HYPERHOMOCYSTEINEMIA IN CIRRHOSIS OF THE LIVER

Juraev Bobur Rakhmonovich *Bukhara State Medical Institute named after Abu Ali ibn Sina, Uzbekistan, Bukhara, A. Navoi St. 1 Phone number: +998 (65) 223-00-50*
e-mail: jorayev.bobur@bsmi.uz

Abstract:*Numerous clinical and epidemiological studies have identified elevated plasma homocysteine levels as a risk factor for atherosclerotic vascular diseases and thromboembolism. Hyperhomocysteinemia may develop due to defects in genes that metabolize homocysteine; dietary conditions that lead to a deficiency of vitamins B6, B12, or folic acid; or chronic alcohol use. Homocysteine is an intermediate product of methionine metabolism, which occurs mainly in the liver. Impaired liver function leads to changes in the metabolism of methionine and homocysteine; however, the molecular basis of such changes is not fully understood. In addition, the mechanisms of cellular toxicity caused by homocysteineare not fully defined.*

Keywords: *homocysteine, metabolism, liver, cirrhosis, vitamins B6, B12.*

Introduction

Homocysteine is a sulfur-containing amino acid that is formed as an intermediary in the metabolism of methionine. 1 Extensive data indicate that elevated plasma concentrations of HC, reflecting impaired cellular metabolism, can be considered as an independent risk factor for atherothrombotic vascular disease (reviewed by Refsum et al2) [1-4]. This condition was observed in 20-30% of patients with premature atherosclerosis and in 21% of the general population over a certain age.3,4 Three enzymes use HC as a substrate: methionine synthase (MS) and betaine-homocysteine methyltransferase. (BHMT), which convert homocysteine back to methionine, and cystathionine-β-synthase (CBS), the first enzyme in the trans-sulfuration pathway. 1 The distribution of HC between them depends on metabolic conditions: when methionine is relatively deficient, remethylation of HC favors 1,2 S-adenosylmethionine (AdoMet), the first methionine metabolite, modulates the flow of HC through these metabolic pathways: elevated levels of AdoMet activate CBS and inhibit MS and BHMT activity.1.5 Impaired remethylation or transsulfuration of HC leads to hyperhomocystinemia [5-9]. Such situations may develop due to genetic defects of the MS, CBS or methylenetetrahydrofolate reductase enzymes (an enzyme that synthesizes the MS cosubstrate 5-methyltetrahydrofolate).2,3 Lack of vitamin B6, a cofactor of CBS or folate, and vitamin B12, a cosubstrate and cofactor of multiple sclerosis, can also lead, along with impaired renal function, to hyperhomocystinemia [10-15].

The liver plays a central role in the synthesis and metabolism of homocysteine, given the fact that most of the dietary methionine is metabolized in this organ, where about 85% of the entire body's transmethylation capacity is located. Accordingly, the liver exhibits a specific expression of genes involved in methionine and homocysteine metabolism [16-19]. There are 2 genes encoding methionineadenosyltransferase (MAT), an enzyme that converts methionine to AdoMet, one (MAT1A) is expressed exclusively in the liver, and the second gene (MAT2A) is expressed in all tissues.5 The expression of BHMT and CBS is limited. mainly in the liver, while multiple sclerosis is widespread (Figure 1) [20-25] .1 Thus, it is quite possible that in the case of liver damage, changes in HC may occur. In fact, hyperhomocysteinemia has been reported in chronic alcoholics and patients with alcoholic cirrhosis, as well as in experimental models of liver damage.6-10 Although there is extensive data on the aforementioned genetic and nutritional determinants ofhyperhomocystinemia, knowledge of the molecular basis of changes in HC metabolism in liver damage is still limited [26-30].

In this report, we describe our attempt to gain further understanding of the mechanisms of hyperhomocysteinemia associated with liver damage, as well as the molecular basis of HC interference in normal cell function.

Material and methods

DL-HZ was from Sigma Chemical Co.. Cell culture media, fetal bovine serum, and antibiotics were from GIBCO-BRL. The monoclonal antibody against tissue metalloproteinase inhibitor-1 (TIMP-1) was from Calbiochem. A monoclonal antibody against proliferating cell nuclear antigen (PCNA) was produced by Santa Cruz Biotechnology. The radioactive isotopes were purchased from Amersham. Restriction enzymes were obtained from Boehringer Mannheim, M-MLV reverse transcriptase was from GIBCO-BRL, and Bio Taq DNA polymerase was from Bioline.

Patients

A group of 26 patients (17 men and 9 women; mean age 54±8.5 years) with liver cirrhosis of various etiologies (13 - hepatitis C virus-induced cirrhosis, 10-alcoholic cirrhosis, 1-hepatitis B virus-induced cirrhosis, 1-cryptogenetic cirrhosis, and 1 primary biliary cirrhosis). The control group consisted of 10 patients who underwent cholecystectomy to treat symptomatic cholelithiasis and who agreed to a liver biopsy during the surgical procedure.

Liver samples were immediately frozen and stored at -80°C until processed. In the control group, both liver function tests and liver biopsy were normal. The study was approved by the Human Research Committee of the University of Navarre.

Serum methionine and determination of total HC content

Determination of serum methionine and total serum HC (t HZ = protein bound and free HZ) was performed as described.20

Results obtained

For the first time, we measured the expression of liver genes involved in methionine metabolism. According to the level of expression in comparison with the control liver, cirrhotic patients were divided into 3 groups: group 1 — patients with very low or undetectable expression; group 2-patients with an expression level lower than in the control (50% of the expression found in the control); group 3-patients with an expression level similar to control. Human serum albumin (HSA) expression was also measured.

Individual patients with a marked decrease in the expression of this gene (group 1) tended to be in the same group for all tested genes. To assess whether a decrease in the expression of various genes involved in methionine metabolism is associated with the severity of the disease expressed on the Child-Pugh scale, 21 patients were divided into 2 groups. One group (Group A) included patients with normal or only reduced mRNA levels for at least 3 of the 5 analyzed genes that are involved in methionine metabolism. The second group (Group B) included patients with very low or undetectable mRNA levels of all or 4 of the 5 analyzed genes involved in methionine metabolism. The mean Child-Pugh score was significantly higher in patients with cirrhosis of the liver in group B (9.0±0.7) than in patients with cirrhosis of the liver in group A (7.1±0.4, $P<0.03$).

The mean value of serum HC was significantly higher in patients with cirrhosis of the liver in group B than in group A (Figure 3). Accordingly, the mean concentration of HC was significantly higher for all patients with cirrhosis of the liver (14.1±1.3 mmol/l) than for the control group (8.1±0.9 mmol/ l, $P<0.03$). According to previous publications5, 22 fasting serum methionine was higher in patients with cirrhosis of the liver (106.3±34.7 mmol/L) than in the control group (30.8±4.8 mmol/L, $P<0.01$). Differences in the concentration of methionine in patients with cirrhosis of the liver in groups A and B were also statistically significant.

The second goal of this work was to improve our knowledge of the molecular basis of the cellular effects of HC [30-35]. We observed that treatment with GC of dormant human SGMCS induces expression of PCNA, a marker of cell proliferation (Fig. This effect was also observed when HC was administered to mice and PCNA expression in aortic tissue was determined (Figure 4B).

We looked for other genes whose expression could be altered by GC in cultured human SGMCS using DDPCR. The cells were brought to rest by serum deprivation, and then treated with 100 mmol/ l HZ, a concentration compatible with intermediate hyperhomocystinemia2, for 24 hours [36-39]. Using DDPCR analysis, we determined that TIMP-1 is activated in response to HC treatment (2-fold induction) (Figures 5A and 5B). This effect was also observed in SGMCS cultured in pig aorta (Figure 5C). Moreover, intraperitoneal administration of HC to mice also induced TIMP-1 expression in the aorta (Figure 5D). TIMP-1 plays an important role in regulating

extracellular matrix homeostasis (ECM), 23 which is important not only for the vascular wall, but also in the liver. 24 Therefore, we studied the expression of TIMP-1 and α1 (I)procollagen. in HSC rats, observing that both genes are induced by GC as a function of time and dose in this cell type (Figures 6A and 6B). This effect of GC on TIMP-1 expression was also extended to at hepatocytes and HepG2 cells (Figures 6C and 6D).

Conclusions

Our results show that changes **in HC metabolism** in human liver cirrhosis can be partially explained by a marked decrease in the expression of the main genes involved in its metabolism, namely MS, BHMT, and CBS. The expression of these genes was always more impaired than that of HSA and was associated with the severity of the disease expressed on the Child - Pugh scale. We observe reduced expression **of HC metabolizing genes**in both alcoholism and hepatitis C virus-induced liver cirrhosis.It has been suggested that impaired **HC metabolism** in cirrhosis may also be associated with reduced availability or use of vitamins B6, B12, or folate, 8 which is possible. However, our current data on hyperhomocysteinemia in cirrhosis of the liver have been confirmed in other human studies and experimental studies in which hyperhomocysteinemia was not associated with altered plasma levels of the aforementioned vitamins.25,26 We also observed a decrease in MAT1A expression in cirrhotic liver, which contributes to the reported hypermethioninemia and impaired AdoMet synthesis in this condition.5 Previously, we showed that treatment with AdoMet in rats with cirrhosis of the liver reduces elevated levels **of HC** in plasma.10 Reduced AdoMet levels plus impaired CBS expression in cirrhosis may result in reduced **HC flux** through the transsulfuration pathway and contribute to hyperhomocysteinemia associated with this condition.

Literature

1. Finkelstein JD. Methionine metabolism in mammals. J Nutr Biochem. 1990; *1*: 228–236.
2. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Medicine. 1998; *49*: 31–62.
3. Mayer EL, Jacobsen DW, Robinson K. Homocysteine and coronary atherosclerosis. J Am Coll Cardiol. 1996; *27*: 517–527.
4. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly populations. J Am Med Assoc. 1993; *270*: 2693– 2698.
5. Mukhammadieva M.I. (2022). Modern Clinical and Biochemical Characteristics of Liver Cirrhosis Patients of Viral Etiology with Spontaneous Bacterial Peritonitis //Texas Journal of Medical Science. – 2022.- P. 86-90.

6. Oblokulov Abdurashid Rakhimovich Mukhammadieva Musharraf Ibrokhimovna Sanokulova Sitora Avazovna Khadieva Dora Isakovna. (2023).CLINICAL AND LABORATORY FEATURESOF SPONTANEOUS BACTERIAL PERITONITIS IN PATIENTS WITH VIRAL LIVER CIRRHOSIS. Journal of Advanced Zoology, 44(S2), 3744–3750. from http://www.jazindia.com/index.php/jaz/article/view/1716
7. Oblokulov A.A., Oblokulov A.R., Ergashov M.M. Clinical and laboratory criteria for spontaneous bacterial peritonitis in liver cirrhosis of viral etiology. Central Asian journal of medical and natural sciences Volume 03, Issue 03, may-jun 2022 172-177. ISSN: 2660-4159.
8. Ibrokhimovna, M. M. . (2024). Improvement of Primary Prophylaxis and Treatment of Spontaneous Bacterial Peritonitis Complicated in Virus Etiology Liver Cirrhosis. Journal of Intellectual Property and Human Rights, 3(4), 19–25. Retrieved from http://journals.academiczone.net/index.php/jiphr/article/view/2506
9. Elmurodova A.A. (2023). Viral Hepatitis Delta: An Underestimated Threat. *Texas Journal of Medical Science*, *26*, 1–3. Retrieved from https://zienjournals.com/index.php/tjms/article/view/4610
10. Oblokulov Abdurashid Rakhimovich Mukhammadieva Musharraf Ibrokhimovna Sanokulova Sitora Avazovna Khadieva Dora Isakovna. (2023). CLINICAL AND LABORATORY FEATURES OF SPONTANEOUS BACTERIAL PERITONITIS IN PATIENTS WITH VIRAL LIVER CIRRHOSIS. Journal of Advanced Zoology, 44(S2), 3744–3750. from http://www.jazindia.com/index.php/jaz/article/view/1716
11. Mukhammadieva M.I. (2022). Modern clinical and biochemical characteristics of liver cirrhosis patients of viral etiology with spontaneous bacterial peritonitis //Texas Journal of Medical Science. – 2022.- P. 86-90
12. Abdulloev Mukhriddin Ziyodulloevich. (2023). Modern Therapy of Viral Hepatitis. Texas Journal of Medical Science, 26, 66–69. Retrieved from https://www.zienjournals.com/index.php/tjms/article/view/4636
13. Abdulloev Mukhriddin Ziyodulloevich. (2023). Modern Therapy of Viral Hepatitis. Texas Journal of Medical Science, 26, 66–69. Retrieved from https://www.zienjournals.com/index.php/tjms/article/view/4636
14. Mukhammadieva M.I. (2023). Virus etiology : zhigar cirrhosis bemorlarida spontaneous bacterial peritonitis bilan asoratlanishning prophylaxis ва davosini takomillashtirish/ / Oriental Renaissance: Innovative, educational, natural and social sciences. - 2023.-P.947-953.
15. Oblokulov A.R., M.I.Mukhammadieva.(2022). Clinical and biochemical characteristics of liver cirrhosis patients of viral etiology with spontaneous bacterial peritonitis//Academicia Globe: Indersciense Research.-2022.- P. 210-216.

16. M. R., M. ., & S. A., S. . (2022). Features of the Course of Contagious Giardiasis Associated with Giardiasis. Miasto Przyszłości, 26, 186–189. Retrieved from https://miastoprzyszlosci.com.pl/index.php/mp/article/view/464
17. Sanokulova Sitora Avazovna. (2023). COVID-19 IN CHILDREN. Academia Science Repository, 4(06), 25–32. Retrieved from https://academiascience.com/index.php/repo/article/view/843
18. Sanokulova Sitora Avazovna. (2023). Acute Kidney Injury in Critically Ill Cirrhotic Patients with Spontaneous Bacterial Peritonitis. Texas Journal of Medical Science, 25, 65–70. Retrieved from https://zienjournals.com/index.php/tjms/article/view/4502
1. 19. Sanokulova Sitora Avazovna. (2023). Factors of Development of Hepatorenal Syndrome in Patients with Liver Cirrhosis of Viral Etiology. Texas Journal of Medical Science, 26, 4–9.from https://www.zienjournals.com/index.php/tjms/article/view/4611
19. Avazovna, S. S. (2023). Factors Of Development Of Hepatorenal Syndrome In Patients With Cirrhosis Of The Liver Of Viral Etiology. AMALIY VA TIBBIYOT FANLARI ILMIY JURNALI, 2(12), 1–8. Retrieved from https://www.sciencebox.uz/index.php/amaltibbiyot/article/view/8673
20. Sanokulova, Sitora Avazovna FEATURES OF THE COURSE OF CONTAGIOUS HELMINTHS ASSOCIATED WITH GIARDIASIS / / ORIENSS. 2023. №2. URL:https://cyberleninka.ru/article/n/ocobennosti-techeniya-kontogioznyh-gelmintov -assotsiirovannogo-s-lyambliozom (дата обращения: 12.12.2023).
21. Oblokulova Z.I, Oblokulov A.R, & Jalilova A.S. (2022). Diagnostic Significance of Hepatic Fibrosis in Patients with Extrahepatic Chronic Viral Hepatitis C. Central Asian Journal of Medical and Natural Science, 3(3), 438-443. Retrieved from https://www.cajmns.centralasianstudies.org/index.php/CAJMNS/article/view/806
22. Абдуллоев М. и др. Взгляд Инфекциониста На Течении Кора У Детей //AMALIY VA TIBBIYOT FANLARI ILMIY JURNALI. – 2023. – Т. 2. – №. 12. – С. 930-939.
23. Абдуллоев М. З. Особенности Течении Кори У Детей //Journal of Intellectual Property and Human Rights. – 2024. – Т. 3. – №. 4. – С. 26-33.
24. Абдуллоев М. З. ВЕТРЯНАЯ ОСПА У ВЗРОСЛЫХ И ДЕТЕЙ //TA'LIM VA RIVOJLANISH TAHLILI ONLAYN ILMIY JURNALI. – 2023. – Т. 3. – №. 12. – С. 410-418.
25. Amirkulovna A. G. Clinical and Epidemiological Features of Measles in Adults //Central Asian Journal of Medical and Natural Science. – 2022. – Т. 3. – №. 3. – С. 80-86.