SciMedCentral

JSM Enzymology and Protein Science

Short Communication

Beneficial Effect of Homocysteine Lowering on Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Level in Hyperhomocysteinemic Mice

Asma Tlili[1]*, Leanne de Koning[2], Thierry Dubois[2], Bart De Geest[3], and Nathalie Janel[1]*

[1]*Université Paris Diderot, Sorbonne Paris Cité, Biologie Fonctionnelle et Adaptative (BFA), France*

[2]*Department of Translational Research, Institut Curie, France*

[3]*Center for Molecular and Vascular Biology, University of Leuven, Belgium*

***Corresponding author**

N Janel, Université Paris Diderot, Sorbonne Paris Cité, Biologie Fonctionnelle et Adaptative (BFA), 75205 Paris cedex 13, France, Tel: 00-331-5727-8360; Fax: 00-331-5727-8354; Email: janel@univ-paris-diderot.fr

Submitted: 15 November 2016

Accepted: 05 December 2016

Published: 07 December 2016

Keywords

- Hyperhomocysteinemia
- LOX-1
- DYRK1A
- Polyphenols
- Adenovirus

Abstract

Background: Hyperhomocysteinemia often results from vitamin deficiency and/or an unhealthy lifestyle. Because the condition is a risk factor for developing cerebrovascular disease or atherosclerosis, approaches that decrease plasma homocysteine level are needed to alleviate this public health issue. Unfortunately, as the standard treatment of supplementation with B vitamins has shown limited benefit, novel therapies must be explored. We have recently focus on two novel approaches, the first being a preventive approach through diet by supplementation with polyphenols, and the other a reinforcement of homocysteine metabolism by targeting the principal organ of homocysteine metabolism, the liver. Chronic supplementation of polyphenols decreased plasma homocysteine and LOX-1 level in hyperhomocysteinemic mice. Plasma homocysteine level also significantly decreased after hepatocyte-specific DYRK1A gene transfer in hyperhomocysteinemic mice, DYK1A being an enzyme implicated in different aspects of homocysteine metabolism.

Aim: We aimed to extend our previous findings by analyzing the effect of hepatocyte-specific Dyrk1a gene transfer on plasma LOX-1 level in hyperhomocysteinemic mice.

Materials and methods: Plasma LOX-1 level and some signalling pathways in aorta were assessed by ELISA and RPPA.

Results: Hepatocyte-specific DYRK1A gene transfer restored plasma LOX-1 level and Pi3K/Akt/mT or pathway in aorta of hyperhomocysteinemic mice.

Discussion: Hepatocyte-specific DYRK1A gene transfer makes it possible to normalize plasma homocysteine level and its associated endothelial dysfunction by restoring LOX-1 level in hyperhomocysteinemic mice as previously found with polyphenols supplementation.

ABBREVIATIONS

CBS: Cystathionine Beta Synthase; DYRK1A: Dual-Specificity Tyrosine-Phosphorylation-Regulated Kinase 1A; GSK3 : Glycogen Synthase Kinase 3; Hhcy: Hyperhomocysteinemia; Hcy: Homocysteine; LOX-1: Lectin-like Oxidized Low-Density Lipoprotein Receptor-1; Oxldl: Oxidized Low Density Lipoprotein Particles; PON-1: Paraoxonase-1

INTRODUCTION

Since its discovery and toxicity in human disease, the metabolism of homocysteine (hcy) and its genetic defects have been extensively explored [1]. The main therapeutic approach to treating Hcy defects is supplementation with B vitamins, in combination with protein restriction, and cysteine supplementation [2-4]. Although treatments can be effective, some patients with hyperhomocysteinemia (Hhcy) are unresponsive to conventional treatment with B vitamins [5]. Therefore, new therapeutic approaches have been sought in recent years to reduce the plasma level of this amino acid, which is considered to be an independent risk factor for the progression of vascular disease [6]. We have recently focus on two major new therapeutic approaches, the first being a preventive approach through diet by supplementation with polyphenols, and the other a reinforcement of Hcy metabolism by genetic manipulation. Both approaches have been designed to improve the health of vessels in patients by targeting the principal organ of Hcy metabolism, the liver. Indeed, impairment of hepatic Hcy

metabolism can lead to higher intracellular concentrations and export to the blood. Hence, plasma Hcy level is an important reflection of hepatic methionine metabolism and of the rate of processes modified by B vitamins. We found, on the one way, that chronic supplementation of polyphenolic extract from red wine in Hhcy mice due to cystathionine beta synthase (CBS) deficiency and fed a high-methionine enriched diet decreased plasma Hcy level [7]. Similar results were observed with a diet that was supplemented with purified catechin [8,9] or epicatechin [9]. On the other way, our results implicated the dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A), an enzyme that is thought to play a role in signaling pathways regulating proliferation and differenciation, in different aspects of Hcy metabolism, with its hepatic protein expression negatively correlated with plasma Hcy level [10-12]. DYRK1A has been therefore showed to be a good candidate for gene therapy to normalize Hcy levels. However, a large body of evidence also implicates DYRK1A in altering synaptic plasticity and facilitating neurodegeneration and dementia [13]. Thus, therapy must be targeted rather than broad. We therefore implemented targeted gene therapy to overexpress DYRK1A specifically in liver [14,15]. For this, a specific adenoviral vector was used to rescue Dyrk1a expression in the liver of Hhcy mice, and plasma Hcy levels significantly decreased after hepatocyte-specific Dyrk1a gene transfer in Hhcy mice [14,15].

One of the key xenobiotic metabolizing enzymes (XME) affected by Hcy is Paraoxonase-1 (PON-1). PON-1 is a phase I XME associated with serum high density lipoprotein (HDL), and is synthesized in the liver [16]. Hhcy mice exhibit a decrease of liver and plasma PON-1 activity, with a strong correlation with plasma Hcy level [17-19]. Supplementation with polyphenolic extract or purified catechin in Hhcy mice induces increased activity of PON-1 in liver and in plasma [7-9]. Targeted hepatic rescue of expression of Dyrk1a also resulted in elevated activity of plasma PON-1 [14,15], which plays a major role in the protective function of HDL against endothelial dysfunction. People with Hhcy exhibit increased expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in mononuclear cells of peripheral blood [20]. LOX-1 activation allows oxidized low density lipoprotein particles (OxLDL) to penetrate macrophages and induce their transformation into foam cells, playing a vital role in regulating the progression of atherosclerotic lesions. Hhcy mice exhibit increased aortic expression and serum amount of LOX-1 [7,9]. Supplementation with polyphenolic extract, catechin or epicatechin, induces a decrease of LOX-1 in aorta and plasma of Hhcy mice [7,9]. It has been demonstrated that LOX-1 activation causes endothelial apoptosis and inflammation, and polyphenol supplementation has beneficial effects on biochemical markers of endothelial dysfunction due to Hhcy [7,9,21]. Commensurate with the effect on PON-1 activity in plasma of Hhcy mice, targeted hepatic Dyrk1a gene transfer can abolish the negative effect of Hhcy on signaling pathways implicated when compromised in impaired endothelial function [14]. Therefore, we aimed to extend our previous findings by analyzing the effect of hepatocyte-specific Dyrk1a gene transfer on plasma LOX-1 level in HHcy mice.

MATERIALS AND METHODS

Experimental animals

All procedures were carried out in accordance with the ethical standards of French and European regulations (European Communities Council Directive, 86/609/EEC). Official authorization from the French Ministry of Agriculture was granted to perform research and experiments on animals (authorization number 75-369), and the experimental protocol was approved by the institutional animal care and use committee of the Paris Diderot University (CEEA40). Mice were housed in a controlled environment with unlimited access to food and water on 12-h light/dark cycle. Number of mice and suffering were minimized as possible. Mice heterozygous for targeted disruption of the Cbs gene (Cbs +/-) were generously donated by Dr. N. Maeda (Department of Pathology, University of North Carolina, Chapel Hill, NC, USA) [22]. Cbs +/- mice, on a C57BL/6 background, were obtained by mating male Cbs +/- mice with female wild-type C57BL/6 (Cbs +/+) mice. The E1E3E4-deleted adenoviral vector "AdDYRK1A" was constructed to induce hepatocyte specific overexpression of DYRK1A, and injected by the retro-orbital sinus to have 2 x 10^{12} adenoviral particles/kg body weight as described previously [14].

Preparation of serum samples, tissue collection, and ELISA assay

When mice were euthanized, blood samples were collected into tubes containing a 1/10 volume of 3.8% sodium citrate and placed on ice immediately. Plasma was isolated by centrifugation at 2,500 x g for 15 min at 4°C. Aorta were harvested, snap-frozen, and stored at -80°C until use. Levels of plasma LOX-1 were determined using an ELISA from R&D Systems, Inc. (R&D Systems Europe, Lille, France).

Protein extraction and reverse phase protein array analysis

Protein extraction from liver and aorta and quantification by slot blotting and reverse phase protein array analysis (RPPA) were performed as described [14].

Data analysis

Statistical analysis was done with one-way analysis of variance (ANOVA) followed by Fisher post-hoc test using Statview software. The results are expressed as medians with interquartile ranges. Data were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Effect on Hcy lowering on plasma LOX-1 levels

We previously found an increased plasma level of soluble LOX-1 in Cbs +/- mice fed a high-methionine diet [7,9]. We confirmed this increase in plasma of Cbs +/- mice compared to wild type Cbs +/+ mice (Figure 1). Conversely, hepatic DYRK1A protein level was decreased in Cbs +/- mice compared to wild type Cbs +/+ mice (64.7 ± 5.9 vs 111 ± 7.5; $p < 0.0005$; n=8 mice for each group) as previously described [11]. Injection of AdDYRK1A resulted in a significant decrease in plasma LOX-1 level (Figure 1). Conversely, hepatic DYRK1A protein level was increased in Cbs +/- mice injected with AdDYRK1A compared to Cbs +/- mice (389 ± 78

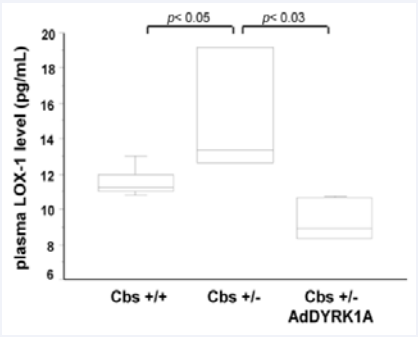

Figure 1 Effect of hepatocyte-specific Dyrk1a gene transfer (AdDYRK1A) on plasma LOX-1 level. n = 8 mice for each group.

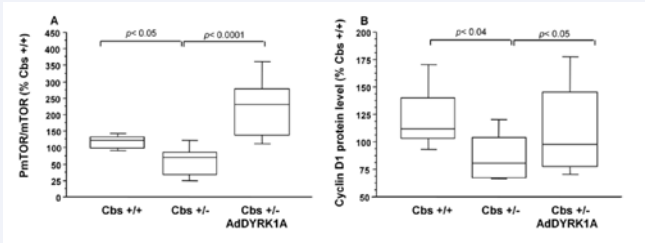

Figure 2 Effect of hepatocyte-specific Dyrk1a gene transfer (AdDYRK1A) on aortic phospho-mTOR and cyclin D1 levels. (A) – Phosphorylation of mTOR determined by RPPA. Relative protein level was determined by normalization from PmTOR with that of total mTOR. (B) – cyclin D1 protein level determined by RPPA. Data were normalized to the mean of wild-type mice (Cbs+/+). n = 8 mice for each group.

vs 64.7 ± 5.9 p <0.0001; n=8 mice for each group) as previously described [14]. Therefore, in relation to our previous work which underlines the effect of supplementation with polyphenols and of hepatocyte-specific Dyrk1a gene transfer on plasma Hcy level [7,8,9,14,15], the decrease in plasma Hcy shows a beneficial effect on plasma LOX-1 level in HHcy mice. Polyphenols can act at several locations to counteract the cytotoxicity of Hcy, thereby preventing vascular pathology related to liver phenotypes. A healthy liver supports vascular health. The down-regulation of LOX-1 may be a potential treatment to protect against ox-LDL-induced endothelial cell injury in high-risk vascular diseases. Ox-LDL-induced endothelial cell apoptosis can be reduced by inhibiting LOX-1 expression and by increasing the activation of PI3k/Akt signaling pathways [23]. The PI3K/Akt pathway is a pivotal signal pathway involved in cell survival and metabolism [24]. PI3K/Akt has been shown to affect several important endothelial functions to promote endothelial cells survival [25]. We previously found a decrease of phospho-Akt in aorta of HHcy mice, which is restored by hepatocyte-specific Dyrk1a gene transfer [14].

Effect on Hcy lowering on Pi3K/Akt/mTor pathway in aorta

Given the fact that PI3K/Akt/mTOR pathway plays a key role in cellular homeostasis through its role in regulation of apoptosis, cell growth, cell cycle and angiogenesis, we also analysed mTOR phosphorylation. We found a decreased phosphorylation of mTOR in aorta of Cbs +/- mice (Figure 2A), and injection of AdDYRK1A resulted in a significant increase (Figure 2A). Previous results demonstrated the role of Pi3K for upregulation of cyclin D1 [26]. Akt also promotes cyclin D1 translation via indirect activation of mTOR [27], and prevents phosphorylation and degradation of cyclin D1 by phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) at Ser9 [28]. Hepatocyte-specific Dyrk1a gene transfer not only resulted in PI3K/Akt activation but also in GSK3 inhibition in aorta of Hhcy mice [14]. We therefore analysed the protein level of cyclin D1, and found a decrease in aorta of Cbs +/- mice (Figure 2B), which is abolished after injection of AdDYRK1A to Cbs +/- mice (Figure 2B). Previous results demonstrated the ability of Hcy to decrease mRNA expression of cyclin D1 in human umbilical vein endothelial cells [29]. Further, hepatocyte-specific Dyrk1a gene transfer rescues Akt/GSK3/cyclin D1 signaling pathways not only in aorta but also in liver of HHcy mice [15]. Taken together, Hcy lowering by AdDYRK1A injection abolishes the negative effect on PI3K/Akt/mTOR pathway and on cyclin D1 protein level.

CONCLUSION

In conclusion, taken together with our previous works, we found that two different approaches one preventive and based on chronic supplementation of polyphenols, and the other genetic and based on restoring hepatic function make it possible to normalize plasma Hcy level and its associated endothelial dysfunction by restoring LOX-1 level in Hhcy mice.

ACKNOWLEDGEMENTS

We thank Dr. N. Maeda (Department of Pathology, University of North Carolina, Chapel Hill, NC) for providing heterozygous Cbs-null mice. We thank D. Quintas for technical assistance. We acknowledge the platform accommodation and animal testing of the animal house at the Institute Jacques-Monod (University Paris Diderot) and the FlexStation3 Facility of the Functional and Adaptative Biology (BFA) laboratory. This work was supported by the Association Gaspard Félix (L'AGAFE). Leanne de koning and the Institut Curie RPPA platform are supported by the Cancéropole Ile-de-France.

REFERENCES

1. Schalinske KL, Smazal AL. Homocysteine imbalance: a pathological metabolic marker. Adv Nutr. 2012; 3: 755-762.
2. Wilcken DE, Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. J Inherit Metab Dis. 1997; 20: 295-300.
3. Yap S, Naughten ER, Wilcken B, Wilcken DE, Boers GH. Vascular complications of severe hyperhomocysteinemia in patients with homocystinuria due to cystathionine beta-synthase deficiency: effects of homocysteine-lowering therapy. Semin Thromb Hemost. 2000; 26: 335-340.
4. Yap S, Boers GH, Wilcken B, Wilcken DE, Brenton DP, Lee PJ, et al. Vascular outcome in patients with homocystinuria due to cystathionine beta-synthase deficiency treated chronically: a multicenter observational study. Arterioscler Thromb Vasc Biol. 2001; 21: 2080-2085.
5. Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet. 1985; 37: 1-31.

6. Heneghan HM, Sultan S. Homocysteine, the cholesterol of the 21st century. Impact of hyperhomocysteinemia on patency and amputation-free survival after intervention for critical limb ischemia. J Endovasc Ther. 2008; 15: 399-407.

7. Noll C, Hamelet J, Matulewicz E, Paul J-L, Delabar J-M, Janel N. Effects of red wine polyphenolic compounds on paraoxonase-1 and lectin-like oxidized low-density lipoprotein receptor-1 in hyperhomocysteinemic mice. J Nutr Biochem. 2009; 20: 586-596.

8. Hamelet J, Demuth K, Dairou J, Ledru A, Paul JL, Dupret JM, et al. Effects of catechin on homocysteine metabolism in hyperhomocysteinemic mice. Biochem Biophys Res Commun. 2007; 355: 221-227.

9. Noll C, Lameth J, Paul J-L, Janel N. Effect of catechin/epicatechin dietary intake on endothelial dysfunction biomarkers and proinflammatory cytokines in aorta of hyperhomocysteinemic mice. Eur J Nutr. 2013; 52: 1243-1250.

10. Noll C, Planque C, Ripoll C, Guedj F, Diez A, Ducros V, et al. DYRK1A, a novel determinant of the methionine-homocysteine cycle in different mouse models overexpressing this Down-syndrome-associated kinase. PLoS ONE. 2009; 4: 7540.

11. Hamelet J, Noll C, Ripoll C, Paul JL, Janel N, Delabar JM. Effect of hyperhomocysteinemia on the protein kinase DYRK1A in liver of mice. Biochem Biophys Res Commun. 2009; 378: 673-677.

12. Delabar JM, Latour A, Noll C, Renon M, Salameh S, Paul JL, et al. One-carbon cycle alterations induced by Dyrk1a dosage. Mol Genet Metab Rep. 2014; 1: 487-492.

13. de la Torre R, de Sola S, Hernandez G, Farré M, Pujol J, Rodriguez J, et al. Safety and efficacy of cognitive training plus epigallocatechin-3-gallate in young adults with Down's syndrome (TESDAD): a double-blind, randomised, placebo-controlled, phase 2 trial. Lancet Neurol. 2016; 15: 801-810.

14. Tlili A, Jacobs F, de Koning L, Mohamed S, Bui LC, Dairou J, et al. Hepatocyte-specific Dyrk1a gene transfer rescues plasma apolipoprotein A-I levels and aortic Akt/GSK3 pathways in hyperhomocysteinemic mice. Biochim Biophys Acta. 2013; 1832: 718-728.

15. Latour A, Salameh S, Carbonne C, Daubigney F, Paul JL, Kergoat M, et al. Corrective effects of hepatotoxicity by hepatic Dyrk1a gene delivery in mice with intermediate hyperhomocysteinemia. Mol Genet Metab Reports. 2015; 2: 51-60.

16. Chambers JE. PON1 multitasks to protect health. Proc Natl Acad Sci U S A. 2008; 105: 12639-12640.

17. Robert K, Chassé JF, Santiard-Baron D, Vayssettes C, Chabli A, Aupetit J, et al. Altered gene expression in liver from a murine model of hyperhomocysteinemia. J Biol Chem. 2003; 278: 31504-31511.

18. Janel N, Robert K, Chabert C, Ledru A, Gouédard C, Barouki R, et al. Mouse liver paraoxonase-1 gene expression is downregulated in hyperhomocysteinemia. Thromb Haemost. 2004; 92: 221-222.

19. Hamelet J, Aït-Yahya-Graison E, Matulewicz E, Noll C, Badel-Chagnon A, Camproux AC, et al. Homocysteine threshold value based on cystathionine beta synthase and paraoxonase 1 activities in mice. Eur J Clin Invest. 2007; 37: 933-938.

20. Holven KB, Scholz H, Halvorsen B, Aukrust P, Ose L, Nenseter MS. Hyperhomocysteinemic subjects have enhanced expression of lectin-like oxidized LDL receptor-1 in mononuclear cells. J Nutr. 2003; 133: 3588-3591.

21. Hung CH, Chan SH, Chu PM, Tsai KL. Homocysteine facilitates LOX-1 activation and endothelial death through the PKCβ and SIRT1/HSF1 mechanism: relevance to human hyperhomocysteinaemia. Clin Sci (Lond). 2015; 129: 477-487.

22. Watanabe M, Osada J, Aratani Y, Kluckman K, Reddick R, Malinow MR, et al. Mice deficient in cystathionine beta-synthase: animal models for mild and severe homocyst(e)inemia. Proc Natl Acad Sci U S A. 1995; 92: 1585-1589.

23. Qin M, Luo Y, Meng XB, Wang M, Wang HW, Song SY, et al. Myricitrin attenuates endothelial cell apoptosis to prevent atherosclerosis: An insight into PI3K/Akt activation and STAT3 signaling pathways. Vascul Pharmacol. 2015; 70: 23-34.

24. García-Cardeña G, Anderson KR, Mauri L, Gimbrone MA Jr. Distinct mechanical stimuli differentially regulate the PI3K/Akt survival pathway in endothelial cells. Ann N Y Acad Sci. 2000; 902: 294-297.

25. Tie G, Yan J, Yang Y, Park BD, Messina JA, Raffai RL, et al. Oxidized low-density lipoprotein induces apoptosis in endothelial progenitor cells by inactivating the phosphoinositide 3-kinase/Akt pathway. J Vasc Res. 2010; 47: 519-530.

26. Takuwa N, Fukui Y, Takuwa Y. Cyclin D1 expression mediated by phosphatidylinositol 3-kinase through mTOR-p70(S6K)-independent signaling in growth factor-stimulated NIH 3T3 fibroblasts. Mol Cell Biol. 1999; 19: 1346-1358.

27. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004; 18: 1926-1945.

28. Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Mol Cancer. 2007; 6: 24.

29. Outinen PA, Sood SK, Pfeifer SI, Pamidi S, Podor TJ, Li J, et al. Homocysteine-induced endoplasmic reticulum stress and growth arrest leads to specific changes in gene expression in human vascular endothelial cells. Blood. 1999; 94: 959-967.

Cite this article

Tlili A, de Koning L, Dubois T, De Geest B, Janel N (2016) Beneficial Effect of Homocysteine Lowering on Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 Level in Hyperhomocysteinemic Mice. JSM Enzymol Protein Sci 1(1): 1008.