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Waterborne beclomethasone dipropionate affects the physiology of fish while its metabolite beclomethasone is not taken up

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HIGHLIGHTS

- BDP, a commonly used glucocorticoid has physiological effects in rainbow trout exposed via water.
- As BDP is expected to be metabolized, environmental risks from excretion are likely to be small.
- Effects observed of BDP in fish were similar to effects of corticosteroids observed in man.
- Effects were observed at plasma concentrations similar to those reached in humans.
- This adds confidence to read-across approaches between humans and fish.

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ABSTRACT

Asthma is commonly treated with inhalable glucocorticosteroids, including beclomethasone dipropionate (BDP). This is a synthetic prodrug which is metabolized to the more active monopropionate (BMP) and free beclomethasone in humans. To evaluate potential effects of residual drugs on fish, we conducted a 14 day flow-through exposure experiment with BDP and beclomethasone using rainbow trout, and analyzed effects on plasma glucose, hepatic glutathione and catalase activity together with water and body concentrations of the BDP, BMP and beclomethasone. We also analyzed hepatic gene expression in BDP-exposed fish by microarray and quantitative PCR. Beclomethasone (up to 0.65 μg/L) was not taken up in the fish while BDP (0.65 and 0.07 μg/L) resulted in accumulation of both beclomethasone, BMP and BDP in plasma, reaching levels up to those found in humans during therapy. Accordingly, exposure to 0.65 μg/L of BDP significantly increased blood glucose as well as oxidized glutathione levels and catalase activity in the liver. Exposure to beclomethasone or the low concentration of BDP had no effect on these endpoints. Both exposure concentrations of BDP resulted in significantly higher transcript abundance of phosphoenolpyruvate carboxykinase involved in gluconeogenesis, and of genes involved in immune responses. As only the rapidly metabolized prodrug was potent in fish, the environmental risks associated with the use of BDP are probably small. However, the observed physiological effects in fish of BDP at plasma concentrations known to affect human physiology provides valuable input to the development of read-across approaches in the identification of pharmaceuticals of environmental concern.

1. Introduction

Synthetic glucocorticoids are a class of pharmaceuticals with wide applications for the treatment of human disease as well as in veterinary medicine. Lately, this class of pharmaceuticals has been recognized as being of high concern regarding potential effects in the environment (Kugathas and Sumpter, 2011; Kugathas et al., 2012). Glucocorticoids are important hormones in all vertebrates, involved in e.g. stress responses and control of metabolism, cell growth, bone density and

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vasoconstriction. Glucocorticosteroids are also known to have immunosuppressive effects and for this reason, they are often used to treat immune diseases such as asthma and rheumatoid arthritis. Glucocorticoids act by binding to glucocorticoid receptors (GR), present in the cytoplasm of most cell types, thereby initiating transcription via glucocorticoid response elements (Zhou and Cidlowski, 2005). Inhibition of the transcription factors nuclear factor κB (NF-κB) and activator protein-1 (AP-1), via protein–protein interactions, mediates their anti-inflammatory effects thereby decreasing expression of genes involved in inflammatory responses (Hayashi et al., 2004). It is known that treatment with glucocorticoids can result in hyperglycemia (Melby, 1977) via induction of the production of phosphoenolpyruvate carboxykinase (PEPCK), an enzyme which controls the rate limiting step in gluconeogenesis (Phuc Le et al., 2005).

Several different glucocorticoids, including both natural and synthetic steroids, are found in treated sewage effluents, leading to contamination of waterways (Chang et al., 2007; Fan et al., 2011; Gilbert, 2011; Schriks et al., 2010). This class of drugs may be administered to patients via inhalers, oral formulations or topical applications. As drugs administered on the skin may be washed off without any prior metabolism, this administration route may, in relative terms, lead to higher risks for contamination of the external environment than the same amounts of orally ingested drugs (Daughton and Ruhoy, 2009). Concern about direct releases of corticosteroids from manufacturing sites and effects on fish has also been raised (Sanchez et al., 2011; Gilbert, 2011).

Two glucocorticoid receptors have been identified in rainbow trout (*Oncorhynchus mykiss*), known as GR1 and GR2 (Bury et al., 2003). In teleost fish, cortisol is the principle glucocorticoid, and is produced in the head kidney under the control of the hypothalamus–pituitary–interrenal axis (Mommsen et al., 1999). Fish lack mineralcorticoids, so cortisol fills both glucocorticoid and mineralcorticoid functions, and plays important roles in metabolism and immune function (Mommsen et al., 1999). Glucocorticoids also facilitate the acquisition of osmoregulatory abilities, e.g. during smoltification in anadromous species like rainbow trout, and have a permissive action on thyroid hormones during larval metamorphosis in fish (Wada, 2008), which suggests a possibility for interactions with exogenous corticosteroids on different processes. Expression of GR mRNA is regulated via negative feedback, i.e. mRNA levels decrease with increasing GR protein levels, in rainbow trout hepatocytes (Aluru and Vijayan, 2007; Sathiyaa and Vijayan, 2003a), as is also the case in humans. The mechanisms regulating this feedback are not known, but evidence indicates that regulation of proteasomal degradation of the GR receptor may be involved (Sathiyaa and Vijayan, 2003b). Exposure of fish to the synthetic corticosteroid dexamethasone has been reported to result in impaired reproduction, growth, and development, at least at high exposure concentrations (500 μg/L) (LaLone et al., 2012; Lee et al., 1992; Overturf et al., 2012).

Beclomethasone is a synthetic glucocorticoid which is primarily used as an inhalant in treatment of asthma and rhinitis. Beclomethasone is administered as the more lipophilic prodrug beclomethasone-dipropionate (BDP) which is metabolized to beclomethasone-17-monopropionate (BMP), beclomethasone-21-monopropionate (inactive) and free beclomethasone in humans via esterases present in numerous tissues of the body (Daley-Yates et al., 2001). In fish, the metabolic enzymes are unknown. Beclomethasone and BMP are active metabolites of the prodrug BDP, though in humans, beclomethasone has 18 times lower affinity to the glucocorticoid receptor (GR) than BMP (Würthwein and Rohdewald, 1990). However, although beclomethasone is considered to be a relatively inactive metabolite, its binding affinity to the human GR is still comparable to that of dexamethasone (Würthwein and Rohdewald, 1990). In accordance, both these corticosteroids induce transcriptional activity at similar concentrations in a rainbow trout GR2 assay in vitro (Kugathas and Sumpter, 2011). Environmental monitoring of glucocorticoids has focused primarily on dexamethasone (Chang et al., 2007; LaLone et al., 2012) though other substances, including BDP and its metabolites, may also be of concern (Kugathas and Sumpter, 2011; Kugathas et al., 2012).

Dose-related systemic adverse effects of glucocorticoids on humans have been established and include adrenal suppression, reduction in growth rate, and adverse effects on skin, bone and eyes (Lipworth, 1999). An inhaled dose of 0.8 mg/day of the prodrug BDP yields a maximum plasma concentration of the most active metabolite, BMP, of 0.33 ng/mL (Lipworth, 1999; Mortimer et al., 2006). Doses twice as high or above were associated with marked adrenal suppression and posterior subcapsular cataracts in humans, with a considerable degree of inter-individual susceptibility, indicating that there are systemic effects of the drug (Lipworth, 1999). In fish, exposure to nominal concentrations of waterborne BDP increases plasma glucose levels (0.1 μg/L), decreases white blood cell counts (1 μg/L), and reduces plasma levels of egg yolk proteins (10 μg/L) (Kugathas et al., 2013a; Kugathas and Sumpter, 2011).

In this study, we aimed to compare the uptake and effect of both the prodrug (BDP) and of beclomethasone, as we expect that the latter would be more likely to be found in the environment. In addition, this study allows us to test the applicability of the fish plasma model (Huggett et al., 2003). This is a conceptual model where risks for pharmacological interaction in fish are assessed, based on the assumption that approximately similar plasma levels would be required in both fish and humans to elicit effects. This model has potentially important uses for identifying drugs of environmental concern, but it needs empirical validation for different types of drugs. We are aware of a few previous studies that have specifically addressed the applicability of this conceptual model by comparing plasma concentrations in fish with different pharmacological endpoints relating to non-steroidal anti-inflammatory drugs (Cuklev et al., 2012; Cuklev et al., 2011), a 5α-reductase inhibitor (Margiotta-Casaluci et al., 2013) and antidepressants (Grabicova et al., 2014; Holmberg et al., 2011; Valenti et al., 2012).

2. Materials and methods

2.1. Fish

In the first exposure setup, juvenile rainbow trout (*O. mykiss*) weighing approximately 60 g were obtained from Antens Fiskodling AB, Sweden. For the second exposure setup, fish weighing approximately 85 g were obtained from Vänneåns Fiskodling AB, Sweden, due to limitations in availability of suitable fish at Anten. To allow time for the fish to acclimatize to laboratory conditions, fish were kept in separate 500 L tanks with aeration and recirculating preconditioned tap water (12.0 ± 1 °C; 100% oxygen saturation) for 2 weeks prior to the start of each experiment. The water was recirculated through a biological filter and treated by UV light. Water from the same system was used in subsequent exposures but then the water was not recirculated. The fish were fed every other day with commercial fish pellets prior to the start of the experiments but not during the exposure study to reduce differences in the analyzed endpoints related to individual differences in food intake. Rainbow trout tolerates food deprivation for this time period well, and liver glycogen content is preserved (Kullgren et al., 2010). This experiment and handing of the fish was conducted in accordance with the ethical requirements as described by the Swedish animal welfare act and the University of Gothenburg, Sweden, ethical permission diary numbers and 36—2007 and 216—2010.

2.2. Exposure experiments

Fish were moved to 50 L glass aquaria, where they were arbitrarily assigned to each tank. Water in the tanks was aerated and flow was 250 mL/min, temperature was constant at 12.0 ± 1 °C, and fish were kept on a 12:12 hour light:dark cycle. In the first exposure, 12 individuals were placed in each aquarium. In the second exposure 8 individuals were placed in each aquarium.