and its metabolites as well as their toxicity and bioaccumulation.

We report here on the biodegradability of the calcium channel antagonist verapamil and the identification of a