ZEBRAFISH AS A MODEL FOR UNDERSTANDING THE ROLE OF ENVIRONMENTAL CHEMICALS IN OBESITY

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Introduction

Obesity is a serious health risk that has grown to epidemic proportions globally. Obesity has historically been considered a disorder of energy imbalance (too much intake, too little expenditure) imposed on a background of genetic disposition. Increasing evidence exists, however, which shows that nutritional and environmental factors during early life influence the development of obesity in the long term. In particular, early developmental exposures to environmental chemicals may play a role in the onset of adult obesity. While the underlying mechanisms are unclear, environmental chemicals may disturb epigenetic, structural and functional adaptive responses responsible for regulating energy metabolism and adipogenesis. We have recently initiated a project using the zebrafish *Danio rerio* to test the hypothesis that early exposure to environmental chemicals leads to adult onset of obesity, which may also be passed on over generations. The zebrafish is one of the most important models in environmental toxicology and developmental biology, and is rapidly becoming a major model for studies in human health and disease. The potential to perform high throughput screens in the zebrafish embryo allows for the development of methods to rapidly test environmental chemicals for their "obesogenic" potential. This project will determine causality and multigenerational effects of early exposure to environmental chemicals, as well as examine the molecular targets and cellular mechanisms underlying obesogenic effects of environmental chemicals. In addition, the simultaneous execution of this project together with the EU project OBELIX[1] allows for comparison of the established mouse model for obesity with this up-and-coming zebrafish model. The first studies in this project will be described here, in which our objective was to determine if exposure to known obesogenic chemicals affects lipid metabolism in the embryo.

Materials and methods

Adult and embryonic zebrafish were maintained as described before[2]. Embryos were exposed to bisphenol A (BPA) and tributytin (TBT), which were dissolved in DMSO and added to embryo medium (Dutch standard water) at a volume of 0.01%[2]. The method of lipid staining described by Jones and collegues (2008)[3] was adapted to environmental chemical exposure. Briefly, zebrafish larvae were incubated with daily refreshing of nile red containing embryo medium starting from 3 days post-fertilization (dpf) and continuing with daily refreshing until 7 dpf. Fluorescent lipid staining was visualized as described previously[3]. In situ hybridization of an RNA probe peroxisome proliferator activator receptor gamma (PPARγ) as described previously[2].

Results and discussion:

Preliminary experiments indicate that exposure of zebrafish embryos to BPA and TBT leads to enhanced lipid accumulation in 7 dpf embryos (Figure 1). We are currently investigating if this enhanced lipid accumulation is due to enhanced uptake of lipids from the yolk and/or due to stimulation of adipocyte differentiation. We have confirmed by in situ hybridization analysis that PPARγ, an important regulator of adipogenesis, is expressed in zebrafish in the gut and pancreas at 7 dpf (Figure 2). Accordingly, exposure of zebrafish embryos to the pharmacological PPARγ activator troglitazone also resulted in enhanced lipid staining using Nile Red (data not shown).

The chemicals tested in this study have all been implicated in animal studies as being potential obesogens. Current studies in the OBELIX project have shown that perinatal exposure of mice to BPA leads to significantly higher weight gain in males[4]. In addition, TBT is known to induce differentiation of mouse pre-adipocytes to adipocytes in vitro[5,6]. Fluorescent lipid staining in zebrafish embryos appears to be a promising method to screen chemicals for their potential to disrupt lipid metabolism. Further studies are ongoing to develop a high throughput screen, as well as to examine if early life exposure to chemicals leads to effects in adult zebrafish. In

addition, studies are ongoing to examine biological mechanisms underlying altered programming by early life stage exposure to chemicals, in particular changes in DNA methylation.

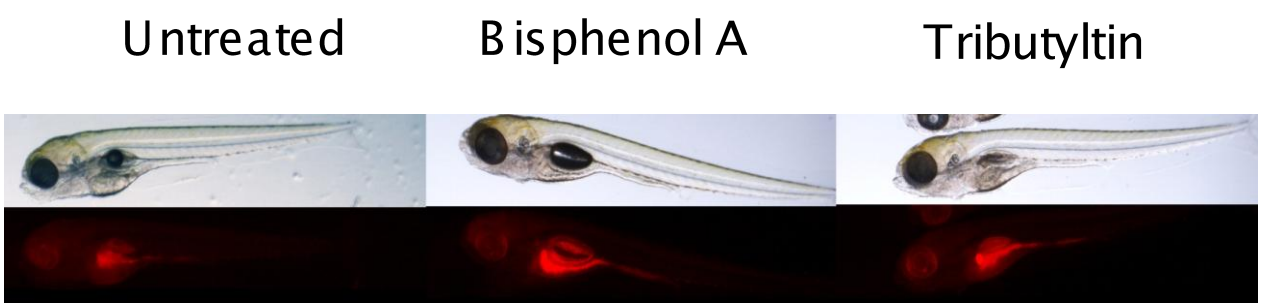

Figure 1: Enhanced lipid accumulation in zebrafish embryos exposed to environmental chemicals. Zebrafish (7 days post fertilization) were stained with fluorescent lipid stain Nile Red.

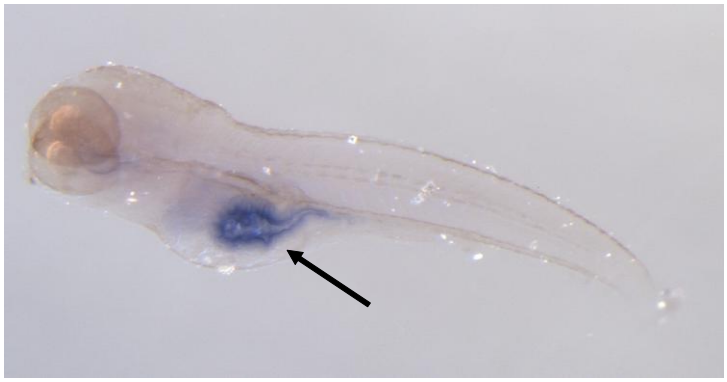

Figure 2: Zebrafish embryos (7 dpf) express PPARγ mRNA in gut and pancreas (blue staining, arrow).

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