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BLT-1, a specific inhibitor of the HDL receptor SR-BI, induces a copper-dependent phenotype during zebrafish development

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Abstract

Block lipid transport-1 (BLT-1) is a small chemical widely used to inhibit the transfer of lipids between high-density lipoproteins (HDL) and cells mediated by scavenger receptor B, type 1 (SR-BI). This study demonstrated that BLT-1 induced in zebrafish (*Danio rerio*) embryos a copper-dependent phenotype with a twisted notochord, brain ventricle enlargement, and absence of melanisation, phenocopying neocuproine-treated, or *calamity* mutants. This finding supports an unexpected link between copper availability and SR-BI activity. The copper-chelating activity of BLT-1, revealed by its dramatic effect during embryo development, should be considered in any evaluation of the pharmacological effect of this thiosemicarbazone derivative on SR-BI activity and the potential therapeutic value of this molecule.

Keywords: BLT-1; Copper; High-density lipoproteins; SCARB1; Scavenger receptor class B type 1; Zebrafish

1. Introduction

Scavenger receptor class B, type 1 (SR-BI) (also known as SCARB1, CD36L1 or CLA1), like CD36 and lysosomal membrane protein II, classified as a member of the class B scavenger receptor family, plays a major role in controlling cholesterol homeostasis in mammals, and is a physiologically relevant high-density lipoprotein (HDL) receptor (Rhainds and Brissette, 2004; Greaves and Gordon, 2005; Van Eck et al., 2005). Members of this family are deduced to have horseshoe-like membrane topologies with the bulk of the protein in a heavily N-glycosylated, disulfide-containing extracellular loop (Krieger, 2001; Rhainds and Brissette, 2004). HDL are protective against cardiovascular disease, due to their important role in the reverse cholesterol transport pathway. SR-BI facilitates the uptake of HDL cholesteryl esters (CE) in a two-step process, involving binding HDL to its extracellular domain and transferring HDL core CE to a metabolically active membrane pool. This process does not involve the sequential internalization of an intact lipoprotein particle and its subsequent degradation. SR-BI also mediates the bidirectional flux of unesterified cholesterol and phospholipids between HDL and cells. It also acts as a low-density lipoprotein (LDL)

Abbreviations: apoA-I, apolipoprotein A-I; BLT-1, block lipid transport-1; CE, cholesteryl esters; DIC, differential interference contrast; DMSO, dimethylsulfoxide; HDL, high-density lipoproteins; hpf, hours post-fertilization; LDL, low-density lipoproteins; SR-BI, scavenger receptor class B type 1; YSL, yolk syncytial layer

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receptor, as well as a receptor of modified lipoproteins (including oxidized LDL, acetylated LDL, and oxidized HDL), anionic phospholipids, serum amyloid A, maleylated bovine serum albumin, and advanced glycation end-products (Krieger, 2001; Van der Westhuyzen et al., 2005). SR-BI has been shown to be involved in other physiological systems (Krieger, 2001), such as mediating transport of lipophilic vitamin E across enterocytes (Reboul et al., 2006), is a key partner for the initial high affinity cholesterol ligand binding process in the intestinal cholesterol absorption pathway (Labonté et al., 2007) and its overexpression may accelerate intestinal lipid absorption in mice (Bietrix et al., 2006).

Block lipid transporter-1 (BLT-1) is a small molecule, identified as the most potent chemical inhibitor of the SR-BI-mediated selective transfer of lipids (Nieland et al., 2002). Although the mechanisms underlying BLT-1 activity are currently unknown, the effect of this compound is considered highly specific to the SR-BI pathway, as it does not interfere with receptor-mediated endocytosis or other forms of intracellular vesicular traffic. BLT-1, at concentrations ranging from 1 to 100 μM, is currently used as a tool for analysing the molecular and cellular mechanisms involved in SR-BI activity, as well as the physiological functions of this HDL receptor (Nieland et al., 2002; Van der Westhuyzen et al., 2005; Duong et al., 2006; Örtegren et al., 2006; Osada et al., 2006; Sahoo et al., 2007; Sun et al., 2006; Zimetti et al., 2006).

In this paper, we show that BLT-1, commonly used as a specific SCARB1/SR-BI inhibitor in mammals, induces embryonic malformations in zebrafish, similar to those observed with neocuproine, a copper chelator, or *calamity*, a mutant defective in the ortholog of the Menkes disease gene (Mendelsohn et al., 2006). The BLT-1-induced phenotype was rescued by treatment with copper chloride, supporting the hypothesis that there is a link between copper availability and SR-BI activity.

2. Materials and methods

2.1. Zebrafish maintenance and analysis

Zebrafish (*Danio rerio*) embryos and larvae were obtained by natural mating and raised at 28.5 °C, as described at http://zfin.org/zf_info/zfbook/cont.html. Developmental stages (Kimmel et al., 1995) were reported in hours post-fertilization (hpf). Phenotypes were recorded as exhibited by each animal at the indicated drug dose and developmental stage. Live embryos and larvae were examined with a Nikon SMZ 1500 stereomicroscope. Embryos and larvae were fixed in 4% paraformaldehyde overnight at 4 °C, followed by several washes in phosphate-buffered saline, and gradually transferred to 90% glycerol. As all embryos treated with BLT-1 were unhatched, they were removed from their chorion with fine forceps before observation. Differential interference contrast (DIC) images were obtained using a Nikon Eclipse E1000 microscope fitted with Nomarski optics. Images were acquired with a Nikon DXM1200 camera and LUCIA G version 4.81 software.

2.2. Experimental solutions

Neocuproine, copper chloride ($CuCl_2$), and dimethylsulfoxide (DMSO) were purchased from Sigma (St. Louis, MO, USA). BLT-1 was obtained from Chembridge Corporation (San Diego, CA, USA) and prepared as 5 mg/ml stock solution in 100% DMSO. Neocuproine and copper chloride experimental solutions were directly prepared in embryo water: 90 μg/ml Instant Ocean (Aquarium Systems, Sarrebourg, France), 0.58 mM $CaSO_4$, $2H_2O$, dissolved in reverse osmosis water. The following exposure concentrations of $CuCl_2$ were tested: 2, 4, 6, 8, 10 and 25 μM. The experimental solutions of copper chloride, neocuproine (10 μM) and BLT-1 (8 μM), were prepared immediately before applying them to zebrafish embryos. Embryo water and embryo water containing 0.04% DMSO were used as control solutions for neocuproine and BLT-1, respectively; the latter treatment had no discernable effect on embryo development.

2.3. Chemical treatments

Within 2 h after spawning, embryos were transferred to 100 ml glass beakers to start continuous exposure to the experimental solutions. By 4 hpf, blastula stage developing embryos were selected and transferred to plastic 12-well plates (1 embryo per well with 2.5 ml medium). The embryos were reared in a dark incubator at 28.5 °C and checked every 12 h for 4 days. The total number of embryos exposed to BLT-1 and neocuproine was 100 with four replicates and 60 with three replicates, respectively. The dose–response studies with exposure to copper alone or with BLT-1 were conducted with nine embryos per dosage group.

3. Results and discussion

Zebrafish is a very valuable model for investigating chemical developmental toxicity (Hill et al., 2005; Lieschke and Currie, 2007). An earlier developmental screen, using synthetic small molecules, reported that zebrafish embryos treated with the small molecule 33M20 showed folds in the notochord along the axis of the trunk (Peterson et al., 2000). Interestingly, 33M20 and BLT-1 are, in fact, different names for the same molecule, 2-hexyl-1-cyclopentanone thiosemicarbazone. Thiosemicarbazones have powerful chelating properties and are complexing agents for copper (Stünzi, 1982; Lhuachan et al., 2003; Tang et al., 2003; Chandra