Effects of Lipoproteins on CycloOxygenase and Lipoxygenase Pathways in Human Platelets

Pages with reference to book, From 84 To 88

Sheikh A. Saeed, Riaz A, Memon, Anwar H. Gilani, Bukhtiar H. Shah (Department of Physiology and Pharmacology, Faculty of Health Sciences, The Aga Khan University, Karachi.)

Abstract

The products of arachidonic acid (AA) metabolism in platelets play an important role in platelet shape change, adhesion and aggregation which may participate in the pathogenesis of ischemic heart disease and thrombosis. Since lipoproteins are also involved in the pathogenesis of thrombo-embolic disordets, the effects of human lipoproteins (HDL, LDL, VLDL) on AA metabolism in human platelets was investigated. Lipoproteins were separated by density gradient zonal ultracentrifugation. The effects of lipoproteins on production of AA metabolites in human platelets i.e., thromboxane A2 (TXA2) and hydroxy-eicosatetraenoic acids (HETEs) were examined using radiometric thin layer chromatography coupled with automated data integrator system. In human platelets, HDL inhibited 12-HETE and TXA 2 fonnation in a concentration-dependent manner. LDL had a strong inhibitory effect on TXA 2 production and a weak inhibitory effect on 12-HETE production. VLDL had no effect on platelet AA metabolism. These findings point to a new facet of lipoproteins action and suggest.that lipoproteins may have a physiological role in the regulation of AA metabolism in platelets (JPMA 47:84,1997).

Introduction

The pmducts of arachidonic acid (AA) metabolism in platelets include thromboxane A_2 (TXA_2) and hydroxy-eicosatetraenoic acids (HETEs). These metabolites are well characterized mediators of inflammatory and immunological reactions in the pathophysiology of several disorders, where they may act in an autocrine or paracrine fashion[1,2]. TXA_2 is a vasoconstrictor and exerts a strong pro-agglegatory effect on platelet agpegation whereas 12-HETE is a potent chemotactic factor[1,2]. TXA 2 receptors on platelets are coupled with guanme nucleotidebindingproteins (G-proteins) of the class Gq family[3]. Activation of Gq proteins generates second messengers, inositol triphosphate (IP 3) and diacyiglycerol (DAG) which in turn causes release of intracellular calcium and activation of protein kinase C, respectively[4].

Lipoproteins are macromolecular complexes canying various lipids and proteins in the plasma. The principal biologic function of the lipoproteins is the transport of water-insoluble lipids within the bloodstreamfrom the sites of absorption or synthesis to the sites of storage or metabolic utilization[5]. The lipoproteins are subdivided into chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). These lipoprotein classes vary in their density, size, source ofongin, lipid and protein compositionand functions[6]. The major function of chylomicrons is transport of dietary triglycerides whereas VLDL is primarily involved intransport of endogenously synthesized triglycerides and some cholesterol. LDL is mainly responsible for the transport of cholesterol esters of hepatic and intravascular origin to peripheral cells and is a major risk factor for coronary heart disease[7]. On the other hand, HDL is involved in 'reverse cholesterol transport' whereby excess cholesterol from peripheral cells is returned to liverforcatabolism andby virtue of this effect HDL is considered a protective factor against coronary heart disease[7]. Recent studies have demonstrated that lipoprotems may also have other functions. Beitz and Forster have shown that HDL

stimulates, whereas LDL inhibits the prostacyclin synthetase activity of pig aortic microsomes[8]. On the other hand, Shakhov et at[9] have recently demonstrated that both LDL and HDL stimulate the formation of 6-keto-PGF la (a stable degradation product of prostacyclin) in cultured human and rabbit aortic smooth muscle cells. We have also shown that LDL. HDL and VLDL inhibited the biosynthesis of PGE 2 and 6-keto-PGFla in bovine seminal vesicle microsomes[10].

While all of these studies have focused on the effects of lipoproteins on cyclooxygenase pathway of AA metabolism, very little is known about the effects of lipoproteins on the lipoxygenase pathway. Platelets are a unique model for studying A.A metabolism as both cyclo-oxvgenase and lipoxygenase pathways can be simultaneously studied. In human platelets, AA is primarily metabolized by cyclo-oxygenase and lipoxygenase pathways into TXA_2 and 12-HETE respectively TXA 2 is very unstable and is rapidly hydrolyzed to a stable metabolite TXB 2 which can be measured and reflects the rate of TXA_2 formation. In order to further define the effects of human lipoproteins on AA metabolism, in this study we have examined the effects of HDL, LDL and VLDL on metabolic disposition of radio-labelled AA in human platelets.

Material and Methods

Materials

Arachidonic acid (grade 1: 99% pure) and reduced glutathione were purchased from Sigm Chemical Company (St. Louis, MO, USA). C] AA (sp. act. 58 mci/mmol), [S3H] thromboxane B2 (sp. act. SA 120 Ci/mmol) and 12-hydroxy-~3 H]-eicosatetmenoic acid .(12-HETE) (sp. act.
100 Ci/mmol) were obtained from Amersham International plc, U.K. All other chemicals used were of analytical grade.

Preparation of Lipoproteins

Blood obtained from 12 hours fasted healthy volunteers (age range 20-30 years) was collected in EDTA (1 mg/ml) containing tubes. The plasma lipoproteins were separated by sequeritial density gradient ultracentrifuption using the method of Havel et al11 as described earlier 2 After isolation, each lipoprotein fraction was sterilized by filtration through a Millipore (0.45 um) filter (Millipore Corp, Bedford, MA) and stored at 4°C. The protein content of each lipoprotein fraction was determined by the method of Lowry et al13 3using human serum albumin as standard and adjusted to initial protein concentration for each batch. The homogeneity of each lipoprotein fraction was confirmed by agarose gel. electrophoresis and the purified lipoproteins were used within one month of preparation.

Arachidonic acid metabolism by human platelets

Human blood platelets from donors were obtained in plastic bags containing 30-40 ml of concentrated platelet-rich plasma (PRP) from The Aga Khan University Hospital
Laboratory. The PRP was centrifuged at 100 g for 5 min and the supernatant was discarded. The remaining PRP was then centrifuged at 1200 g for 20 min and the sedimented platelets were washed twice with ice-cold 50 mM. phosphate buffer (pH 7.4) containing 0.15 M sodium chloride and 0.2 mM EDTA.

After centrifugation platelets were resuspended in the same. buffer without EDTA at the initial PRP cell concentration. The PRP suspension was homogenized at 4°C using a polytron homogenizer for 15 seconds and the homogenate was centrifuged at 1,200 g for 20 min. AA metabolism in human platelets was studied as described in our earlier publications14,15. Briefly, 300 uL of the supematant (containing 0.4 mg of protein) was incubated with 10 ug unlabelled AA and 0.1 uCi [1-14C] AA in the absence and presence of different concentrations of HDL, LDL and VLDL. After 15 min with gentle shaking at 37c the reaction was stopped by addition of citric acid and ethyl acetate. The reaction mixture was vortexed and centrifuged at 600 g for 5 min at 4C, the organic layer was separated and evaporated to dryness

under nitrogen,
The residues were dissolved in 50 ul of et hanol and 20 ul were applied to silica gel G thin layer chromatography (TLC) plates (Analtech, Delaware, USA). The AA, TXB2 (a stable degradation product of TXA2) and 12-HETE standards were spotted separately. The plates were developed in ether/petroleum ether/acetic acid (50:50:1 by volume) to a distance of 17 cm. By use of this solvent system the various lipoxy genase products (HETEs) were separated with TXB2 and PGs remaining at the origin16. The solvent system used for the separation of various PGs and TXB2 in dried organic extracts of platelets was ethyl acetate: isoctane: water: acetic acid (11:5:10:2, v/v,upper phase). Radioactive zones were located and quantified by the use of a Berthold TLC linear analyzer and chromatography data system (Model LB 511, Berthold, Germany).

Statistics

The data are presented as means+s.e.m. The control values were compared with those obtained in the presence of various lipoproteins between two groups was calculated by using students unpaired t-test. Comparisons among more than two groups were performed by one-way analysis of variance (ANOVA) using Crunch statistical software (Oakland, CA) as described earlier[17].

Results

The effect of different concentration of HDL on [1-14C] AA metabolism in human platelets is presented in Figure 1.

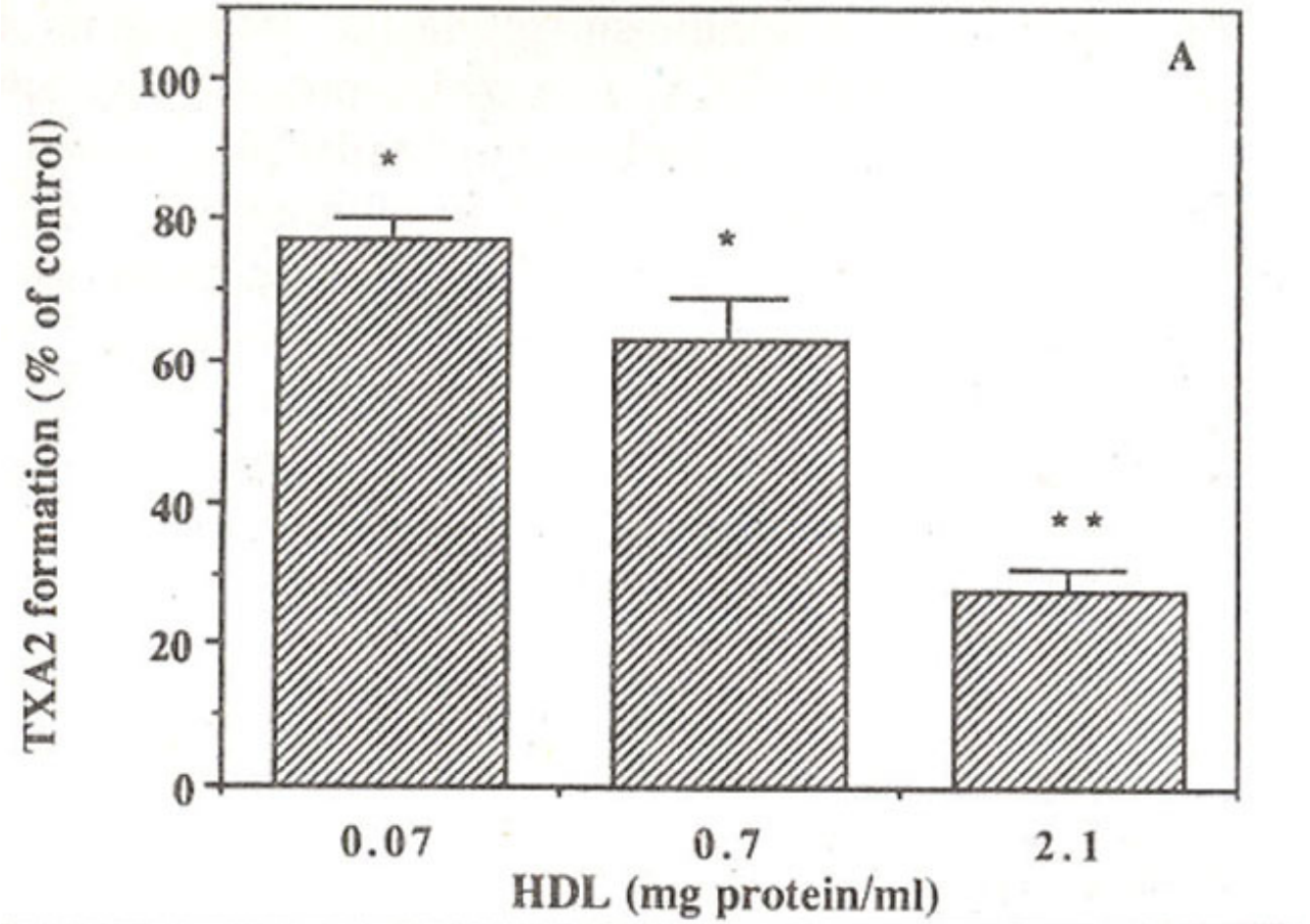

Figure 1 (A). Effect of different concentrations of high density lipoproteins on TXA_2.

HDL decreased TXA2 formation by 23+3 % ($p<0.05$), 37+6 % (<0.01) and 72+3 % ($p<0.001$) at the

protein concentrations of 0.07, 0.7 and 2.1(mg protein/ml assay volume) respectively (Figure 1A). HDL also had a concentration dependent inhibitory effect on 12- HETE formation in platelets. A decrease of 12+1 % ($P<0.05$), 58+4 % ($P<0.01$) and 81±3 % ($P<0.001$) in 12-HETE formation at similar protein concentrations was observed with HDL (Figure 1B).

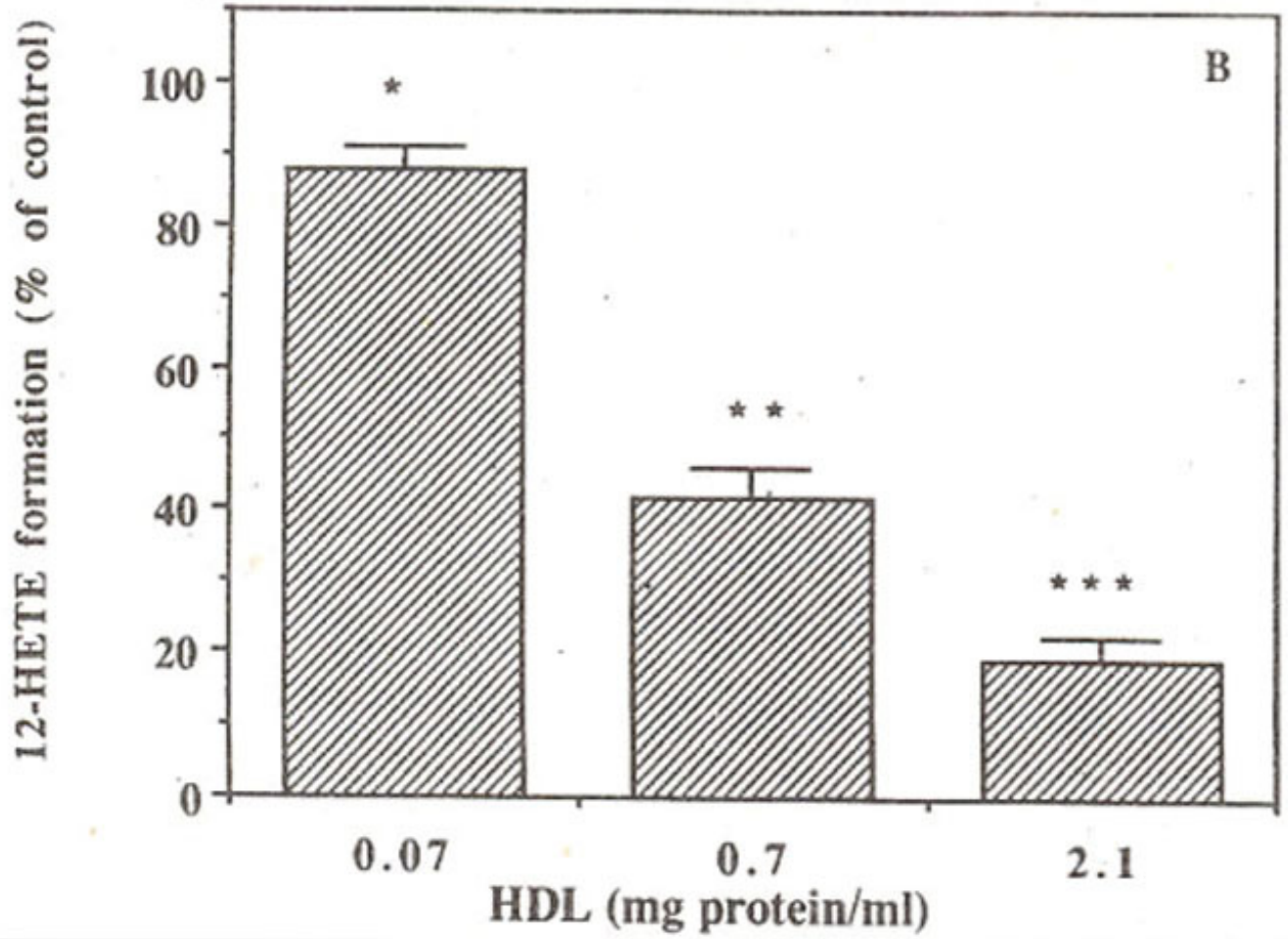

Figure 1(B). Effect of different concentrations of high density lipoproteins on 12-HETE formation in human platelets. The data is presented as mean±s.e.m. for four determinations. TXA_2 and 12-HETE were measured as described under methods.

The effect of different concentration of LDL on -14C] AA metabolism in human platelets is presented in Figure 2.

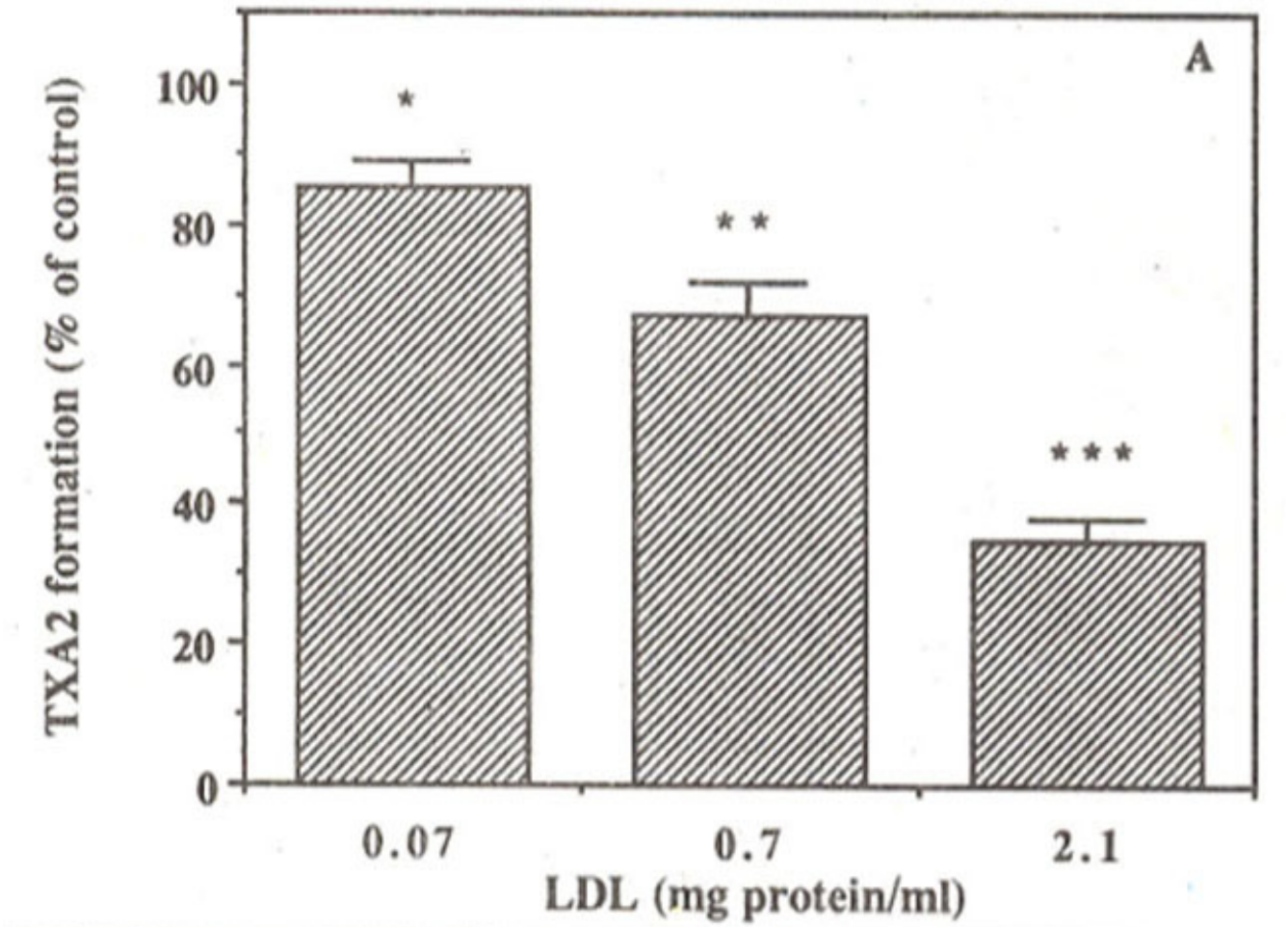

Figure 2 (A). Effect of different concentrations of low density lipoproteins on TXA_2.

LDL had a concentration-dependent inhibitory effect on TXA2 formation (Figure 2A) which was decreased by 15±2 %(p

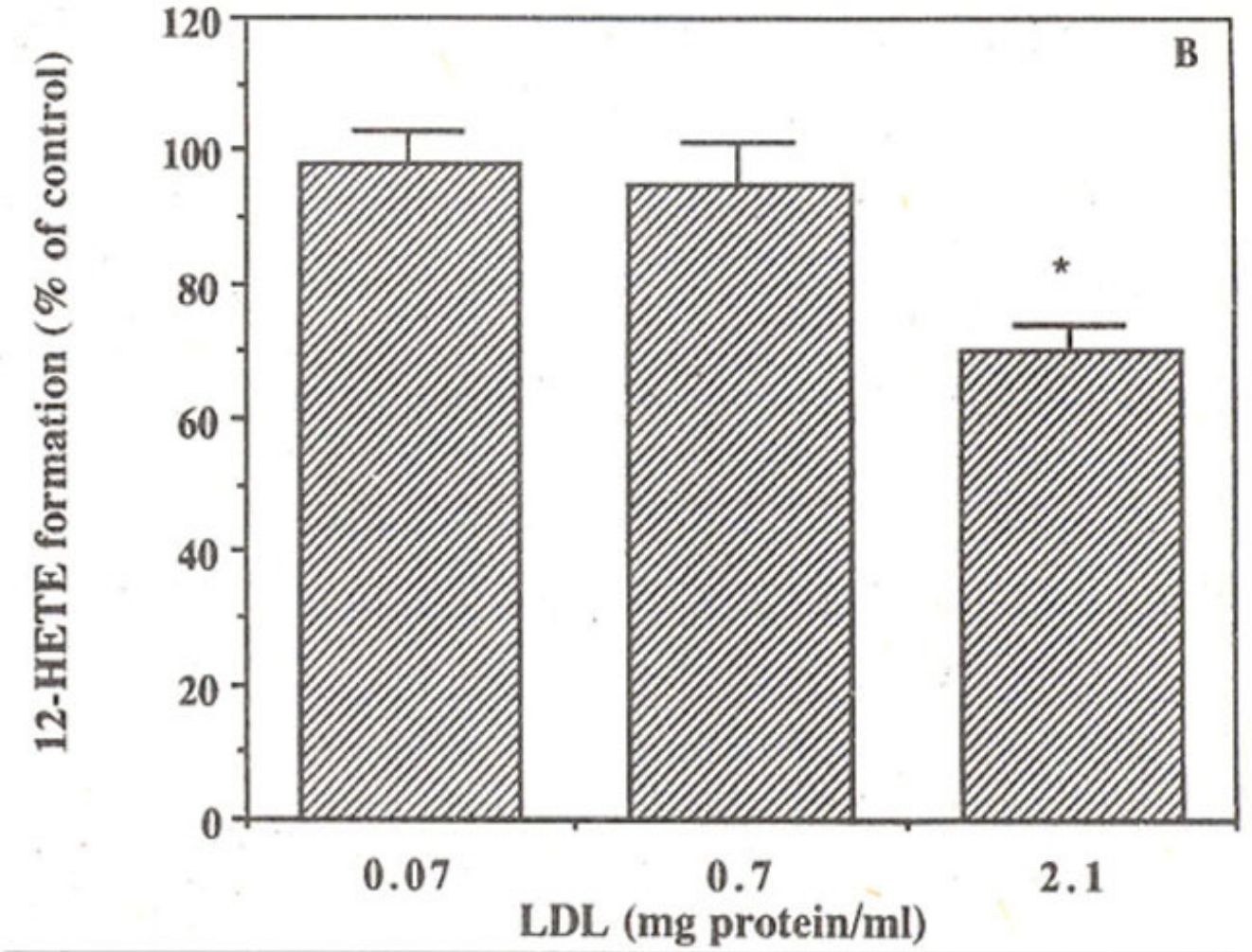

Figure 2 (B). Effect of different concentrations of low density lipoproteins on 12-HETE formation in human platelets. The data is presented as mean±s.e.m. for four determinations. TXA_2 and 12-HETE were measured as described under methods.

The effect of . different concentration of VLDL on AA metabolism in humanpiatelets is shown in Figure 3.

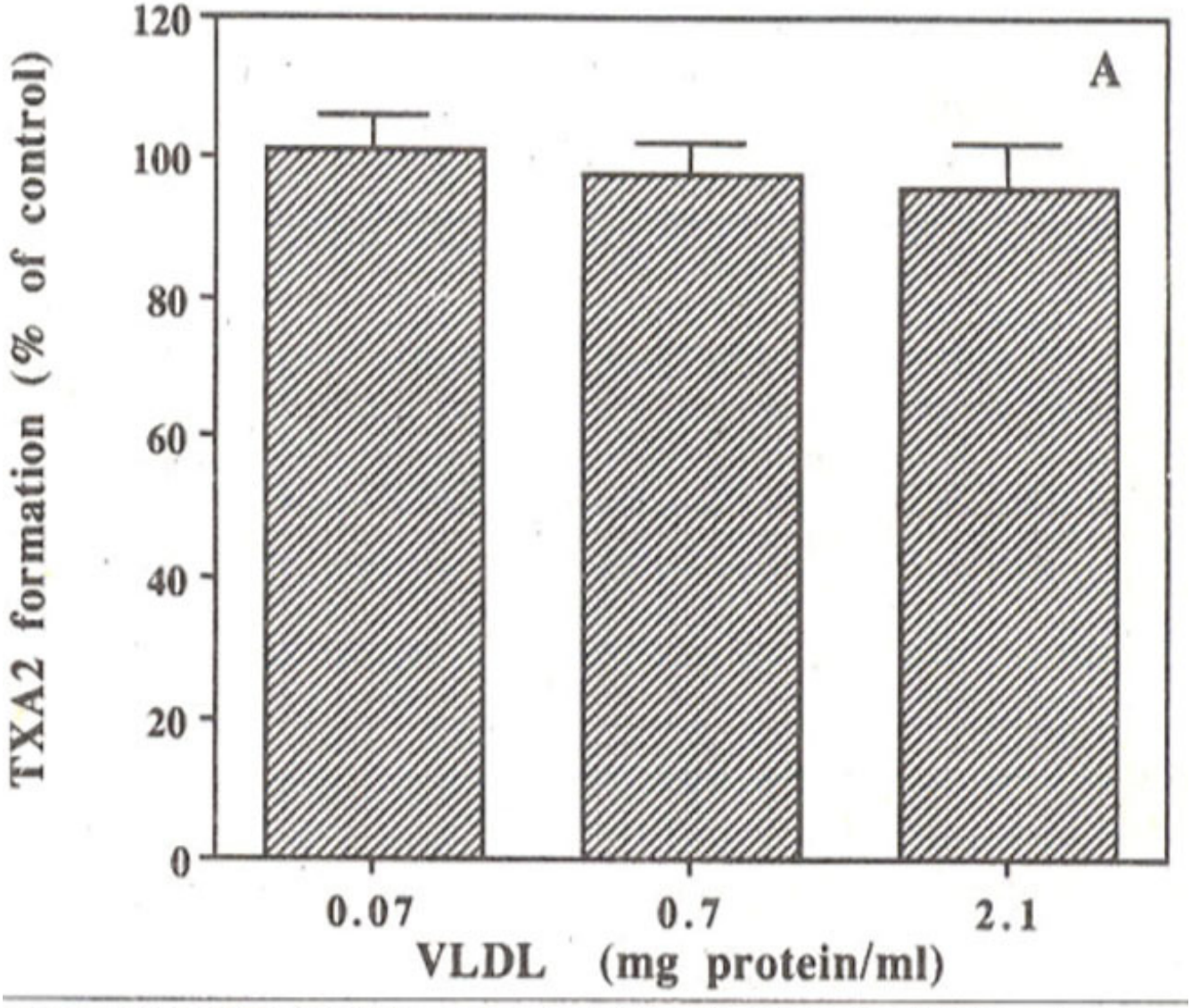

Figure 3 (A). Effect of different concentrations of very low density lipoproteins on TXA_2.

There was no significant inhibitory or stimulatoty effect of VLDL at similar protein concentrations of 0.07, 0.7 and 2.1 (mg protein/mi assay volume) on cyclo-oxygenase (Figure 3A) or lipo-oxygenase metabolites (Figure 3B) in human platelets.

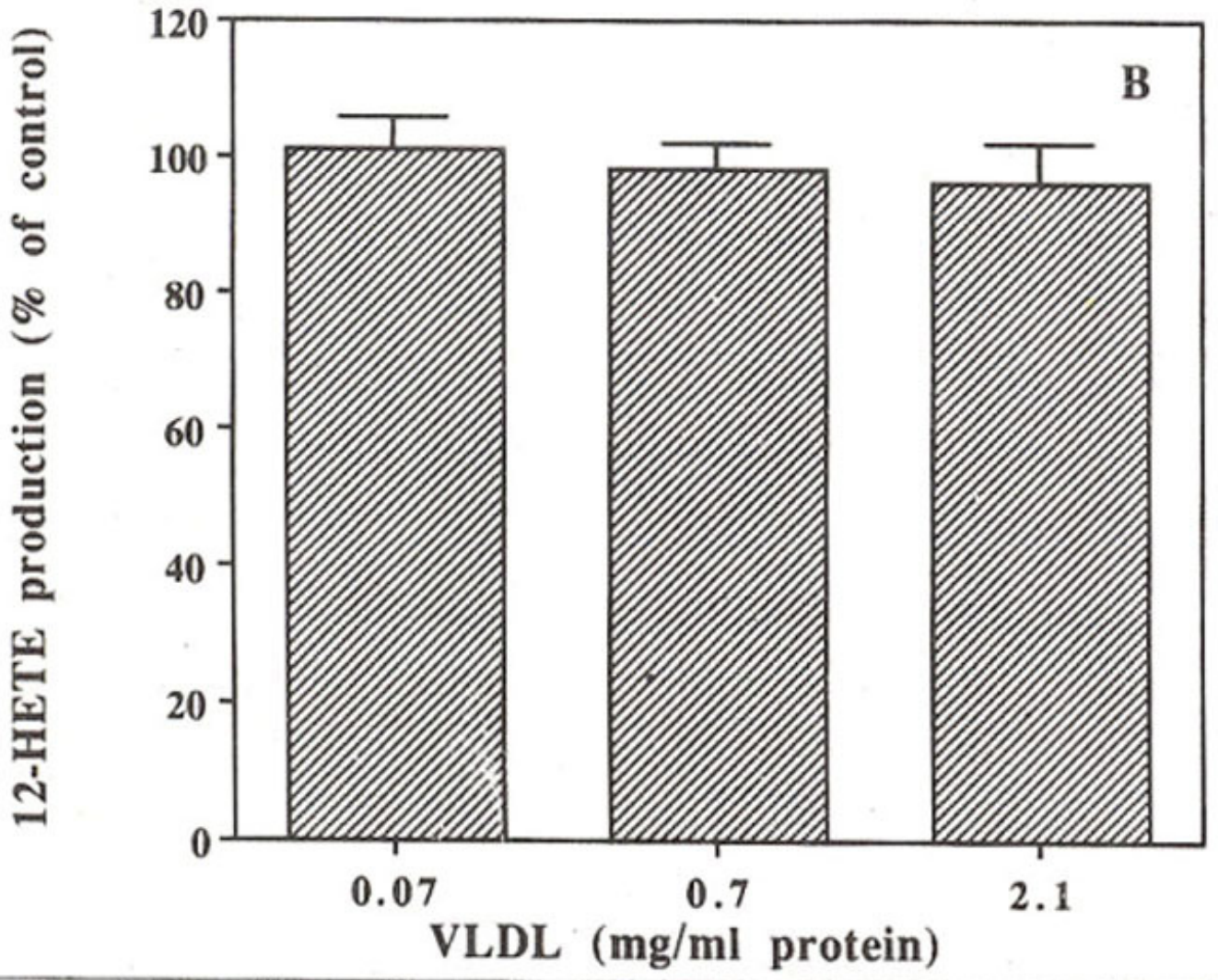

Figure 3 (B)). Effect of different concentrations of very low density lipoproteins on 12-HETE formation in human platelets. The data is presented as mean±s.e.m. for four determinations. TXA_2 and 12-HETE were measured as described under methods.

Discussion

The results of the present study demonstrate that HDL and LDL exert inhibitory effects on AA metabolism in human platelets. HDL decreased the fOrmation of TXA_2 and 12-HETE in platelets suggesting that it has an inhibitory effect on both cyclo- oxygenase and lipoxygenase pathways of AA metabolism. The magnitude of inhibition was dependent upon the protein concentration of HDL. Like HDL, LDL decreased the fonnation of TXA_2 in a concentration dependent-manner indicating that LDL also has inhibitory effect on cyclo-oxygenase pathways of AA metabolism. On the other hand, LDL had a weak inhibitory effect on 12-HETE formation in human platelets which was only observed at high protein concentrations.

In contrast to HDL and LDL, VLDL had no significant effect on either TXA_2 or 12-HETE formation in human platelets. The reason for this lack of VLDL effect on AA metabolism in platelets is not clear, however, it could be related to the composition of different lipoproteins. VLDL consists of mainly triglycerides as compared to LDL and HDL which contain cholesterol as their primary lipid

component[5,6]. Moreover, the major apoproteins associated with HDL, LDL and also different and have different functional roles[5,6]. The differences in the apoproteins associated with LDL and HDL may be responsible for the differences in the rñagmtude of their effects on lipooxygenase metabolites. Thus, it is likely that the differential effects of lipoproteins on AA metabolism may be related to the unique composition of individual lipoproteins. Both TXA2 and 12-HETE play an important role in platelet shape change, adhesion and aggregation[1,18] and these changes play a significant role in the progression of atherosclerosis[19]. Wile TXA_2 is a major pro- aggregatory agent, the role of 12-HETE has been confined to platelet shape change and chemotaxis[20]. High HDL levels are considered a protective factor against coronary heart disease because of their role in 'reverse cholesterol transport' and are associated witha decreased risk of atherosclerosis[21]. It has been shown by Coil et al[22] that HDL can inhibit platelet aggregation induced by a variety of aggregating agents. The inhibitory effects of HDL on TXA2 and 12-HETE formation shown in this study suggestanew beneficial and protective role forHDL whereby, it may decrease the formation of pro-aggregatoiy and chemotactic cicosanoids which are involved in the progression of atherogenesis[20]. The significance of the inhibitory effects of LDL on TXA2 formation in humans platelets is not clear as raised plasma levels of LDL are considered a positive risk factor for coronary heart disease[7]. However, these studies were conducted on lipoproteins isolated from normal healthy volunteers whose plasma lipoprotein levels were in the normal range. It is likely that LDL isolated from patients with coronary heart disease may behave differently as these patients have a substantial increase in oxidized LDL which is mainly responsible for increasing the atherogenic potential of LDL[23].

In addition to theirrole in lipid transport and modulatory effects on AA metabolism, lipoproteins have been shown to exert other effects. Several studies have now reported that chylomicrons, VLDL, LDL and HDL can bind to endotoxin and prevent its ability to induce fever, hypotension or death in rodents[24-26]. On quantitative basis, HDL appears to be the most important contributor to host defence against bacterial endotoxin[27]. Lipoproteins can also decrease endotoxin-induced cytokine secretion by macrophagcs[27,28] and thereby decrease their toxic effects[30]. The ability of HDL and LDL to inhibit the production of TXA2 and 12-FIETE which are the mediators ofinflaniniatory responses may be an additional non-specific mechanism which may be operative in host defence in several disease states and inflammatory conditions where lipopmteins are usually elevated. In summary our results indicate that HDL and LDL have significant inhibitory effects on TXA2 and 12-HETE fonnation suggesting that lipoproteins may have a physiological role in the regulation of AA metabolism in platelets. Since lipopmteins levels are elevated in several conditions such as coronary heart disease, chronic renal failure, diabetes mellitus and chronic infections[7,30-32] it is likely that they may modulate a wide range of biological effects of different AA metabolites in the pathophysiology of these diseases.

References

1. Smith, W.L. The eicosanoids and their biochemical mechanisms of action. Biochem. J.. 1989;259:31 5-324.
2. Marcus. A.J. and Hajjar, D.P. Vascular transcellular signaling. J. Lipid Res., 1993;34;2017-2031.
3. Knezevic, I., Borg, C. and Breton, G.C. Identification of G, as one of the G-proteins which co-purify with human platelet thromboxane Axprostaglandin H2 receptors. 3. Biol. Chem, 1993;268:26011-26017.
4. Shah, B.H. and Milligan, G. The gonadotrophin-releasing hormone receptor of a T3-1 pituitary cells regulates cellular levels of both of the phosphoinositidase C-linked proteins Gqa and G11a equally.Mol. Pharmacol., 1994;46:1-7.

4. Memon, R.A. and Gilani, A.H. An update on hyperlipidemia and its management. J. Pak. Med. Assoc., 1995,45:275-282.
5. Weinberg, R.B. Lipoprotein metabolism: Hormonal regulation. Hosp. Pract., 1987,22:223-243.
6. Grundy, SM. Cholesterol and coronary heart disease. JAMA., 1990;264:3053-3059.
7. Beitz, J. and Forster, W. Influence of human low and high density lipoprotein cholesterol on the in vitro prostaglandin I2 synthetase activity. Biochem. Biophys. Acts., 1980;620:352-355.
8. Shakhov, Y., Larrue, J., Perova, N. et a!. Effectoflipoproteins on prostaglandins in cultured aortic smooth muscles. J. Mol. Cell. Cardiol., 1989;21 :461-468.
9. Saeed, S.A., Simjee, RU., Mahmood, F. et al. Differential effects of human lipoproteins on prostagiandin biosynthesis. Indian J. Physiol. Allied Sci., 1993;47:26-31.
10. Havel, R.J., Eder, H.A. and Bragdon, J.H. The distribution and composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest., 1955;34:1345-1353.
11. Feingold, KR., Hardardottir, I., Memon, R.A. et al. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. J. Lipid Res., 1993;34:21 47-2158.
13. Lowry, OH., Rosebrough, N.J., Fan; AL. eta!. Protein measurement with the folin phenol reagent. J. Biol. Chem., 1951:93:265-274.
14. Saced, S.A.,Karimi, S. J. and Suria, A. Differential effects of dimethy l-sulfoxide on human platelet aggregation and arachidonic acid metabolism. Biochem. Med. Metab. Biol., 1988;40:143-150.
15. Saeed, S.A., Simjee, RU., Shamim, G. et a!. Eugenol: A dual inhibitor of platelet-activating factor and arachidonic acid metabolism. Phytomedicine, 1995;2:23-28.
16. Saeed, S.A., Simjee, RU., Mahniood, F. et a!. Dual inhibition of platelet-activating factor and arachidonic acid metabolism by ajmaline and effect on carrageenan induced rat paw oedema. J. Pharm. Pharmaco!., 1993;45:715-719.
17. Memon, R.A., Feingold, K.R., Moser, A.H. et al. Differential effects of interleukin-1 and tumor necrosis factor on ketogenesis. Am.J. Physiol., 1992;263 :E301 -E309.
18. Saeed, S.A. and Gilani, A.H. Mechanisms of platelet aggregation and the anti-aggregatory effect of human plasma. Asian J. ExpI. Sci., 1988;4: 1-7.
19. Pomerantz, K. and Hajjar, D.P. Signal transduction in atherosclerosis: Integration of cytokines and eicosanoid network. FASEB. J., 1992;6:2933-2941.
20. Sekiya, F., Takagi, J., Usui, T et a!. 12- hydroxyeicosatetraenoic acid plays a central role in the regulation of platelet activation. Biochem. Biophys. Res. Commun., 1991;179:345-351.
21. Steinberg, D. and Witztum, 3. Lipoproteins and atherogenesis. JAMA., 1990;264:3047-3052.
22. Coli, S., Maderna, P., Tremoli, B. et a!. Prostacyclin- lipoprotein interactions: Studies on human platelet aggregation and adenylate cyclase.Biochem. Pharmacol., 1985;34:245 1-2457.
23. Steinberg, D., Parthasarathy, S., Carew, T.E. et a!. Beyond cholesterol: Modifications of low density lipoprotein that increase its atherogenecity. N. Engl. 3. Med., 1989;320:9l5-924.
24. Harris, H.W., Grunfeld, C., Feingold, K.R. et a!. Human very low density !ipoproteins and chylomicrons can protect against endotoxin-induced death in mice.J. Clin. Invest., 1990;86:696- 702.
25. Navab, M., Hough, G.P., Vanlenten, B.J. et al. Low density lipoproteinstransfer bacterial lipopolysaccharides across endothelial monolayers in a biologically activeform. 1. Clin. Invest., 1988;81:601-605.
26. Levine, D.M., Parker, T.8., Donnelly, T. lvL In vivo protection against endotoxin by plasma high density lipoprotein. Proc. Nt!. Acad. Sci. USA., 1993;90: 12040-12044.
27. Flegel, WA., Wolpl, A., Mannel, D.N. et a!. Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. Infect. Immun., 1989;57:2237-2245.
28. Cavaillon, J.M, Fitting, C., Cavaillon, N.H. et a!. Cytokine response by monocyte and macrophage to free and lipoprotein bound lipopolysaccharide. Infect. Imrnun,, 1990;58:2375-2382.
29. Memon, R.A., Feingoid, K.R and Grunfeld, C. Role of cytokines in lipidmetabolism and cachexia. In: Human cytokines. Their role in disease and therapy (B. B. Aggarwa!l, RK Puri eds).

Cambridge,Blackwell Science, 1995, pp. 239-251.
30. Grundy, SM. Multifactorial etiology ofhypercholesterolemia: Implications for prevention of coronary heart disease. Arteriosckz. Thromb., 1991;11:1619-1635.
31. Memon, R.A., Grunfeld, C.,Moser, AN. et a!. Fatty acid synthesis in obese insulin resistant diabetic mice. Horm. Metab. Res., 1994;26:85-87.