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Mode of action characterization for adverse effect of propranolol in *Daphnia magna* based on behavior and physiology monitoring and metabolite profiling☆

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ABSTRACT

Studies are underway to gather information about the mode of action (MOA) of emerging pollutants that could guide practical environmental decision making. Previously, we showed that propranolol, an active pharmaceutical ingredient, had adverse effects on *Daphnia magna* that were similar to its pharmaceutical action. In order to characterize the mode of action of propranolol in *D. magna*, which is suspected to be organ-specific pharmaceutical action or baseline toxicity, we performed time-series monitoring of behavior along with heart rate measurements and nuclear magnetic resonance (NMR) based metabolite profiling. Principle component analysis (PCA) and hierarchical clustering were used to categorize the mode of action of propranolol among 5 chemicals with different modes of action. The findings showed that the mode of action of propranolol in *D. magna* is organ-specific and vastly different from those of narcotics, even though metabolite regulation is similar between narcotic and non-narcotic candidates. The method applied in this study seems applicable to rapid characterization of the MOA of other cardiovascular pharmaceutical ingredients.

1. Introduction

Monitoring abnormal responses caused by biological dysfunction in test organisms has been used to distinguish normal and abnormal health states and to study the MOA of various agents in environmental science as well as medical science (McKim et al., 1987; Moore et al., 2004; Krentz and Wong, 2006; Benenson, 2013; Peakall and Shugart, 2013). Diverse aquatic organisms have been examined via response monitoring, and the usefulness of several biological parameters as biomarkers has consequently been proven (Bradbury et al., 1991; Ankley et al., 2007; Segner, 2009; Volz et al., 2011; Jeong et al., 2014). Recent advances in omics techniques allow the acquisition of high-throughput data and have widened the platforms for sub-organismal response monitoring (Dzialowski et al., 2006; Poynton et al., 2008; Steinberg et al., 2008; Nestler et al., 2012). In case of active pharmaceutical ingredients (APIs), whose pharmacological and toxicological information is well established in the drug discovery and development stages, the monitoring data is usually compared with their established background data, using which their MOA in model organisms is deduced (Escher and Hermens, 2002; Escher et al., 2005; Campos et al., 2012).

Use of background and monitoring data to determine the MOAs of APIs for non-target organisms, most of which lack physiological data, is advantageous since it is cost and time effective. As reliable MOA data is being increasingly required for practical decision making when rapid determination is needed, its applicability is widespread. For example, in mixture ecological risk assessment (ERA), MOA information is fundamental for mixture model selection (Escher and Hermens, 2002; SCCS, 2012; Ågerstrand et al., 2015). In our previous study, propranolol showed therapeutic-like effects in the aquatic organism *D. magna*, namely, a reduction in heart rate (Jeong et al., 2015). Propranolol is a β-adrenergic receptor antagonist used to treat cardiovascular diseases in humans and is one of the most toxic and bioaccumulative pharmaceuticals in

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aquatic ecology (Fent et al., 2006; Stanley et al., 2006; Brausch et al., 2012; Maszkowska et al., 2014). In addition, propranolol is also known as an effective antagonist for 5-HT (hydroxytryptamine) receptor which is a potential target receptor in wildlife (Alexander and Wood, 1987). A reduction in heart rate is the main pharmacological action of propranolol in humans, and it is needed to determine whether this agent reacts similarly in non-targeted aquatic organisms (Jeong et al., 2015).

Although the symptoms of propranolol toxicity in *Daphnia* are very similar to its pharmaceutical actions in human, arguments suggest that propranolol's action is not from a specific MOA. The similarity of the pharmaceutical target receptor, β_2-adrenergic receptor, between human and *Daphnia* species is reportedly under 30% based on ortholog prediction (Gunnarsson et al., 2008). Prediction using the quantitative structure-activity relationship (QSAR) model suggests that the mechanism of the acute toxicity of propranolol is baseline toxicity (acute aquatic toxicity MOA by OASIS, QSAR Toolbox 3.2). Additionally, according to the assumption that propranolol is a narcotic in non-target organisms, the heart rate reduction is explainable as a result of physiological retardation. Therefore, in the present study, we suggest a methodology utilizing response monitoring and metabolomics data along with the background pharmacological information for characterization of propranolol's MOA in *D. magna*, from among potential MOAs: baseline toxicity, β-adrenergic receptor antagonism, and 5-hydroxytryptamine (HT) receptor antagonism. Our aim is to characterize the major MOA of propranolol in *D. magna* by comparing results with pharmacological information and results from chemicals known to have the potential MOAs of propranolol. Our finding may show whether or not the MOA of propranolol's action on heart of *Daphnia* is baseline toxicity and provide an example for MOA characterization methodology.

2. Materials and methods

2.1. Organism and chemical agents

D. magna was cultured in compliance with the guidelines of the USEPA (Weber, 1991). The crustacean was fed a mixture of *Raphidocelis subcapitata* and yeast, cereal leaves, and tetramin (YCT) daily as a nutrient source. Hard water was synthesized using Milli-Q water as the medium, and the medium water was renewed three times a week. The temperature and light/dark conditions were 20 ± 1 °C and 16 h/8 h. The media and room conditions were identical for every exposure test set. Chemical ingredients were purchased from Sigma-Aldrich: (±)-propranolol hydrochloride (>99%), 4-chloroaniline (>98%), ethylacetate (99.9%), nadolol (>97%), yohimbine hydrochloride (>98%), and cyproheptadine hydrochloride sesquihydrate (>98%). All chemicals were stored and handled as recommended by the manufacturer.

2.2. Exposure experiments and response observation

We investigated the MOA of propranolol in *D. magna* and whether it is organ specific or baseline toxicity. The study scheme is described in Fig. 1. Before this study, pharmacological information on propranolol was collected, and endpoints related to pharmaceutical action and baseline toxicity were chosen. In addition, multiple chemicals suspected to have the potential MOAs of propranolol in non-targeted organisms were selected for the exposure study. Swimming activity and heart rate were monitored and metabolite profiling was performed on exposure to chemicals. MOA was finally characterized by translating results based on pharmacological data and comparison with other chemicals. Because narcotics non-specifically inhibit biological activity, we assumed that narcotics result in whole-body depression rather than heart-specific inhibition. With regard to metabolite profile, we suspected that chemicals with a similar MOA would regulate metabolites similarly. The chemicals chosen for the exposure test are known to have one of the potential MOAs of propranolol in *D. magna*: narcosis (4-chloroaniline), β-adrenergic receptor inhibition (nadolol), and 5-HT receptor antagonism (yohimbine and cyproheptadine). Information on the pharmaceutical action of propranolol, nadolol, yohimbine, and cyproheptadine was from Drugbank's database, and information on the narcotic effect of 4-chloroaniline was obtained from previous studies (Bradbury et al., 1989; Veith and Broderius, 1990; Law et al., 2014).

For the exposure tests and data acquisition, three sets of chemical exposure were established for examining swimming activity, heart rate, and metabolite profiles. In the first two sets, an

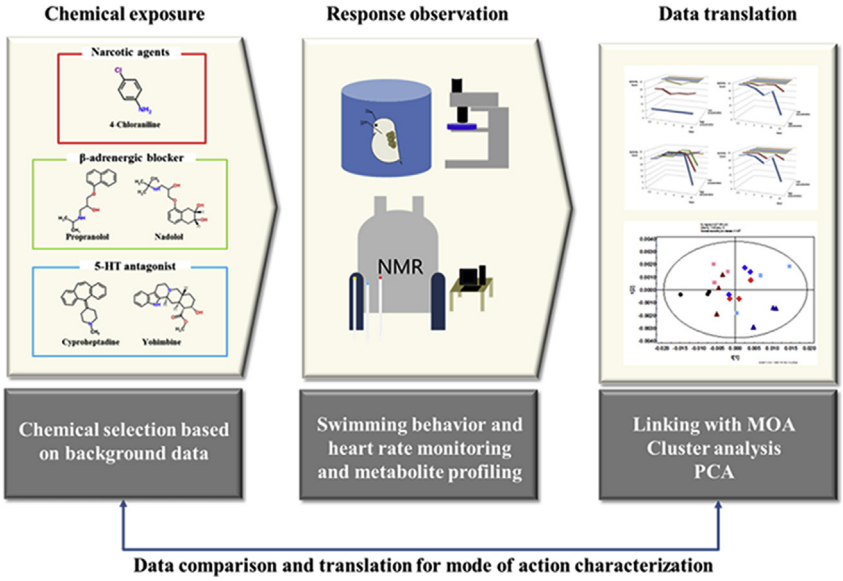

Fig. 1. Overall design of study.