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NUTRITION

Counteract of bone marrow of blotchy mice against the increases of plasma copper levels induced by high-fat diets in LDLR−/− mice

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ABSTRACT

Background: Bone marrow of blotchy mouse (blotchy marrow) reflects the function of transmembrane domain and relevant intramembrane sites of ATP7A in myeloid cells. By chronic infusion of angiotensin II, we previously found that blotchy marrow plays a minor role in regulating plasma copper. Moreover, the recipients of blotchy marrow presented a moderate reduction of plasma lipids and inflammatory mediator production. Little is known about whether these changes are a specific response to angiotensin II or reveal a more general role of ATP7A.

Objective and design: We investigated if blotchy marrow reduces plasma lipids and inflammatory mediators induced by high-fat diets. To test this hypothesis, blotchy and control marrows were reconstituted to the recipient mice (irradiated male LDLR−/− mice), followed by high-fat-diet feeding for 4 months. At the end points, plasma metals (copper, zinc and iron), lipid profiling (cholesterol, triglyceride, phospholipids and lipoprotein) and six inflammatory mediators (lymphotacin, MCP3, MCP5, TIMP1, VEGF-A and IP-10) were measured. Parallel experiments were performed using male LDLR−/− mice fed either high-fat diets or chow diets for 4 months.

Results: In addition to hyperlipidemia and low-grade inflammation, high-fat diets selectively increased plasma copper concentration compared to chow diets in LDLR−/− mice. After high-fat-diet feeding, the recipients with blotchy marrow showed a decrease in plasma copper ($p < 0.01$) and an increase in plasma iron ($p < 0.05$). The recipients with blotchy marrow also presented decreases in cholesterol ($p < 0.01$) and phospholipids ($p < 0.05$) in plasma. Surprisingly, plasma levels of MCP3 ($p < 0.05$), MCP5 ($p < 0.05$), TIMP1 ($p < 0.01$), VEGF-A ($p < 0.01$) and IP-10 ($p < 0.01$) were significantly increased in the recipients with blotchy marrow compared to controls; the increased levels of MCP3, MCP5 and TIMP1 were more than 50%.

Conclusion: Our studies showed that blotchy marrow counteracts the increased copper levels induced by high-fat diets, indicating that circulating myeloid cells can regulate blood copper levels via ATP7A. Moreover, transplantation of blotchy marrow followed by high-fat diets leads to a decrease in lipid profile and an increase in inflammatory mediator production. Overall, blotchy marrow mediates divergent responses to angiotensin II and high-fat diets in vivo.

Introduction

The blotchy mouse is an animal model primarily recapitulating the connective tissue defects in human Menkes disease, which is an X-linked genetic disorder caused by loss-of-function mutations of *ATP7A*. *ATP7A* is located at Xq21.1, consists of 23 exons, and encodes a copper-transporting P-type ATPase. Its gene product, ATP7A, a single-chain 178-kDa polypeptide, is localized to the *trans*-Golgi apparatus and, to a lesser extent, plasma membrane, nucleus, lipid rafts and phagosome [1–3]. The similarity between mouse and human ATP7A is high: 88% in nucleotide and 90% in amino acid [4]. The studies from Menkes disease and its animal models reveal that the primary functions of ATP7A are to emigrate copper from cells [5–9] and to deliver dietary copper from

Abbreviations: BMT, bone marrow transplantation; cGy, centiGray; FPLC, fast performance liquid chromatography; GFP, green fluorescent protein; HDL, high density lipoprotein; IP, interferon-γ-inducible protein; LDL, low density lipoprotein; LDLR, low-density lipoprotein receptor; MCP, monocyte chemotactic protein; PCR, polymerase chain reaction; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein; WT, wild type.

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intestine to blood [10–12]. The reduced activity of copper-dependent enzymes, such as dopamine-β-hydroxylase [13,14], cytochrome oxidase [13,15], lysyl oxidase [16,17] and extracellular superoxide dismutase [18–20], are well documented in the tissues of Menkes disease patients and animal models. The reduction appears partly due to *ATP7A* mutations failing to transfer copper to cellular secretory pathways, likely the first site for these enzymes to receive copper in a tightly regulated manner [21], and partly due to overall reduction of copper levels in extracellular space, likely the second site for these excreted enzymes to receive copper for the maintenance of their full activity. The later is important in the homeostasis of vascular walls, because a recent study indicated that elastic laminae in extracellular matrix serve as a major copper reservoir [22,23]. Furthermore, the copper deprivation caused by dysfunctional copper delivery in intestine of Menkes disease overshadows the functions of ATP7A in other tissues, bone marrow transplantation (BMT) offers a bypass around the intestine barrier and directly investigates the function of ATP7A in myeloid cells in vivo.

The functional domains of ATP7A include six metal-binding domains with MXCXXC binding motifs, a *n*ucleotide-binding domain, an *a*ctuator domain, a *p*hosphorylation domain, eight transmembrane domains and a C-terminal tail. These functional domains offer a glimpse how ATP7A functioning as a copper émigré. Copper emigration is efficiently (from few minutes to few hours) regulated via two mechanisms: translocation, ATP7A primarily locates at *trans*-Golgi apparatus at basal levels and has capacity to translocate to plasma membrane under the copper elevation [24]; and catalytic cycle, a cycle of phosphorylation to escalate copper affinity of ATP7A and dephosphorylation to reduce copper affinity [25]. ATP7A translocation can be regulated by metal-binding domains [24], which serves as a sensor of low copper levels [25]. Catalytic cycle contains two important components: the formation of a transient phosphorylated intermediate, affecting copper affinity of ATP7A [25]; and the role of a lumenal loop (Met672–Pro707) between the first two transmembrane domains, binding copper and facilitating copper releasing [21]. In addition, two transmembrane domains (six and seven) contain high affinity sites to copper [26]. Furthermore, the theory of catalytic cycle emphasizes the important role of transmembrane domains, which is further reiterated in blotchy mice with ATP7A mutations targeting to the fourth transmembrane domain [27]. Because the mutations in blotchy mice moderately reduced tissue amount of ATP7A (hypomorphic), but not change the protein size, the finding from bone marrow of blotchy mice (blotchy marrow) primarily represents the function of transmembrane domain and intramembrane sites of ATP7A in myeloid cells. In addition, we found that certain stimulants can gradually increase ATP7A protein levels within a few days, likely associated with increased copper emigration [8]. Thus, increasing protein levels may serve as a chronic mechanism to regulate copper emigration via an increase in functional domain availability to stimulate protein translocation and/or to enhance the copper affinity constantly.

Because blotchy mice develop aortic aneurysm and rupture [28], we recently studied the contribution of blotchy marrow to aortic pathologies in a well-accepted model of aortic aneurysm and dissection (chronic infusion of angiotensin II) [29]. This study revealed that blotchy marrow has less influence on plasma copper and aortic aneurysm and dissection, although there was an increased tendency of aortic rupture. Moreover, this study revealed novel functions of blotchy marrow – the recipients of blotchy marrow present a moderate reduction of plasma triglycerides and a subset of inflammatory mediators. However, little is known about whether these changes are a specific response to angiotensin II or reveal a more general role of blotchy marrow. We therefore investigated how blotchy marrow regulates plasma lipids and inflammatory mediator production under the induction of high-fat diets. Surprisingly, this study also revealed an opposite regulation of plasma copper levels by high-fat diets and blotchy marrow.

Materials and methods

Mice: ATP7A-deficient heterozygous female mice (B6Ei.Cg-*Atp7a*$^{Mo-blo}$), green fluorescent protein (GFP) transgenic mice [C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ] and low-density lipoprotein receptor (LDLR)−/− mice (B6.129S7-*Ldlr*^{tm1Her}) were obtained from the Jackson Laboratory (Bar Harbor, ME). ATP7A-deficient female heterozygotes were crossed with GFP transgenic males to produce two new mouse strains: blotchy; GFP and wild type (WT); GFP mice [29]. A normal chow diet and an atherogenic high-fat diet (TD.88137) were used (Table 1). The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio.

Genotyping: Genomic DNAs were obtained from either mouse-tail snips or blood samples. Polymerase chain reaction (PCR) was employed for the genotyping of LDLR−/− mice and GFP transgenic mice, whereas PCR followed by restriction enzyme (AvaII, New England Biolabs, Ipswich, MA) digestion was conducted for the genotyping of blotchy mice and mice reconstituted with blotchy marrow as described [29].

Bone marrow transplantation: Blotchy; GFP or WT; GFP mice at 3–4 months of age (donors) and LDLR−/− mice at 2–3 months of age (recipients) were used to create chimeras. One wk prior to radiation, the recipient mice were given acidified (pH 2.6) water containing neomycin (0.2 mg/mL; Sigma–Aldrich, St. Louis, MO) and polymyxin B (0.02 mg/mL; Sigma–Aldrich). During radiation, a total of 900 centiGrays (cGy) was administered at a rate of 98.1 cGy/min to eradicate bone marrow from the recipient mice using a ^{137}Cesium source. Bone marrow was isolated from the donor mice by flushing their femurs with Dulbecco's modified Eagle's medium (GIBCO^{TM}, Invitrogen, Carlsbad, CA), followed by disrupting the marrow through a 25-gauge needle and filtering through a 40-μm-cell strainer (BD Biosciences, Franklin Lakes, NJ). Following centrifugation at 250 × *g* for 5 min, the pellet was resuspended in Hank's balanced salt solution (Invitrogen). Four hrs after radiation, these marrow cells (5×10^6) were injected into the tail veins of recipient mice. The mice were kept on antibiotic water for 4 wks post-radiation. There was no significant difference in

Table 1
Comparison of dietary composition between the chow diets and high-fat diets used in this study.

Dietary composition	Unit	Chow diet[a] (Harlan 7012)	High-fat diet[a] (TD.88137)
Protein	% by weight	19	17
Fat (ether extract)	% by weight	6	49
Carbohydrate	% by weight	44	21
Energy density	kcal/g	3	5
Calories from protein	%	25	15
Calories from fat	%	17	42
Calories from carbohydrate	%	58	43
Total saturated fatty acid	% of diet	1	13
Total monounsaturated fatty acid	% of diet	1	6
Total polyunstaturated fatty acid	% of diet	3	1
Copper	mg/kg	23	6
Zinc	mg/kg	63	35
Iron	mg/kg	240	36

[a] The data were collected from datasheet of 7012 and TD.88137 as well as personal communication (Dr. Ramesh Khanal) from Harlan Laboratories, Inc., Madison, WT 53744.