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Molecular docking: A potential tool to aid ecotoxicity testing in environmental risk assessment of pharmaceuticals

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HIGHLIGHTS

- Molecular docking could aid environmental risk assessment of pharmaceuticals.
- *O. mykiss* and *X. tropicalis* COX2 homologues bind diclofenac and ibuprofen.
- *X. laevis* & *D. rerio* progesterone receptor homologues bind levonorgestrel.
- Molecular docking can aid sensitive species selection in ecotoxicity tests for ERA.
- Mode of action ecotoxicity test end points can be selected using molecular docking.

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ABSTRACT

A cocktail of human pharmaceuticals pollute aquatic environments and there is considerable scientific uncertainty about the effects that this may have on aquatic organisms. Human drug target proteins can be highly conserved in non target species suggesting that similar modes of action (MoA) may occur. The aim of this work was to explore whether molecular docking offers a potential tool to predict the effects of pharmaceutical compounds on non target organisms. Three highly prescribed drugs, diclofenac, ibuprofen and levonorgestrel which regularly pollute freshwater environments were used as examples. Their primary drug targets are cyclooxygenase 2 (COX2) and progesterone receptor (PR). Molecular docking experiments were performed using these drugs and their primary drug target homologues for *Danio rerio*, *Salmo salar*, *Oncorhynchus mykiss*, *Xenopus tropicalis*, *Xenopus laevis* and *Daphnia pulex*. The results show that fish and frog COX2 enzymes are likely to bind diclofenac and ibuprofen in the same way as humans but that *D. pulex* would not. Binding will probably lead to inhibition of COX function and reduced prostaglandin production. Levonorgestrel was found to bind in the same binding pocket of the progesterone receptor in frogs and fish as the human form. This suggests implications for the fecundity of fish and frogs which are exposed to levonorgestrel. Chronic ecotoxicological effects of these drugs reported in the literature support these findings. Molecular docking may provide a valuable tool for ecotoxicity tests by guiding selection of test species and incorporating the MoA of drugs for relevant chronic test end points in environmental risk assessments.

1. Introduction

Substantial mixtures of human pharmaceuticals have been detected in surface waters at concentrations in the ng L^{-1} to low mg L^{-1} range for each individual compound (Ashton et al., 2004; Redshaw et al., 2008; Santos et al., 2009; Pal et al., 2010; Bound and Voulvoulis, 2006). A number of reviews have been published which summarise known ecotoxicological effects of human pharmaceuticals, for example, Santos et al. (2009) and Fent et al. (2006). However, there is still considerable uncertainty as to the effects that pharmaceuticals, their metabolites and transformation products may have on aquatic organisms (Fent et al., 2006; Kummerer, 2009; Fatta-Kassinos et al., 2011a). Aquatic organisms are exposed to a continuous cocktail of human pharmaceuticals, at least a dozen different pharmaceuticals have been measured in a single surface water sample (Daughton and Brooks, 2011). This is highly likely to be a substantial underestimate because of limitations in analysis. Human pharmaceuticals can often disrupt key biological functions in aquatic organisms such as reproduction and growth (Fent et al., 2006). The presence of the synthetic hormone contraceptive 17α ethinylestradiol (EE2) in sewage effluent and surface waters has been clearly linked with the endocrine disruption of fish and frogs (Desbrow et al., 1998; Jobling et al., 2002; Caldwell et al., 2008; Gyllenhammar et al., 2009). Fish are particularly sensitive to EE2, the predicted no effect concentration (PNEC)

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for EE2 is <1 ng L^{-1} (Lange et al., 2001; Caldwell et al., 2008). This detrimental effect on aquatic organisms was not foreseen, despite the human mode of action (MoA) for EE2 being via the oestrogen receptor which is highly conserved in other vertebrates such as fish (Christen et al., 2010).

Veterinary medicines have also been the cause of a dramatic detrimental effect on non target organisms. The use of diclofenac in cattle has caused a major decline in vultures in India and Pakistan. The *Gyps* genus of vulture were surprisingly sensitive to residues of diclofenac in deceased carrion on which they fed, leading to acute renal failure and visceral gout (Oaks et al., 2004). Diclofenac has since been withdrawn as a veterinary medicine in India, Nepal, Bangladesh and Pakistan (Kumar, 2006). However, it is still used widely as an analgesic in human medicine; it is persistent during sewage treatment and is regularly detected in effluent and surface waters around the world (Hoeger et al., 2005).

There are several examples of chronic effects on aquatic organisms at environmentally relevant concentrations. The antidepressant fluoxetine (Prozac) has been shown to effect innate behavioural responses of fish (Painter et al., 2009; Schultz et al., 2011) and alterations in reproduction patterns have also been observed (Brooks et al., 2003).

Although it is widely accepted that some of these compounds are associated with adverse developmental effects at environmentally relevant concentrations (Khetan and Collins, 2007; Fatta-Kassinos et al., 2011a), chronic ecotoxicity data is lacking for most pharmaceuticals. A key difficulty in assessing the toxic effects of these low concentration pollutants is that it is not yet established which organisms and which endpoints are relevant for risk assessment (Fatta-Kassinos et al., 2011b).

The EU the guidelines for environmental risk assessment (ERA) of medicinal products for human use are laid down by EMEA//CHMP/SWP/4447/00, Directive 2004/27/EC. These guidelines state that if the predicted exposure concentration exceeds 10 ng L^{-1}, ecotoxicological tests using OECD guidelines are required. If a drug affects reproduction of vertebrates or lower animals at concentrations lower than 10 ng L^{-1}, then ecotoxicological tests are also required. Three problems have been identified with the standardised ecotoxicity tests specified in the guidelines. The first is that the relatively narrow range of species used i.e. algae, *Daphnia* and one fish, may not include the most sensitive species exposed in natural water courses. Many types of organisms, such as amphibians are not considered.

The second problem is that there is no specification for chronic tests that reflect the MoA of the drug. Pharmaceuticals are designed to have a specific biological effect (Dorne et al., 2007; Christen et al., 2010; Kar and Roy, 2010). They interact with specific human target proteins and metabolic pathways. These may be highly conserved and therefore cause analogous effects in other organisms (Gunnarsson et al., 2008). Many chronic ecotoxicological studies using MoA related end points have revealed effect concentrations that are substantially lower than standardised studies (Crane et al., 2006; Boxall and Greenwood, 2010). Several authors have suggested that testing could be improved by including targeted strategies based on known pharmacological properties and MoA to decrease uncertainties (Fent et al., 2006; Ankley et al., 2007).

The third potential failing of the ecotoxicity tests is that mixture effects are not considered. Several compounds in the aquatic environment may affect the same metabolic pathway or process in non target organisms and may produce additive or synergistic or antagonistic effects (Schnell et al., 2009). This could lead to effects in aquatic organisms that would not occur if exposed to a compound in isolation.

These problems have led many authors to highlight a need for an intelligent ecotoxicity testing strategy for pharmaceuticals (Lange and Dietrich, 2002). This includes the use of information on the MoA of a substance to predict or anticipate effects in a range of species and based on this tailor the tests and select species as part of ERA (Montforts et al., 2007). The use of 'omics' based approaches using extrapolation of evolutionary sequence conservation of drug targets could prove a useful method for guiding such a strategy. In a study by Gunnarsson et al. (2008), a high number of conserved human drug targets were identified in other species. It is important to note that the existence of a similar protein sequence in an organism does not automatically mean that the human MoA of the drug will occur. Further work on the 3D structure of the proteins is needed to predict drug–protein interactions in order to make this information relevant to ecotoxicological tests and ERAs (Gunnarsson et al., 2008). In a recent review of toxicological studies of pharmaceuticals in the aquatic environment, Brausch et al. (2012) highlights the potential and need for further research of computational toxicology approaches. Molecular docking may be a potential tool in the design of an intelligent test strategy as part of the ERA by identifying sensitive species, selecting appropriate test species, selecting MoA related chronic test end points for toxicity studies and interpreting the relevance of existing toxicological data.

Molecular docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Recently there have been several studies which highlight the potential of molecular docking to predict the effects of chemical pollutants. For example Yang et al. (2011) found that molecular docking and post docking analysis can serve as an efficient pre-screening technique for identifying potential chemical estrogens. Wu et al. (2010) found that homology modeling and molecular docking might be a potential tool to predict interactions between contaminants and associated receptors in different trophic levels in a study investigating flame retardants and the androgen receptor in several vertebrate species. Wu et al. (2009) found the results of their AutoDock molecular docking study consistent with those of animal experiments reported in the literature, indicating that molecular docking would have the potential to predict the nuclear hormone receptors of environmental pollutants.

Two analgesics diclofenac and ibuprofen and the synthetic progesterone levonorgestrel were chosen for investigation into the potential for molecular docking to predict ecotoxicological effects of human pharmaceuticals on non target organisms. The rationale for selection of these drugs were their high usage (Roos et al., 2012), high detection frequency in surface waters (Ellis, 2006) and adverse ecotoxicological effects reported in the literature at environmentally relevant concentrations (Cleuvers, 2004; Mehinto et al., 2010; Safholm et al., 2012; Svensson et al., 2013).

In humans, the analgesics diclofenac and ibuprofen act by inhibiting (reversibly or irreversibly) the cyclooxygenase (COX) enzymes which catalyze the synthesis of prostaglandins (Vane and Botting, 1998). In humans prostaglandins are involved in inflammation, pain regulation, regulation of blood circulation especially in the kidney, coagulation processes, synthesis of gastric mucosa, vascular permeability and kidney function including ion retention and ovulation (Mercure and Van Der Kraak, 1996; Sorbera et al., 2001; Fent et al., 2006). There is divided opinion in the literature as to whether these effects occur in non target organisms such as fish (see Section 4).

Levonorgestrel is a potent testosterone-derived progestin which binds to the progesterone receptor (Petit-Topin et al., 2009). It is used, often combined with EE2, as a contraceptive and also for hormone replacement therapy. It mimics the effects of the natural hormone progesterone, involved in regulating the menstrual cycle, pregnancy, and embryogenesis in humans and other species