DE GRUYTER OPEN

Bull Vet Inst Pulawy 59, 327-330, 2015
DOI:10.1515/bvip-2015-0048

Selected biochemical and oxidative stress parameters and ceruloplasmin as acute phase protein associated with bovine leukaemia virus infection in dairy cows

Pınar Peker Akalın[1], Veysel Soydal Ataseven[2], Fırat Doğan[2], Yaşar Ergün[3], Nuri Başpınar[4], Oğuzhan Özcan[5]

[1]Department of Biochemistry, [2]Department of Virology,
[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine,
[5]Department of Biochemistry, Faculty of Medicine,
Mustafa Kemal University, 31000 Hatay, Turkey,
[4]Department of Biochemistry, Faculty of Veterinary Medicine,
Selçuk University, 42030 Konya, Turkey,
pinarpekerakalin@gmail.com

Received: January 5, 2015 *Accepted: September 3, 2015*

Abstract

The aim of this study was to determine the ceruloplasmin (Cp) and vitamin C concentrations, the total antioxidant status (TAS), and selected biochemical parameters in dairy cows spontaneously infected with bovine leukaemia virus (BLV). Of the 27 cows included in the study, 18 animals were seropositive for enzootic bovine leukosis (EBL), whereas nine cows were seronegative and were used as controls. The serum aspartate aminotransferase (AST) ($P = 0.003$) and Cp concentrations ($P = 0.03$) decreased (65.17 ± 5.03 and 7.70 ± 0.72 respectively) in BLV-infected cows, as compared to healthy animals (100.67 ± 11.50 and 10.40 ± 0.70 respectively). A slight insignificant increase in alkaline phosphatase activity and unchanged levels of alanine aminotransferase, lactate dehydrogenase, calcium, magnesium, and TAS were demonstrated in EBL cows. As the TAS and vitamin C levels remained unchanged in EBL cows, it may be suggested that ruminants may compensate for the impaired oxidative/antioxidative balance. The results obtained also indicate that BLV may suppress AST and Cp synthesis or secretion in the liver through an unknown mechanism. The mechanism of action of BLV in hepatocytes, especially on AST and Cp, requires further investigation to elucidate the immune suppression caused by oncogenic retroviruses.

Keywords: cattle, bovine leukaemia virus, aspartate aminotransferase, oxidative stress, ceruloplasmin.

Introduction

Bovine leukaemia virus (BLV) is a ubiquitous oncogenic deltaretrovirus, responsible for enzootic bovine leukosis (EBL), a chronic lymphoproliferative disease in cattle (14, 15, 30). The virus has a genomic organisation similar to human T-cell leukaemia virus (HTLV-1). A genome integration strategy used by both BLV and HTLV-1 is an important step in their sustenance in the host genome (12, 14). Despite infecting different target cells, the two viruses have similar mechanisms for persistence, spread, and transformation (21). Furthermore, EBL infection is generally asymptomatic and manifests itself as either persistent lymphocytosis or a neoplastic B cell leukaemia/lymphoma associated with the clinical stage (14).

Some haematological changes have been reported within the different stages of EBL (25). Altered blood enzyme profile, as well as biochemical and oxidative stress parameters were also reported by different researchers (25, 28, 29). The relevant data vary widely among investigators; an increase in alkaline phosphatase (ALP) activity and inorganic phosphate in cattle with leukosis were reported by Spatar and Tsimbal (29), whereas a decrease in ALP activity was reported by Hisamutdinov *et al.* (8) at the tumour stage of EBL. Increased blood serum LDH activity was

© 2015 Pınar Peker Akalın et al. This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivs license (http://creativecommons.org/licenses/by-nc-nd/3.0/)

reported in sheep which were experimentally infected with EBL virus (23), but reduced LDH levels were determined in spontaneously infected cattle with altered blood cell counts (24). Beside routine biochemical parameters, the oxidative status of the organism has been studied by some researchers in recent years (19, 28). Blood serum glutathione peroxidase activity (GPx) was found to be decreased and total antioxidant status (TAS) was unaltered in BLV-infected dairy cows (28). Ceruloplasmin (Cp) is an acute phase protein, which also serves as an antioxidant in the organism. Acute phase proteins are non-specific indicators of health, synthesised in hepatocytes during acute phase response of the organism, and are mediated by cytokines. During the acute phase response, the concentration of the acute phase proteins in serum changes dramatically (18). To our knowledge, this is the first study to analyse the Cp levels together with vitamin C and other biochemical parameters during BLV-infection. Hence this study aimed to determine the Cp and vitamin C levels, TAS, and some biochemical parameters in dairy cows spontaneously infected with BLV.

Material and Methods

Animals, samples, and the detection of BLV antibody. Blood samples were obtained from 27 non-pregnant Holstein-Friesian cows up to 2.5 years of age (2.206 ± 0.106) that came from a dairy herd located in the East-Mediterranean district of Turkey. Blood was centrifuged at 2000 g x 10 min to obtain serum and was stored at -86ºC until further analyses. The presence of BLV infection was determined on the basis of the BLV-antibody status. The sera were diluted 1:20 and tested using a commercial BLV antibody ELISA kit (IDEXX, France). All results were evaluated spectrophotometrically at 450 nm absorbance wavelength (ELISA Reader, Biotek Instruments, USA). A panel of nine BLV-free Holstein-Friesian cows was used as negative control; they tested negative by both PCR, using primer pairs (nucleotides 2464 to 2621) amplifying pol gene of BLV described elsewhere (27), and ELISA for BLV. None of the animals included in this study were positive for BVDV antigen or antibodies to BHV-1 and paratuberculosis.

Determination of total antioxidant status and selected biochemical parameters. Total antioxidant status (TAS) was determined colorometrically using a commercial kit (Rel Assay Diagnostics, Gaziantep, Turkey). The results of the assay were calculated as μmol Trolox equivalent/L.

The assays of serum LDH, AST, ALP, ALT, Ca, and Mg were performed according to the standard procedures, using commercially available diagnostic kits (Abbott Diagnostics, Germany) with an automated clinical chemistry analyser (Architect c8000, Clinical Chemistry Analyzer, Abbott, USA).

Measuring serum ceruloplasmin and vitamin C concentrations. The serum was incubated with p-phenylenediamine dichloride in optimum acetate buffer. The enzymatic oxidation of p-phenylenediamine dichloride resulted in the formation of a pink product with the maximum absorption at 546 nm in spectrophotometer (UV 2100 UV–VIS Recording Spectrophotometer Shimadzu, Japan). Cp concentration was calculated using the formula described by Colombo and Richterich (5).

Vitamin C is oxidised to dehydroascorbic acid and reacts with 2,4-dinitrophenylhydrazine. Treating dehydroascorbic acid with H_2SO_4, resulted in the formation of a red product with the maximum absorption at 520 nm in spectrophotometer (7).

Statistical analysis. Statistical analyses were performed with the SPSS software (SPSS Inc., Chicago, IL) and analysis of the differences among the treatments was performed with unpaired t-test. The results obtained were expressed as mean ± SEM. Statistical significance was set at $P < 0.05$.

Results

The investigated biochemical and oxidative parameters in healthy and BLV-infected cows are shown in Table 1. The serum AST ($P = 0.003$) and Cp levels ($P = 0.03$) were decreased (65.17 ± 5.03 and 7.70 ± 0.72 respectively) in BLV-infected cows compared to healthy animals (100.67 ± 11.50 and 10.40 ± 0.70 respectively). There were no significant differences among the other parameters between BLV-infected and healthy cows.

Table 1. Biochemical and oxidative parameters in BLV-infected and healthy cows (mean ± SEM)

	Healthy (n = 9)	BLV (n = 18)
ALT (U/L)	30.67 ± 4.10	30.39 ± 2.80
AST (U/L)	100.67 ± 11.50	65.17 ± 5.03**
LDH (U/L)	1160.90 ± 72.68	1184.50 ± 51.48
ALP (U/L)	41.63 ± 5.70	51.42 ± 5.99
Ca (mg/dL)	10.57 ± 0.32	10.23 ± 0.20
Mg (mg/dL)	3.13 ± 0.13	3.08 ± 0.12
TAS (μmol/L)	1.45 ± 0.07	1.51 ± 0.03
Cp (mg/dL)	10.40 ± 0.70	7.70 ± 0.72*
Vit C (μg/mL)	0.92 ± 0.17	1.23 ± 0.17

ALT - alanine aminotransferase, AST - aspartate aminotransferase, LDH - lactate dehydrogenase, ALP - alkaline phosphatase, TAS - total antioxidant status, Cp – ceruloplasmin, * P = 0.03, **P = 0.003

Discussion

Oxidative stress is a central issue in the process of aging and in the transformation or death of living cells. Viruses may alter the oxidative status by increasing the formations of iron and nitric oxide or by inhibiting the synthesis of enzymes involved in the oxidative defence within a host cell (26). In this study, some biochemical

variables, the total antioxidant status, and serum concentrations of Cp and vitamin C in BLV-infected dairy cows were investigated.

A slightly insignificant increase in ALP and unchanged ALT levels were determined, which is in accordance with the results of other researchers (25), whereas unchanged Ca levels were incompatible. In addition, the ALT, LDH and Mg levels were not significantly changed in this study. Sandev *et al.* (25) suggested that the development of hypocalcaemia was related to the altered permeability of the cell membranes, which is regulated by Ca; however, in this study, the Ca and Mg levels were unchanged, suggesting normal cell membrane permeability and normal neuromuscular transmission (3).

AST levels were significantly reduced in BLV-infected cows compared to healthy animals in the current study, in accordance with Sandev *et al.* (25) who also reported decreased AST levels ($P < 0.05$) in dairy cows seropositive for leukosis with both negative ($n = 21$) and positive ($n = 15$) haemogramme compared to healthy animals. In humans, the most important metabolic cause of decreased AST levels was reported to be vitamin B6 deficiency (22). Some drugs, anorexia, and severe weight loss are also responsible for decreased AST levels (31). To our knowledge, no infectious diseases were related to decreased AST levels in animals. The reduction in AST levels in BVL-infected cows requires further investigation.

We determined decreased levels of Cp ($P = 0.03$; Table 1), and unaltered vitamin C levels. BLV has a genomic organisation similar to HTLV-1. Despite infecting different target cells (BLV affects B-lymphocytes and HTVL-1 affects T-lymphocytes), the two viruses persist, spread and transform through similar mechanisms (21). The antioxidant system is suppressed in HTLV-1-infection, and vitamin supplementation (vitamins C, A and E) was shown to result in a significant decrease in the levels of modified DNA bases, a reduction in TBARS, and the restoration of the activity of the antioxidant enzymes (10). Decreased levels of ROS were reported in B-cells isolated from BLV-infected sheep, *ex vivo* (4). In the current study, the total antioxidant status levels were unchanged, confirming the results of Souza *et al.* (28), who reported unchanged serum TAS and MDA levels in BLV-infected cows. As ruminants synthesize vitamin C in their tissues (32), the results obtained in the current study indicate that unaltered vitamin C levels may reflect the compensation of impaired antioxidant/oxidant status in ruminants. Cp is an acute phase protein that also serves as an antioxidant in the organism. It is produced mainly in the liver as a single polypeptide chain and secreted into plasma with Cu. Cp exhibits ferroxidase activity, suggesting anti/pro-oxidant properties (16). Some studies suggest that Cp may play a role in the host-protective function. Several cytokines and other factors, such as interferon gamma (13), interleukin 1-6 (20) and TNF-α (6) were shown to induce Cp synthesis in the liver, indicating a link between this protein and the immune function. Although Cp is generally considered a serum protein secreted by the liver, an extrahepatic expression has also been observed (1, 9, 11). It was reported that in human peripheral blood lymphocytes, the two distinct Cp transcripts, namely the secreted and the membrane-bound glycosyl-phosphatidylinositol (GPI)-anchored isoforms, are constitutively expressed (2). The Cp transcript was also reported in CD4 cells, CD8 cells, and B-lymphoblasts (17). Reductions in the levels of Cp in BVL-infected cows may suggest a link between Cp and the immune system. Leukocytosis and lymphocytosis in BLV-infected cows have been documented in seropositive leukosis with positive haemogrammes (25). Infections of immune system cells with BLV may disrupt Cp synthesis and secretion because of a proviral DNA formation. The relation between Cp and immune system cells may highlight a host defence system in oncogenic retrovirus infections, such as HTLV-1-infection.

In conclusion, as TAS and vitamin C levels were unaltered in EBL cows, the results obtained in the present study indicate that ruminants may compensate impaired oxidative/antioxidative balance, whereas BLV may suppress AST and Cp synthesis or secretion through an unknown mechanism. The mechanism of action of BLV in hepatocytes, especially on AST and Cp, requires further investigation to understand the immune suppression caused by oncogenic retroviruses.

Conflict of Interests Statement: The authors declare that they have no conflict of interests regarding the publication of this article.

Animal Rights Statement: This study was approved by Mustafa Kemal University Veterinary Faculty Local Animal Research Ethics Committee.

References

1. Aldred A.R., Grimes A., Schreiber G., Mercer J.F.: Rat ceruloplasmin: Molecular cloning and gene expression in liver, choroid plexus, yolk sac, placenta, and testis. J Biol Chem 1987, 262, 2875–2878.
2. Banha J., Liliana Marques L., Oliveira R., Martins M.F., Paixão E., Pereira D., Malhó R., Penque D., Costa L.: Ceruloplasmin expression by human peripheral blood lymphocytes: A new link between immunity and iron metabolism. Free Radical Bio Med 2008, 44, 483–492.
3. Başpınar N.: Bioelements. In: *Biochemistry*, 1st edition, edited by Kalaycıoğlu L., Serpek B., NizamLıoğlu M., Başpınar N., Tiftik A.M., pp: 43–49. Faculty of Veterinary Medicine, Selcuk University Press, Konya, Turkey, 1998.
4. Bouzar A.B., Boxus M., Florins A., François C., Reichert M., Willems L.: Reduced levels of reactive oxygen species correlate with inhibition of apoptosis, rise in thioredoxin expression and increased bovine leukemia virus proviral loads. Retrovirology 2009, 6, 102–112.
5. Colombo J.P., Richterich R.: Zur bestimmung des caeruloplasmin im plasma. Schweiz Med Wschr 1964, 94, 715–720.

6. Fleming R.E., Whitman I.P., Gitlin J.D.: Induction of ceruloplasmin gene expression in rat lung during inflammation and hyperoxia. Am J Physiol 1991, 260, 68–74.
7. Hagg W., Hofmann W.: Vitamin C content in blood plasma and leukocytes of cattle. Tierarztl Umschau 1987, 42, 956–963.
8. Hisamutdinov F.F.: On changes of blood serum activity of alkaline phosphatase, aspartate aminotransferase in bovine leukosis. Theor Pract Vet Med 1979, 2, 123–126.
9. Jaeger J.L., Shimizu N., Gitlin J.D.: Tissue-specific ceruloplasmin gene expression in the mammary gland. Biochem J 1991, 280, 671–677.
10. Jaruga P., Jaruga B., Gackowski D., Olczak A., Halota W., Pawlowska M., Olinski R.: Supplemetation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients. Free Radic Bio Med 2002, 32, 414–420.
11. Klomp L.W., Gitlin J.D.: Expression of the ceruloplasmin gene in the human retina and brain: implications for a pathogenic model in aceruloplasminemia. Hum Mol Genet 1996, 5, 1989–1996.
12. Lewinski M.K., Bushman F.D.: Retroviral DNA integration-mechanism and consequences. Adv Genet 2005, 55, 147–181.
13. Mazumder B., Mukhopadhyay C.K., Prok A. Cathcart M.K., Fox P.L.: Induction of ceruloplasmin synthesis by IFN-gamma in human monocytic cells. J Immunol 1997, 159, 1938–1944.
14. Miyasaka T., Oguma K., Sentsui H.: Distribution and characteristics of bovine leukemia virus integration sites in the host genome at three different clinical stages of infection. Arch Virol 2015, 161, 39–42.
15. Murakami H., Yamada T., Suzuki M., Nakahara Y., Suzuki K., Sentsui H.: Bovine leukemia virus integration site selection in cattle that develop leukemia. Virus Res 2011, 156, 107–112.
16. Osaki S., Johnson D.A.: Mobilization of liver iron by ferroxidase (ceruloplasmin). J Biol Chem 1969, 244, 5757–5758.
17. Pan Y., Katula K., Failla M.L.: Expression of ceruloplasmin gene in human and rat lymphocytes. Biochim. Biophys Acta 1996, 1307, 233–238.
18. Petersen H.H., Nielsen J.P., Heegaard P.M.H.: Application of acute phase protein measurements in veterinary clinical chemistry. Vet Res 2004, 35, 163–187.
19. Rajeshwari U., Shobha I. Raghunatha R. Andallu B.: Oxidative stress and antioxidant status in acute and chronic myeloid leukemia patients. Open J Blood Dis 2013, 3, 17–22
20. Ramadori G., Van Damme J. Rieder H. Meyer zum Buschenfelde K.H.: Interleukin 6, the third mediator of acute–phase reaction, modulateshepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. Eur J Immunol 1988, 18, 1259–1264.
21. Rodriguez S.M., Florins A. Gillet N. Brogniez A. Sanchez-Alcaraz M.T., Boxus M., Boulanger F., Gutierrez G., Trono K., Alvarez I., Vagnoni L., Willems L.: Preventive and therapeutic strategies for bovine leukemia virus: Lessons for HTLV. Viruses 2011, 3, 1210–1248.
22. Rossouw JE., Labadarios D., Davis M., Williams R.: Vitamin B6 and aspartate aminotransferase activity in chronic liver disease. S Afr Med J 1978, 53, 436–438.
23. Sandev N.: Investigations on some epizootological and immunological features of enzootic bovine leukosis. Doctoral thesis, Stara Zagora (In Bulgarian), 1988.
24. Sandev N., Binev R., Katsarova R., Hvarchilkov V.: Investigations on some haematological and blood biochemical parameters in bovine leukosis. Proceedings of the Union of Scientists - Rousse, 1999, 4, 28–31.
25. Sandev N., Dimitrinka Z., Ivanka S., Nikolina R., Teodora M.: Investigation of some haematological and blood biochemical parameters in cattle spontaneously infected with bovine leukosis virus. Mac Vet Rev 2013, 36, 107–110.
26. Schwarz K.B.: Oxidative stress during viral infection: A review. Free Radic Bio Med 1996, 21, 641–649.
27. Sherman M.P., Ehrlich G.D., Ferrer J.F., Sninsky J.J., Zandomeni R., Dock N.L., Poiesz B.J.: Amplification and analysis of specific DNA and RNA sequences of Bovine Leukaemia Virus from infected cows by polymerase chain reaction. J Clin Microb, 1992, 30, 185–191.
28. Souza F.N., Monterio A.M., Santos P.R., Ramos-Sanchez E.M., Blagitz M.G., Latorre A.O., Neto A.M.F., Gidlung M., Libera A.M.M.P.: Antioxidant status and biomarkers of oxidative stress in bovine leukemia virus–infected dairy cows. Vet Immunol Immunopathol 2011, 143, 162–166.
29. Spatar F.V., Tsimbal G.L.: Blood biochemical parameters in bovine leukosis. Animal Husbandry, Shtinitsa, Kishinev, 1978, 118–123.
30. Trono K.G., Perez-Filgueira D.M., Duffy S., Borca M.V., Carrillo C.: Seroprevalence of bovine leukemia virus in dairy cattle in Argentina: comparison of sensitivity and spesifity of different detection methods. Vet Microbiol 2001, 83, 235–248.
31. Waner T., Nyska A.: The toxicological significance of decreased activities of blood alanine and aspartate aminotransferase. Vet Res Commun 1991, 15, 73–78.
32. Weiss B.: Update on vitamin nutrition of dairy cows. Available at: http://dairy.osu.edu/resource/feed/Vitamin%20update.pdf (accessed 27 October 2014), 2005.