Chemosphere 183 (2017) 147–155

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Kinetics of glucocorticoid exposure in developing zebrafish: A tracer study

Peter Johannes Steenbergen [a, b], Nabila Bardine, PhD [c], Faiza Sharif, PhD [a, d, *]

[a] Department of Integrative Zoology, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands
[b] Department of Medical Pharmacology, Leiden/Amsterdam Centre for Drug Research, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands
[c] Department of Cell Biology, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands
[d] Interdisciplinary Reseach Centre in Biomedical Materials, COMSATS Institute of Information Technology Lahore, Pakistan

HIGHLIGHTS

- Measuring uptake of small compounds by chorionated embryos creates an overestimation.
- 1 Hour uptake of 3H-cortisol by embryonic tissue has an efficiency of 60%.
- After hatching, the uptake of glucocorticoids in zebrafish larvae is around 20%.
- At early stages uptake occurs through the embryo surface mainly by passive diffusion.

ARTICLE INFO

Article history:
Received 30 December 2016
Received in revised form
2 May 2017
Accepted 10 May 2017
Available online 12 May 2017

Handling Editor: Shane Snyder

Keywords:
Zebrafish
Chorion
Glucocorticoids
Cortisol
Dexamethasone
Chemical uptake

ABSTRACT

In the current study the dynamics of glucocorticoid uptake by zebrafish chorionated embryos from the surrounding medium were studied, using 2.5 μM cortisol or dexamethasone solutions complemented with their tritiated variant. We measured the uptake of radioactive cortisol by embryos during a 1 h submersion. Interestingly, the signal in chorionated embryos was 85% (exposure: 1–2 hpf) or 78% (exposure: 48–49 hpf) of the signal present in an equal volume medium. By comparing embryos measured without chorion, we found that 18–20% of the radioactivity present in chorionated embryos is actually bound to the chorion or located in the perivitelline space. Consequently, embryonic tissue contains radioactivity levels of 60% of a similar volume of medium after 1 h incubation. During early developmental stages (1–48 hpf) exposure of more than 24 h in cortisol was needed to achieve radioactivity levels similar to an equal volume of medium within the embryonic tissue and more than 48 h for dexamethasone. In glucocorticoid-free medium, radioactivity dropped rapidly below 10% for both glucocorticoids, suggesting that the major portion of the embryonic radioactivity was a result of simple diffusion. During later developmental stages (48–96 hpf) initial uptake dynamics were similar, but showed a decrease of tissue radioactivity to 20% of an equal volume of medium after hatching, probably due to development and activation of the hypothalamic pituitary interrenal axis. Uptake is dependent on the developmental stage of the embryo. Furthermore, the presence of the chorion during exposure should be taken into account even when small lipophilic molecules are being tested.

© 2017 Published by Elsevier Ltd.

1. Introduction

The zebrafish (*Danio rerio*) has become a very popular laboratory animal model and is used in many major research fields to complement research done in rodents (Lieschke and Currie, 2007; Rihel et al., 2010; Rubinstein, 2003; Steenbergen et al., 2011). Recent developments in regulation on animal use (Braunbeck and Lammer, 2006; "EU (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)," 2006) stimulate the use of zebrafish embryos and young larvae in aquatic toxicology and environmental risk assessment where larvae are submersed in

* Corresponding author. Interdisciplinary Research Centre in Biomedical Materials, COMSATS Institute of Information Technology, Defence Road, Off Raiwind Road, Lahore, Pakistan.
E-mail address: faizasharif@ciitlahore.edu.pk (F. Sharif).

http://dx.doi.org/10.1016/j.chemosphere.2017.05.059
0045-6535/© 2017 Published by Elsevier Ltd.

solutions containing potential harmful chemicals and screened for detrimental effects (Ali et al., 2012; Brittijn et al., 2009; Hill et al., 2005). Also in the field of compound screening, where large numbers of substances are screened for novel potential applications, zebrafish embryos have proved very promising (Brannen et al., 2010; Parng, 2005; Selderslaghs et al., 2009; Teraoka et al., 2003; Zon and Peterson, 2005). One of the main advantages of zebrafish as an experimental model in toxicity or compound screening (reviewed in (Truong et al., 2011), is the large number of embryos available from a single breeding pair (Goolish et al., 1998; Laale, 1977; Lawrence, 2007; Spence et al., 2008). The eggs are laid and fertilized externally, thus making them accessible for experimentally controlled conditions (Nüsslein-Volhard, 1994). Automation using robotics to manipulate and image the embryos is possible due to the presence of the chorion which protects the embryo from mechanical damage (Braunbeck et al., 2005; Graf et al., 2011).

Recently it has become clear that the increasing concentrations of (synthetic) glucocorticoids in waste and surface waters have the potential to influence the normal development of vertebrates in general and their endocrine systems in particular (Hidasi, 2016). For example, it has been shown that environmentally relevant concentrations of prednisolone (Chen et al., 2016; McNeil et al., 2016), triamcinolone and dexamethasone (Chen et al., 2016) or deflazacort (Kumar et al., 2013) influence zebrafish development and behaviour. More specifically, changes in heart function, hatching rate, spontaneous muscle contractions and the locomotor response of larvae in response to a mechanical stimulus were altered in larvae after exposure to prednisolone. The zebrafish endocrine system and glucocorticoid related behaviours are well described (Kalueff et al., 2014; Steenbergen et al., 2011; Stewart et al., 2014; Wendelaar Bonga, 1997). An increasing amount of studies describe the details of the Hypothalamus Pituitary Interrenal axis (HPI-axis) during development (Alsop and Vijayan, 2008; McGonnell and Fowkes, 2006; Tokarz, 2012; Tokarz et al., 2013) and adulthood (Pavlidis et al., 2015; Wilson et al., 2016). Recently, genetic tools using optogenetic activation or morpholino knowdown have been created to influence endogenous cortisol production (De Marco et al., 2013; Gutierrez-Triana et al., 2015; Wilson et al., 2015, 2013). The HPI-axis in zebrafish becomes functional shortly after hatching, and freely swimming larvae exposed to several types of environmental stressors (mechanical (Alsop and Vijayan, 2008; De Marco et al., 2014), chemical (Yeh et al., 2013) or temperature (Long et al., 2015; Yeh et al., 2013)) actively respond to changes in their environment in order to maintain homeostasis. Moreover, during the early stages of development, maternally derived glucocorticoids orchestrate numerous cellular processes and growth of the embryo and are crucial for normal development (Chatzopoulou et al., 2015; Faught et al., 2016; Mommsen et al., 1999).

We (Sharif et al., 2015; Steenbergen et al., 2011) and others (Chatzopoulou et al., 2015; Chen et al., 2016; Hillegass et al., 2008; Sengupta et al., 2012; Weger et al., 2012; Wilson et al., 2015, 2013) have been pharmacologically influencing larval physiology by exposing zebrafish embryos to solutions containing synthetic or natural glucocorticoids. It has previously been shown that alterations of the HPI-axis using exogenous glucocorticoids can lead to developmental defects and behavioural changes among others (Kumar et al., 2013; McNeil et al., 2016; Wilson et al., 2013). Interestingly, the presence of the chorion during zebrafish development has been proven to be a ‘conditional’ barrier that can prevent even very small molecules present in the bathing solution from reaching the embryo itself (Ali et al., 2012; Braunbeck et al., 2005; Brox et al., 2016; Gellert and Heinrichsdorff, 2001). But, to the best of our knowledge, it has not yet been described in zebrafish how efficient the uptake of glucocorticoids from embryo medium is.

In the present study we therefore examined: (1) dynamics of internal glucocorticoid levels in zebrafish embryos in relation to their environment; (2) whether embryos of different developmental stages incorporate water soluble glucocorticoids according to the same pattern or in a different manner. To address those questions, we raised developing embryos in solutions containing radioactively labelled cortisol, the main endogenous glucocorticoid in teleosts, or the synthetic glucocorticoid dexamethasone. Then we measured the presence of radioactivity in the embryos over time. Firstly, we determined the optimal concentration of cortisol and dexamethasone that did not affect behavioural responses in the well-established light-dark transition challenge. The same concentration did not lead to any developmental abnormalities. Then, the contribution of the chorion during measurement was assessed. For the uptake experiments during two developmental stages, the chorion was first removed before the radioactive signal in the embryos was quantified. In all experiments zebrafish embryos with an intact chorion were used as we wanted to preserve the integrity of the developing embryo at very young stages.

2. Materials and methods

2.1. Statement of ethics on animal use

All experimental procedures were conducted in accordance with The Netherlands Experiments on Animals Act that serves as the implementation of “Guidelines on the protection of experimental animals” by the Council of Europe (1986), Directive 86/609/EC, and were performed only after a positive recommendation of the Animal Experiments Committee of Leiden University had been issued.

2.2. Animal husbandry

Male and female juvenile zebrafish (*Danio rerio*) of AB wild type were purchased from Selecta Aquarium Speciaalzaak (Leiden, The Netherlands). Fish were kept at a maximum density of 12 individuals in plastic 7.5 L tanks (Tecniplast, Germany) containing a plastic plant as tank enrichment in a zebrafish recirculation system (Fleuren & Nooijen, Nederweert, The Netherlands) on a 14 h light:10 h dark cycle. Water and air temperature were maintained at 24 °C and 23 °C respectively. Fish were allowed to adapt to our facility for at least 2 months before being used as adult breeders. Mesh nets were applied to the adult tanks the evening before egg collection. Approximately 100 eggs were collected in 10 cm Petri dishes filled with egg water (0.21 g/L Instant Ocean Sea Salt and 0.0005% (v/v) methyl blue) directly after mating and kept in an incubator on a 13 h light:11 h dark cycle (light on at 7am) and maintained at a temperature of 28 °C or directly transferred to 24-well plates.

2.3. Determination of effective glucocorticoid concentration that does not affect the normal development and basal or stimulus-induced behavioural response of zebrafish larvae

For dose selection, embryos were exposed to 5 different concentrations of cortisol or dexamethasone from 1 to 48 hpf. Directly after mating, 40 chorionated embryos were transferred to the well of a 24-well plate containing cortisol solution (hydrocortisone, water soluble, Sigma Aldrich) or dexamethasone solution (water soluble, Sigma Aldrich). Glucocorticoids were dissolved in egg water and kept at 28 °C. A total volume of 1 mL per well was used. For both glucocorticoids, concentrations of 0, 2.5, 5, 10 and 40 μM were tested (respectively 0, 0.9, 1.8, 3.6 and 14.5 mg/L cortisol and 0, 1.0, 2.0, 4.0 and 15.8 mg/L dexamethasone). During exposure,

Download English Version:

https://daneshyari.com/en/article/5745966

Download Persian Version:

https://daneshyari.com/article/5745966

Daneshyari.com