Zebrafish as a model for screening the effect of polyphenols on lipid metabolism

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INTRODUCTION

Zebrafish (Danio rerio) is a common model that has been widely used in pre-screenings as an intermediate model between in vitro assays and murine models mainly in embryology and toxicology. This model has several advantages that make it ideal for experiments previous to rodents and humans, as its maintenance is inexpensive and its offspring is very abundant. Thus, large screening experiments are possible, genetic manipulation can be easily done and embryos are translucent which allows in vivo observation and manipulation of different biological processes. The objective is to evaluate the suitability of zebrafish model for polyphenol screening, verifying resveratrol absorption and metabolism and quantifying its effect on fat deposit in larvae.

MATERIAL & METHODS

Resveratrol doses and stability

30 larvae (3dpf) were incubated with resveratrol 100, 400 and 1000 µM for 48 hrs and dead larvae number was determined. Resveratrol stability was measured in the media at 0, 24 and 48h, at selected working dose.

Absorption and Metabolism: Resveratrol Metabolites

100 larvae (3dpf) were immersed in Resveratrol 100 µM for 24 h. Resveratrol and its metabolites were extracted with 75:25 MeOH:H2O and analyzed by HPLC.

Morphological Study: Nile red staining

Larvae (3dpf) were immersed in 100 µM Resveratrol during 48h. They were stained by overnight immersion in Nile red. Fluorescence was analyzed by microscopy. All images were taken under same camera conditions and pictures were analyzed by ImageJ and by a novel software. Several versions of the software were developed in order to ameliorate the variability.

RESULTS & DISCUSSION

Resveratrol doses and stability

Resveratrol working dose (100 µM) was selected as the oose in wihc larvae mortality was similar to that of the control group. Resveratrol remained stable during 48h under the experimental conditions. Slight increases in Resveratrol concentration with time are probably due to evaporation of the media in larvae rearing conditions (12h light/12h dark cycle at 27º).

Absorption and Metabolism: Resveratrol Metabolites

The metabolite profile of resveratrol was similar to that found in rats (plasma, tissues and urine) and humans (urine). Therefore, zebrafish seems to be a good model for the study of Resveratrol effect, as it is absorbed and metabolized in a similar way to mammalian species, including human. Other polyphenols have to be evaluated, but probably mechanisms for polyphenol transformation are similar between mammals and zebrafish.

CONCLUSIONS

The present work shows zebrafish as an animal model for screening of polyphenol effects in lipid metabolism thus contributing to a fast, reliable and cost effective research.

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