Brief Report

Cytoprotective and antioxidant effects of human lactoferrin against H_2O_2-induced oxidative stress in human umbilical vein endothelial cells

Leila Safaeian, Shaghayegh Haghjoo Javanmard[1], Yaser Mollanoori, Nasim Dana[1]

Department of Pharmacology and Toxicology, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, [1]Department of Physiology, Applied Physiology Research Center, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: Lactoferrin (LF) is an iron-binding glycoprotein with antioxidant, anti-inflammatory and nitric oxide-dependent vasodilatory properties. In the present study, we investigated the protective and antioxidant effects of LF on H_2O_2-induced oxidative stress in human umbilical vein endothelial cells (HUVECs).

Materials and Methods: HUVECs were pretreated by (6.25–100 μg/ml) LF for 24 h and then exposed to 0.5 mM H_2O_2 for 2 h. Cell viability was assessed by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The intra- and extra-cellular hydroperoxides concentration and ferric reducing antioxidant power (FRAP) were determined in pretreated cells.

Results: Pretreatment of HUVECs with LF at the concentrations of 25–100 μg/ml significantly reduced the cytotoxicity of H_2O_2 in a concentration-dependent manner using MTT assay. LF pretreatment at different concentration ranges also decreased the hydroperoxides level and augmented the FRA*P* value in both intra-and extra-cellular assay.

Conclusion: These findings revealed antioxidant and cytoprotective effects of LF against H_2O_2-induced oxidative stress in HUVECs. With regard to the beneficial vascular activity of LF, further investigations are suggested for understanding its clinical value in human endothelial dysfunction and prevention and/or treatment of CVDs.

Key Words: Antioxidant, human umbilical vein endothelial cells, lactoferrin, oxidative stress

Address for correspondence:
Dr. Leila Safaeian, Department of Pharmacology and Toxicology, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: leila_safaeian@pharm.mui.ac.ir
Received: 31.12.2014, **Accepted:** 27.01.2015

INTRODUCTION

Cardiovascular diseases (CVDs) include any disease that affects the cardiovascular system such as heart, brain, kidney and other organ's blood vessels. CVDs are responsible for significant morbidity and mortality in the world.[1] According to the World Health Organization global report on noncommunicable diseases, 17.3 million people died from CVDs in 2008 that representing 30% of all global deaths.[2] Furthermore, it is estimated that mortality rate from heart disease and stroke will increase to 23.3 million by 2030.[3]

The vascular endothelium is the inner layer of blood vessels which has an important role in the

Access this article online	
Quick Response Code:	Website: www.advbiores.net
	DOI: 10.4103/2277-9175.164010

How to cite this article: Safaeian L, Javanmard SH, Mollanoori Y, Dana N. Cytoprotective and antioxidant effects of human lactoferrin against H_2O_2-induced oxidative stress in human umbilical vein endothelial cells. Adv Biomed Res 2015;4:188.

regulation of vascular function.[4] A growing body of evidence suggests the great role of oxidative stress in the endothelial dysfunction. Risk factors including hypertension, hypercholesterolemia, diabetes, and cigarette smoking are associated with increased oxidative stress and alteration in the endothelial function.[5] Reactive oxygen species (ROS) including superoxide anion, lipid radicals, hydroxyl racial, peroxynitrite and hydrogen peroxide released from various sources are involved in the inactivation of nitric oxide (NO), disruption of NO function and reduction of antioxidant capacity in the vascular system.[5,6] Oxidative stress has a causal role in the oxidation of low density lipoprotein (LDL) and in the development of atherosclerosis,[7] and also in the induction of apoptosis of endothelial and myocardial cells following some stimulus such as hypoxia, ischemia, reperfusion and inflammation.[8]

Recent studies have shown some beneficial effects from antioxidant supplementation in the improvement of NO availability and endothelial function.[9,10]

Lactoferrin (LF), an iron-binding glycoprotein, was first isolated from the bovine milk by Sorensen in 1939.[11] This glycoprotein exists in different biological fluids and in specific granules of neutrophils. In human, LF is found in milk, colostrum, and other mucosal secretions.[12] LF is a critical component in mediation of first-line defense against infections and acts as a regulator of organ morphogenesis and promoter of wound healing and bone growth. It has various physiological and pharmacological activities including antioxidant, anti-infective, anti-inflammatory and anticancer effects.[12-14] LF also possesses beneficial cardiovascular properties such as antihypertensive activity.[15,16]

The present study aimed to evaluate the protective effects of human LF under oxidative stress induced by H_2O_2 in human umbilical vein endothelial cells (HUVECs).

MATERIALS AND METHODS

Reagents

Human umbilical vein endothelial cells were obtained from the National Cell bank of Iran (Pasteur Institute Tehran, Iran). Human LF was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO BRL Life Technologies (Grand Island, USA). Fetal bovine serum (FBS) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assessment kit was purchased from Bioidea Company (Tehran, Iran). The kits for hydroperoxides measurement and ferric reducing antioxidant power (FRAP) assay was obtained from Hakiman Shargh Research Co., (Isfahan, Iran). All other chemicals with analytical grade were purchased from Sigma-Aldrich Co., (St. Louis, MO, USA).

Cell culture

The HUVECs were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were incubated in a 95% humidified atmosphere of 5% CO_2 at 37°C.

Cell viability evaluation

Cell viability was assessed using MTT method based on the reduction of MTT by mitochondrial dehydrogenases of viable cells to a purple formazon product.[17] Briefly, the cell suspension at a concentration of 1×10^5 cells/ml was transferred to 96-well plates and incubated for 24 h at 37°C. At the optimum phase of proliferation, the cells were treated with different concentrations of human LF (6.25, 12.5, 25, 50, 100 μg/ml) for an additional 24 h.[18] After that the medium of each well was removed, and the cells were washed out with phosphate buffered saline (PBS) at pH 7.4. Then, a new medium and 0.5 mM H_2O_2 were added to the wells.[19] After 2 h incubation, medium of each well was removed and the cells were washed out with PBS, and 20 μl MTT (0.5 mg/ml) and 50 μl of new medium were added into the each well and incubated for 3 h at 37°C. Then the MTT-formazan product dissolved in 50 μl of dimethyl sulfoxide and absorbance was measured at 570 nm by microplate reader (BioTek Instruments, PowerWave XS, Wincoski, USA). The wells containing the cells without being exposed to the LF or H_2O_2 were considered as control. Cell viability was determined as a percentage of viable cells of treated samples to control samples, and each experiment was tested in triplicate.

Measurement of extra-and intra-cellular hydroperoxides concentration

The effects of LF on intra-and extra-cellular hydroperoxides level were measured based on ferrous ion oxidation by xylenol orange reagent 1 (FOX-1).[20] The FOX-1 reagent containing ammonium ferric sulfate in an aqueous medium with sorbitol was prepared according to the manufacturer's protocol. After pretreatment of HUVECs with LF, the cells were exposed to the H_2O_2. Then, 10 μl of supernatant of the cells or the cell lysates from each well was added to 190 μl of reagent and incubated for 30 min at 40°C. Absorbance was determined at 540 nm against the blank using a microplate reader/spectrophotometer. The hydroperoxides content of samples were subsequently calculated using a standard curve of H_2O_2 concentrations (0.005–1M).

Measurement of cell-free and intra-and extra-cellular ferric reducing antioxidant power

The effect of LF on total antioxidant capacity of the samples was determined by the evaluation of FRAP.[21] FRA*P* value was measured based on the reduction of ferric-tripyridyltriazine complex to the ferrous form by colorimetric assay. The FRAP reagent containing tripyridyltriazine/ferric chloride/acetate buffer was prepared based on the manufacturer's protocol. For each well, 10 µl of sample was added to 200 µl of FRAP reagent. Different concentrations of LF were evaluated for FRAP in cell-free assay. In cell-based assay, supernatant of the cells or the cell lysates from each well were analyzed. The mixture of sample and reagent was incubated for 40 min at 40°C. Then the absorbance was determined at 570 nm against the blank using a microplate reader/ spectrophotometer. The FRA*P* values of samples were calculated using the standard curve, which was obtained from $FeSO_4 \times 7$ H_2O concentrations (0.1–10 mM) and were expressed as µM of FeII equivalents.

Statistical analysis

The results were represented as mean ± standard error of the mean statistical analyzes were conducted using a one-way analysis of variance, followed by Tukey *post-hoc* test SPSS software version 16.0, (SPSS Ltd, Quarry Bay, Hong Kong). $P < 0.05$ were considered as significant.

RESULTS

Cytoprotective effect of lactoferrin against H_2O_2-induced oxidative stress

Figure 1 shows the cytoprotective effect of LF on the viability of HUVECs, which were exposed to the oxidative damage induced by H_2O_2 using the MTT method. The exposure of HUVECs to H_2O_2 at 0.5 mM for 2 h caused a significant reduction in cell viability ($P < 0.001$). Pretreatment of HUVECs with LF at the concentrations of 25–100 µg/ml reduced the cell death resulted from H_2O_2 in a concentration-dependent manner. The cytoprotective effect was not observed at the concentrations of 6.25 and 12.5 µg/ml of LF.

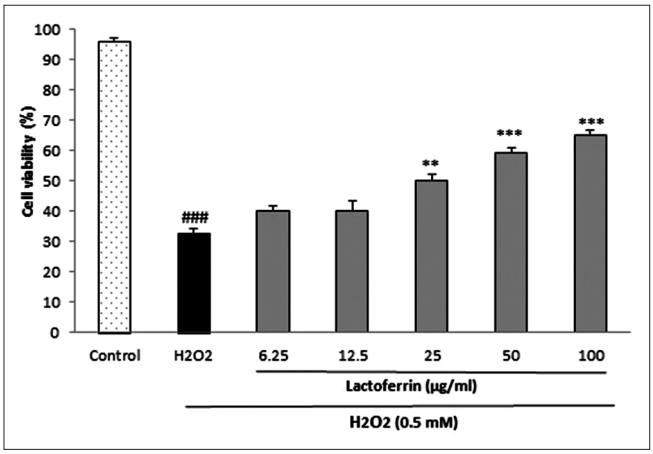

Figure 1: Cytoprotective effect of lactoferrin on H_2O_2-induced oxidative stress in human umbilical vein endothelial cells. Cells were incubated with H_2O_2 (0.5 mM, 2 h) after pretreatment with different concentrations of lactoferrin (6.25–100 µg/ml). The cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Values are means ± standard error of the mean from three independent experiments in triplicate. $^{###}P < 0.001$ versus control (H_2O_2-untreated cells), $^{**}P < 0.01$ and $^{***}P < 0.001$ versus H_2O_2-stimulated cells

Effect of lactoferrin on intra-and extra-cellular hydroperoxides concentration

Figures 2 and 3 are shown the effects of LF on intra-and extra-cellular hydroperoxides concentration in HUVECs culture, which was exposed to the oxidative stress induced by H_2O_2. After pretreatment of HUVECs with LF (6.25–100 µg/ml), the intra-cellular hydroperoxides level were significantly declined compared to the control group. LF pretreatment also significantly reduced the extra-cellular hydroperoxides level at the concentrations of 6.25–100 µg/ml. Increasing the concentration of LF concentration-dependently prevented the increase in hydroperoxides level.

Effect of lactoferrin on cell-free and intra-and extra-cellular ferric reducing antioxidant power value

The FRA*P* value of LF was evaluated in cell-free and in intra-and extra-cellular fluids. In cell-free assay, our data showed increasing trend in FRAP with increasing LF concentrations [Figure 4]. In cell-based assay, LF at concentrations of 6.25–100 µg/ml significantly increased the FRAP levels in intra-cellular fluid [Figure 5] and at the concentrations of 12.5–100 µg/ml in extra-cellular fluid [Figure 6] concentration-dependently.

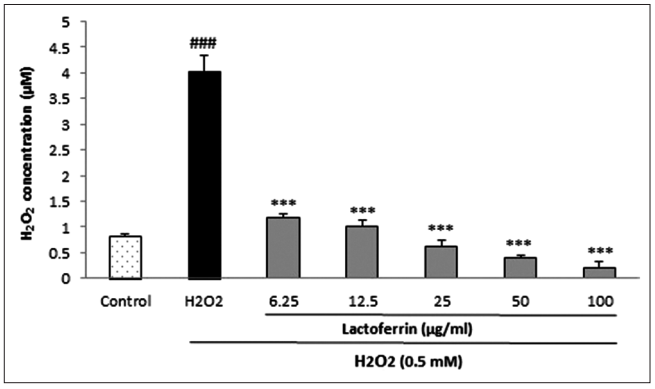

Figure 2: Effect of lactoferrin on intra-cellular hydroperoxides concentration in human umbilical vein endothelial cells. Cells were incubated with H_2O_2 (0.5 mM, 2 h) after pretreatment with different concentrations of lactoferrin (6.25–100 µg/ml). The hydroperoxides concentration was determined by ferrous ion oxidation by xylenol orange method. Values are means ± standard error of the mean from three independent experiments in triplicate. $^{###}P < 0.001$ versus control (H_2O_2-untreated cells), and $^{***}P < 0.001$ versus H_2O_2-stimulated cells

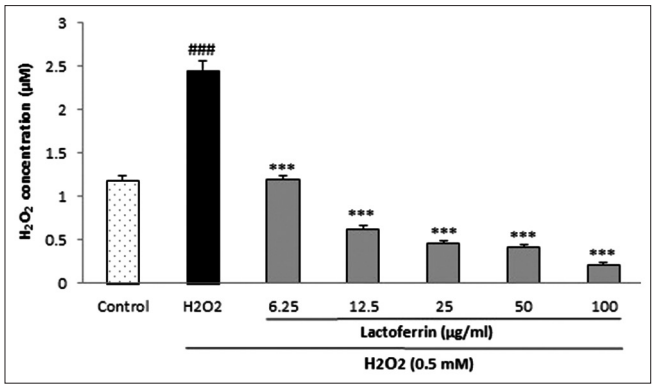

Figure 3: Effect of lactoferrin on extra-cellular hydroperoxides concentration in human umbilical vein endothelial cells. Cells were incubated with H_2O_2 (0.5 mM, 2 h) after pretreatment with different concentrations of lactoferrin (6.25–100 μg/ml). The hydroperoxides concentration was determined by ferrous ion oxidation by xylenol orange method. Values are means ± standard error of the mean from three independent experiments in triplicate. $^{###}P < 0.001$ versus control (H_2O_2-untreated cells), and $^{***}P < 0.001$ versus H_2O_2-stimulated cells

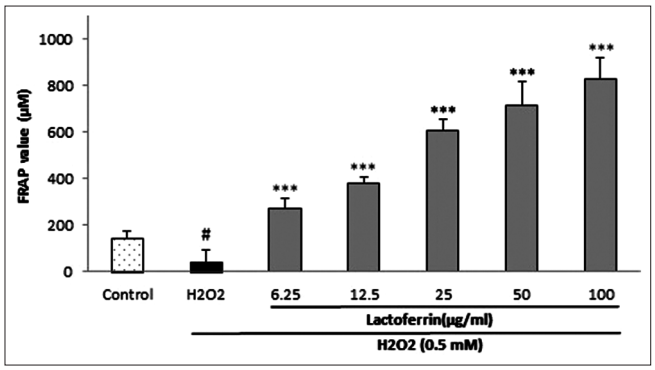

Figure 5: Effect of lactoferrin on intra-cellular ferric reducing antioxidant power value in human umbilical vein endothelial cells. Cells were incubated with H_2O_2 (0.5 mM, 2 h) after pretreatment with different concentrations of lactoferrin (6.25–100 μg/ml). Values are means ± standard error of the mean from three independent experiments in triplicate. $^{##}P < 0.05$ versus control (untreated cells), and $^{***}P < 0.001$ versus H_2O_2 stimulated cells

DISCUSSION

In the present study, our findings revealed cytoprotective effect of LF at the concentration range of 25–100 μg/ml against oxidative stress induced by H_2O_2 in HUVECs. It also decreased hydroperoxides concentration and increased FRA*P* value in both intra-and extra-cellular fluid at different concentration ranges.

Associations between oxidative stress and endothelial dysfunction have been confirmed in numerous studies. Oxidative stress has a crucial role in the impairment of endothelium-dependent vasodilation and also in the induction of hypertrophy, apoptosis and inflammation via activation of many signaling pathways. Excessive ROS result in damage to the endothelial cells through

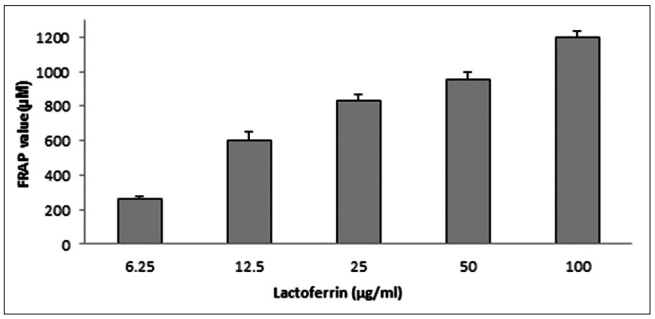

Figure 4: Concentration-dependent ferric reducing antioxidant power values of different concentrations of lactoferrin (6.25–100 μg/ml). Values are means ± standard error of the mean from three independent experiments in triplicate

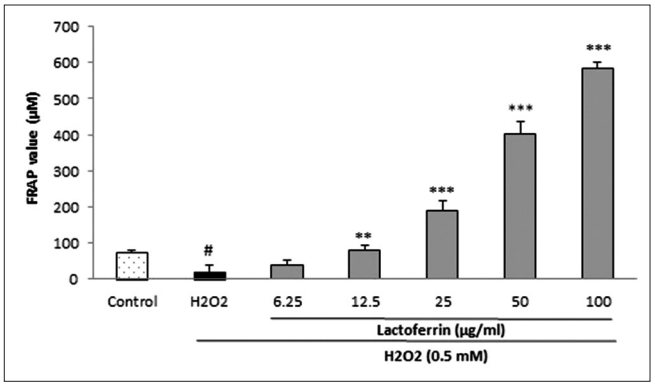

Figure 6: Effect of *Echium amoenum* extract on extra-cellular ferric reducing antioxidant power value in human umbilical vein endothelial cells. Cells were incubated with H_2O_2 (0.5 mM, 2 h) after pretreatment with different concentrations of *E. amoenum* extract (25–1000 μg/ml). Values are means ± standard error of the mean from three independent experiments in triplicate. $^{##}P < 0.05$ versus control (untreated cells), $^{**}P < 0.01$ and $^{***}P < 0.001$ versus H_2O_2 stimulated cells

lipid peroxidation, protein oxidation, and DNA fragmentation.[22] ROS are produced by different oxidase enzymes, however, nicotinamide-adenine dinucleotide phosphate oxidase is a main producer of ROS in the vasculature.[23] Besides inactivation of NO, ROS are associated with down-regulation of endothelial NO synthase (eNOS) through degradation of tetrahydrobiopterin (BH4). BH4 is an essential cofactor for eNOS and its deficiency results in eNOS uncoupling and production of ROS rather than NO.[24]

In this research, pretreatment of HUVECs with LF (6.25–100 μg/ml) significantly reduced the intra-and extra-cellular hydroperoxides level by FOX-1 assay. FOX method is not specific only for H_2O_2, and also is able to detect other hydroperoxides (ROOH). FOX-1 assay is a sensitive method for ROOH estimation due to the consisting of sorbitol, which is a radical scavenger and increases the yield of ferric ion.[25]

Our results also showed significant increasing in total antioxidant capacity in intra-and extra-cellular

fluids after pretreatment of HUVECs with LF. Decreased antioxidant capacity such as superoxide dismutase (SOD), glutathione peroxidase, catalase and Vitamins C and E has been revealed in CVDs. SOD has three enzymatic types including Cu/Zn SOD, Mn SOD, and extracellular SOD. It seems that extracellular SOD has a more important role in NO bioavailability in the vasculature and so in the prevention of atherosclerosis.[26] Administration of Vitamin C has been able to restore impaired endothelium-dependent vasodilation in patients with CVDs.[22]

Lactoferrin is a food-derived multifunctional glycoprotein whose receptors are found in various cell types including endothelial cells. Some of its receptors are involved in LF uptake. In the cerebral endothelial cells, transportation of LF occurs through a receptor-mediated process without any intra-endothelial degradation.[27] In some capillary endothelial cells, LF may transport via a specific LDL receptor-related protein and mediate antioxidant effect.[27]

The potent antioxidant effect of LF and its ability to increase antioxidant capacity have been described in some researches. In healthy humans, LF supplementation has been associated with an increase in the hydrophilic antioxidant capacity.[28] LF has also shown antioxidant effect on erythrocytes through inhibition of lipid peroxidation and hemolysis.[29] Declining intracellular levels of ROS induced by glucose oxidize has been observed following LF treatment suggesting its ability to reduce oxidative stress-induced apoptosis.[12]

Lactoferrin possesses metal ions-binding capacity and, therefore, can exert a protective effect against iron-catalyzed hydroxyl radicals through Fenton reaction, which is an important source of ROS.[30] Therefore, the LF antioxidant activity is most likely related to its iron scavenging ability and inhibition of iron-catalyzed formation of ROS.[11,31]

Furthermore, the beneficial vascular properties of LF have been reported in different studies. LF possesses vasodilator activities through eNOS–dependent pathway, inhibition of angiotensin I-converting enzyme and inhibition of endothelin-converting enzyme.[32-34] LF has been shown anti-inflammatory and protective effects on endothelial cells through chelating lipopolysaccharide, reducing the release of pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha, inhibition of expression of endothelial adhesion molecules and prevention of NF-kB activation.[35] It has been suggested that plasma LF level may be useful as a predictor to endothelial dysfunction in some diseases. LF also has a helpful effect on lipid profile by increasing high-density lipoprotein-cholesterol.[36]

CONCLUSION

The finding of this study revealed the antioxidant and protective effect of LF against H_2O_2 induced oxidative stress in HUVECs. With regard to beneficial vascular activity and safety, LF could be suggested for clinical trial studies for understanding its clinical value.

ACKNOWLEDGMENTS

This study was financially supported by research project No. 393063 from Isfahan University of Medical Sciences.

REFERENCES

1. Lovegrove JA, Gitau R. Personalized nutrition for the prevention of cardiovascular disease: A future perspective. J Hum Nutr Diet 2008;21:306-16.
2. Alwan A. Global status report on non-communicable diseases 2010. Geneva: World Health Organization; 2011.
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006;3:e442.
4. Onat D, Brillon D, Colombo PC, Schmidt AM. Human vascular endothelial cells: A model system for studying vascular inflammation in diabetes and atherosclerosis. Curr Diab Rep 2011;11:193-202.
5. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. Circ Res 2000;87:840-4.
6. Schulz E, Anter E, Keaney JF Jr. Oxidative stress, antioxidants, and endothelial function. Curr Med Chem 2004;11:1093-104.
7. Peluso I, Morabito G, Urban L, Ioannone F, Serafini M. Oxidative stress in atherosclerosis development: The central role of LDL and oxidative burst. Endocr Metab Immune Disord Drug Targets 2012;12:351-60.
8. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: Implications in human hypertension. Hypertens Res 2011;34:5-14.
9. Houston MC. The role of nutrition, nutraceuticals, vitamins, antioxidants, and minerals in the prevention and treatment of hypertension. Altern Ther Health Med 2013;19 Suppl 1:32-49.
10. Manning RD Jr, Tian N, Meng S. Oxidative stress and antioxidant treatment in hypertension and the associated renal damage. Am J Nephrol 2005;25:311-7.
11. Tomita M, Wakabayashi H, Shin K, Yamauchi K, Yaeshima T, Iwatsuki K. Twenty-five years of research on bovine lactoferrin applications. Biochimie 2009;91:52-7.
12. Actor JK, Hwang SA, Kruzel ML. Lactoferrin as a natural immune modulator. Curr Pharm Des 2009;15:1956-73.
13. Baveye S, Elass E, Mazurier J, Spik G, Legrand D. Lactoferrin: A multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin Chem Lab Med 1999;37:281-6.
14. González-Chávez SA, Arévalo-Gallegos S, Rascón-Cruz Q. Lactoferrin: Structure, function and applications. Int J Antimicrob Agents 2009;33:301.e1-8.
15. Safaeian L, Zabolian H. Antihypertensive effect of lactoferrin on dexamethasone-induced hypertension in rat. J Isfahan Med Sch 2013;31:1096-104.
16. Safaeian L, Zabolian H. Antioxidant effects of bovine lactoferrin on dexamethasone-induced hypertension in rat. ISRN Pharmacol 2014;2014:943523.
17. Ma ZC, Hong Q, Wang YG, Tan HL, Xiao CR, Liang QD, *et al.* Ferulic acid

protects lymphocytes from radiation-predisposed oxidative stress through extracellular regulated kinase. Int J Radiat Biol 2011;87:130-40.
18. Damiens E, Mazurier J, el Yazidi I, Masson M, Duthille I, Spik G, *et al.* Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells. Biochim Biophys Acta 1998;1402:277-87.
19. Coyle CH, Kader KN. Mechanisms of H2O2-induced oxidative stress in endothelial cells exposed to physiologic shear stress. ASAIO J 2007;53:17-22.
20. Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator Xylenol orange for measurement of hydroperoxides. Methods Enzymol 1994;233:182-9.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 1996;239:70-6.
22. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. Circ J 2009;73:411-8.
23. Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, *et al.* Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest 1996;97:1916-23.
24. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: Implications for uncoupling endothelial nitric-oxide synthase. J Biol Chem 2003;278:22546-54.
25. Banerjee D, Madhusoodanan UK, Sharanabasappa M, Ghosh S, Jacob J. Measurement of plasma hydroperoxide concentration by FOX-1 assay in conjunction with triphenylphosphine. Clin Chim Acta 2003;337:147-52.
26. Fillebeen C, Descamps L, Dehouck MP, Fenart L, Benaïssa M, Spik G, *et al.* Receptor-mediated transcytosis of lactoferrin through the blood-brain barrier. J Biol Chem 1999;274:7011-7.
27. Gao HL, Pang ZQ, Fan L, Hu KL, Wu BX, Jiang XG. Effect of lactoferrin- and transferrin-conjugated polymersomes in brain targeting: *In vitro* and *in vivo* evaluations. Acta Pharmacol Sin 2010;31:237-43.
28. Mulder AM, Connellan PA, Oliver CJ, Morris CA, Stevenson LM. Bovine lactoferrin supplementation supports immune and antioxidant status in healthy human males. Nutr Res 2008;28:583-9.
29. Maneva A, Taleva B, Maneva L. Lactoferrin-protector against oxidative stress and regulator of glycolysis in human erythrocytes. Z Naturforsch C 2003;58:256-62.
30. Mladenka P, Semecký V, Bobrovová Z, Nachtigal P, Vávrová J, Holecková M, *et al.* The effects of lactoferrin in a rat model of catecholamine cardiotoxicity. Biometals 2009;22:353-61.
31. Lawen A, Lane DJ. Mammalian iron homeostasis in health and disease: Uptake, storage, transport, and molecular mechanisms of action. Antioxid Redox Signal 2013;18:2473-507.
32. Hayashida K, Takeuchi T, Ozaki T, Shimizu H, Ando K, Miyamoto A, *et al.* Bovine lactoferrin has a nitric oxide-dependent hypotensive effect in rats. Am J Physiol Regul Integr Comp Physiol 2004;286:R359-65.
33. Ruiz-Giménez P, Burguete MC, Castelló-Ruiz M, Marcos JF, Salom JB, Vallés S, *et al*. Bovine lactoferrin pepsin hydrolysate exerts inhibitory effect on angiotensin I-converting enzyme-dependent vasoconstriction. Int Dairy J 2007;17:1212-5.
34. Fernández-Musoles R, López-Díez JJ, Torregrosa G, Vallés S, Alborch E, Manzanares P, *et al.* Lactoferricin B-derived peptides with inhibitory effects on ECE-dependent vasoconstriction. Peptides 2010;31:1926-33.
35. Baveye S, Elass E, Fernig DG, Blanquart C, Mazurier J, Legrand D. Human lactoferrin interacts with soluble CD14 and inhibits expression of endothelial adhesion molecules, E-selectin and ICAM-1, induced by the CD14-lipopolysaccharide complex. Infect Immun 2000;68:6519-25.
36. Zakaria A, El Shazly M, Rashed L. Plasma lactoferrin level as a predictor to endothelial dysfunction in patients with obstructive sleep apnea. Egypt J Intern Med 2013;25:86-91.

Source of Support: Financially supported by research project No. 393063 from Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.