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αB-crystallin reduces ristocetin-induced soluble CD40 ligand release in human platelets: Suppression of thromboxane A_2 generation

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Abstract. Our group has previously shown that αB-crystallin (HSPB5), a small heat shock protein, inhibits human platelet aggregation by ristocetin, an activator of glycoprotein Ib/IX/V. In addition, it was demonstrated that glycoprotein Ib/IX/V activation induces soluble CD40 (sCD40) ligand release via thromboxane (TX) A_2. In the present study, the effect of αB-crystallin on the ristocetin-induced sCD40 ligand release in human platelets was investigated. The ristocetin-induced release of sCD40 ligand was suppressed by αB-crystallin. In addition, αB-crystallin reduced the ristocetin-stimulated production of 11-dehydro-TX B_2, a stable metabolite of TXA_2. αB-crystallin did not suppress the platelet aggregation induced by U46619, a TXA_2 receptor agonist. αB-crystallin had little effect on the U46619-induced phosphorylation of p38 mitogen-activated protein kinase or sCD40 ligand release. In addition, αB-crystallin failed to reduce the binding of SZ2, a monoclonal antibody against the sulfated sequence in the α-chain of glycoprotein Ib, to the ristocetin-stimulated platelets. These results strongly suggest that αB-crystallin extracellularly suppresses ristocetin-stimulated release of sCD40 ligand by inhibiting the TXA_2 production in human platelets.

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Key words: αB-crystallin, ristocetin, thromboxane A_2, soluble CD40 ligand, platelet

Introduction

Expression of heat shock proteins (HSPs) is induced in response to various types of biological stress to protect cells against different types of damage as molecular chaperones (1,2). Human HSPs are recently classified into seven groups, HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), HSPB (small HSP), HSPD/E (HSP60/HSP10) and CCT (TRiC) (1). Among them, the small HSP family (HSPB) contains 10 members, including αB-crystallin (HSPB5) (3), HSP27 (HSPB1) (4) and HSP20 (HSPB6) (5) with molecular masses ranging from 15 to 30 kDa. A number of HSPB family members, including αB-crystallin and HSP27, are ubiquitously expressed in cells and tissues, such as skeletal and smooth muscle (1,2). The HSPB family members have a highly homologous structure in their amino acid sequence, termed the α-crystallin domain (2). It is currently recognized that HSPB binds improperly folded proteins and transfers them to the ATP-dependent chaperones, such as HSPA (HSP70), or to the protein degradation machines (6). Accumulating evidence indicates that small HSPs participate in the regulation of numerous intracellular processes in a wide range of cell types and are important in maintaining the function of tissues, such as muscle and nerve tissue (1). However, the exact mechanism underlying HSPB effects on cell function remains to be clarified.

Platelets are important in primary hemostasis and repairing vascular injury, and are initially activated via adhesive receptors, such as glycoprotein Ib/IX/V receptors. Glycoprotein Ib/IX/V receptors mediate rolling and tethering of the platelets to von Willebrand factor at the sites of vascular injury, which is followed by glycoprotein IIb/IIIa activation resulting in platelet aggregation (7,8). In addition, it is generally recognized that shear stress stimulates platelet activation in a physiological or pathological mechanism *in vivo* (7). Under the condition of sheer stress, the activation of platelets is dependent upon the interaction of von Willebrand factor-glycoprotein Ib/IX/V (7,8). Ristocetin, an activator of glycoprotein Ib/IX/V,

potently induces the interaction between von Willebrand factor and glycoprotein Ib/IX/V *in vitro* (9). It has been reported that glycoprotein Ib activation induced by ristocetion leads to thromboxane (TX) A_2 generation by cytosolic phospholipase A2 in platelets (8). Our group recently reported that ristocetin induces the release of soluble CD40 (sCD40) ligand from human platelets via TXA_2 generation (10).

Our group has also demonstrated that human platelets possess specific binding sites for αB-crystallin and that αB-crystallin functions extracellularly and suppresses the human platelet aggregation induced by ristocetin, an activator of glycoprotein Ib/IX/V and thrombin (11,12). In addition, we have recently reported that aB-crystallin suppresses the adenosine diphosphate (ADP)-induced platelet granule secretion by inhibition of HSP27 phosphorylation via p44/p42 MAPK and p38 MAPK (13). However, the exact mechanism underlying the extracellular effect of αB-crystallin on human platelets has not been clarified. The present study aimed to investigate whether αB-crystallin extracellularly affects glycoprotein Ib/IX/V-induced sCD40 ligand release from human platelets.

Materials and methods

Materials. Ristocetin was purchased from Sigma-Aldrich (St. Louis, MO, USA). U46619 was obtained from Enzo Life Sciences International, Inc. (Plymouth Meeting, PA, USA). αB-crystallin, a native protein purified from the bovine eye lens, was purchased from Assay Designs Inc. (Ann Arbor, MI, USA). sCD40 ligand ELISA kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). TXB_2 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Rabbit anti-human monoclonal phospho-specific p38 MAP kinase antibodies (cat. no. 4511) and rabbit anti-human polyclonal p38 MAP kinase antibodies (cat. no. 9212) were obtained from Cell Signaling, Inc. (Beverly, MA, USA). SZ2, a mouse anti-human monoclonal antibody against the sulfated tyrosine/anionic glycoprotein Iba residues Tyr-276-Glu-282 (14), was obtained from Beckman Coulter (Krefeld, Germany; cat. no. IM0409) for immunoprecipitation and from Santa Cruz Biotechnology Inc. (Santa Cruz, CA; cat. no. sc-59052) for western blotting. All other materials and chemicals were obtained from commercial sources.

Preparation of platelets. The present study used seven healthy volunteers (5 male and 2 female) between the ages of 27 and 50 years. All participants were provided a medical check-up at Gifu University Hospital. Human blood (15 ml) was donated from the median cubital vein and and combined with 3.8% sodium citrate (1/10 volume) . Platelet-rich plasma (PRP) was obtained from blood samples by centrifugation at 155 x g for 12 min at room temperature. Platelet-poor plasma was prepared from residual blood by centrifugation at 2,500 x g for 5 min. All participants signed an informed consent agreement after receiving a detailed explanation of the study. The study was approved by the Committee of Ethics in Gifu University Graduate School of Medicine (Gifu, Japan).

Measurement of platelet aggregation induced by ristocetin or U46619. Platelet aggregation using citrated PRP was observed in a PA-200 aggregometer (Kowa Co. Ltd., Tokyo, Japan), which can determine the size of platelet aggregates based upon particle counting using laser scattering methods (small size, 9-25 μm; medium size, 25-50 μm; and large size, 50-70 μm) (15), at 37˚C with a stirring speed of 800 rpm. The platelets were pre-incubated for 1 min, and then platelet aggregation was monitored for 4 min. The percentage of transmittance of the isolated platelets was recorded as 0%, and that of the appropriate platelet-poor plasma (blank) was recorded as 100%. When indicated, PRP was pretreated with αB-crystallin (0.6, 1.8 and 6.0 μg/ml) for 15 min.

Protein preparation after stimulation by ristocetin or U46619. Following stimulation with ristocetin or U46619, platelet aggregation was terminated by the addition of 10 mM ice-cold EDTA (Katayama Chemical Industries Co., Ltd., Osaka, Japan) solution. The mixture was centrifuged at 10,000 x g at 4˚C for 2 min. To measure the levels of sCD40 ligand, PDGF-AB and TXB_2 as described below, the supernatant was isolated and stored at -20˚C for subsequent ELISA. For western blot analysis, the pellet was washed twice with phosphate-buffered saline and then lysed and immediately boiled in a lysis buffer containing 62.5 mM Tris/Cl, pH 6.8; 2% SDS, 50 mM dithiothreitol and 10% glycerol. For immunoprecipitation, the pellet was washed twice with phosphate-buffered saline and lysed in 0.5 ml ice-cold TNE lysis buffer [containing 10 mM Tris-HCl, pH 7.8; 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM sodium fluoride, 1 mM sodium vanadate and protease inhibitor cocktail (all from Roche Applied Science, Mannheim, Germany)]. The lysates were then centrifuged at 10,000 x g at 4˚C for 30 min and the supernatant was collected as TNE-soluble protein.

Measurement of sCD40 ligand and 11-dehydro-TXB_2 levels. The sCD40 ligand and 11-dehydro-TXB2 levels in samples were determined using each ELISA kit according to the manufacturer's instructions.

Western blot analysis. A western blot analysis was performed as described previously (16). Briefly, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to a previous method (17) on a 10% polyacrylamide gel. The proteins in the gel were transferred onto polyvinylidine fluoride (PVDF) membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were then blocked with 5% fat-free dry milk in Tris-buffered saline with 0.1% Tween-20 (TBS-T; 20 mM Tris, pH 7.6; 137 mM NaCl and 0.1% Tween-20) for 2 h prior to incubation with the indicated primary antibodies. The primary antibodies used in this experiment were phospho-specific p38 MAP kinase antibodies or p38 MAP kinase antibodies, respectively. Peroxidase-labeled anti-mouse IgG (cat. no. NA931; 1:1,000; GE Healthcare, Little Chalfont, UK) or anti-rabbit IgG (cat. no. 074-1506; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) antibodies were used as secondary antibodies. The primary and secondary antibodies were diluted to obtain optimum concentrations with 5% fat-free dry milk in TBS-T. Peroxidase activity on PVDF membranes was visualized on X-ray films by means of an enhanced chemiluminescnece western blotting detection system according to the manufacturer's instructions.

Immunoprecipitation. SZ2 (Beckman Coulter) was added to the TNE-soluble proteins, and the mixture was shaken gently for overnight at 4˚C, followed by the addition of 50 ml protein G Dynabeads (Life Technologies, Carlsbad, CA, USA) and a further incubation for 1 h with continuous mixing. Protein immunocomplexes were isolated with the use of a magnetic particle concentrator (6-tube magnetic separation rack; New England BioLabs, Inc., Ipswich, MA, USA). Immunoprecipitated proteins were resuspended in SDS-PAGE loading buffer, heated at 95˚C for 5 min, and analyzed by western blot analysis using SZ2 (Santa Cruz Biotechnology, Inc.) as a primary antibody.

Statistical analysis. The data were analyzed by Student's t-test, and a $P<0.05$ was considered to indicated a statistically significant difference. All data are presented as the mean ± standard error of the mean. All statistical analyses were performed using PASW statistics version 18 (IBM SPSS, Tokyo, Japan).

Results

Effect of αB-crystallin on the ristocetin-stimulated release of sCD40 ligand from human platelets. Our group has recently shown that ristocetin stimulates sCD40 ligand release from human platelets (10). The effect of αB-crystallin on the ristocetin-stimulated sCD40 ligand release from human platelets was examined. αB-crystallin significantly suppressed the ristocetin-stimulated release of sCD40 ligand (Fig. 1). The inhibitory effect was dose dependent and αB-crystallin at 6.0 μg/ml caused an ~80% reduction in the ristocetin-effect (Fig. 1).

Effect of αB-crystallin on the ristocetin-stimulated production of TXA_2 in human platelets. Our group previously demonstrated that ristocetin induces TXA_2 generation, which leads to the release of sCD40 ligand from human platelets (10). In the present study, the ristocetin (1.5 mg/ml)-stimulated TXB_2 production was significantly reduced by αB-crystallin, which was determined by measuring the generation of 11-dehyro-TXB_2, a stable TXA_2 metabolite (18) (Fig. 2). The suppressive effect of αB-crystallin on the TXB2 production was dose-dependent and 6.0 μg/ml αB-crystallin caused ~90% reduction in the ristocetin-effect (Fig. 2).

Effect of αB-crystallin on platelet aggregation by U46619. Our group previously demonstrated that ristocetin stimulates the release of sCD40 ligand through TXA_2 production as an autacoid (10). However, αB-crystallin did not affect the platelet aggregation induced by U46619, which is a selective TXA_2 receptor (TP) agonist (19). In the present study, αB-crystallin had little effect on the distribution of aggregated particle sizes (small size, medium size or large size) even when the platelets were treated with 6.0 μg/ml αB-crystallin (Fig. 3).

Effects of αB-crystallin on the U46619-stimulated release of sCD40 ligand and phosphorylation of p38 MAP kinase in human platelets. Our group have previously demonstrated that TXA_2 receptor activation induces the release of the sCD40 ligand via MAP kinases, such as p38 MAP kinase in human platelets (10). Thus, in the present study, the effect of αB-crystallin on the release of sCD40 ligand stimulated by U46619 from human platelets was analyzed. αB-crystallin was not observed to significantly reduce the U46619-induced release

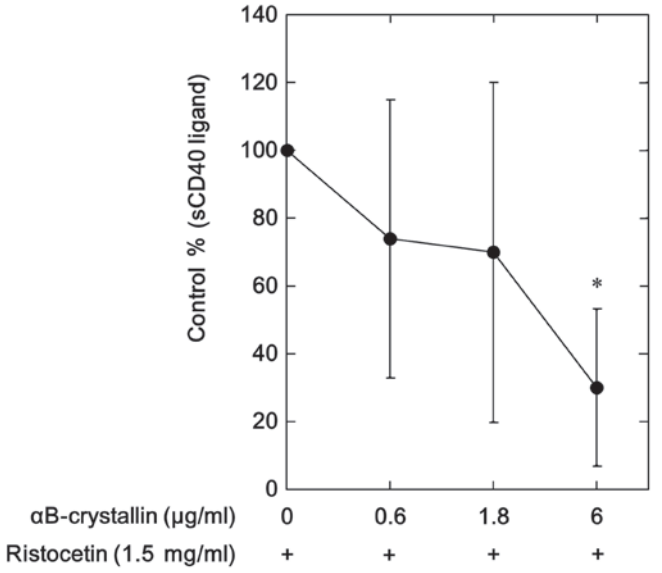


Figure 1. Effect of αB-crystallin on ristocetin-induced sCD40 ligand release in human platelets. Platelet-rich plasma wase pretreated with various doses of αB-crystallin at 37˚C for 15 min, and then stimulated by 1.5 mg/ml ristocetin for 30 min. The reaction was terminated by the addition of ice-cold EDTA (10 mM) solution. The mixture was centrifuged at 10,000 x g at 4˚C and the supernatants were then subjected to ELISA for sCD40 ligand. The net increase in levels of ristocetin alone were presented as 100%. Representative results from five individuals are shown. Each value is represented as the mean ± standard error of the mean. *P<0.05, compared with the value of control.

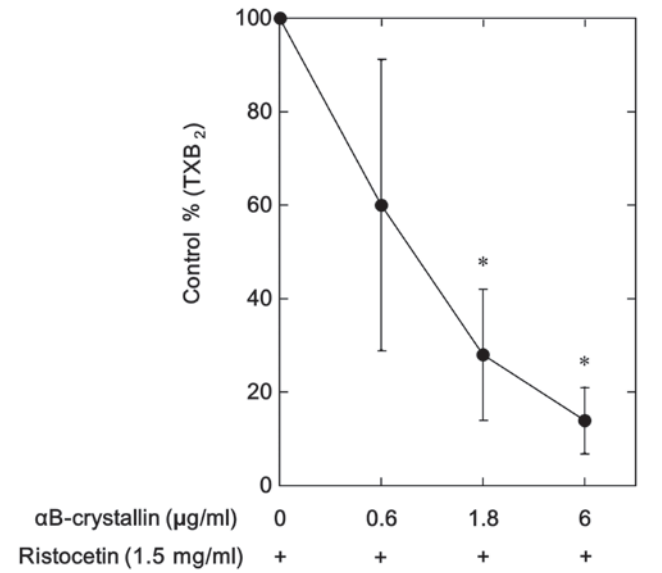
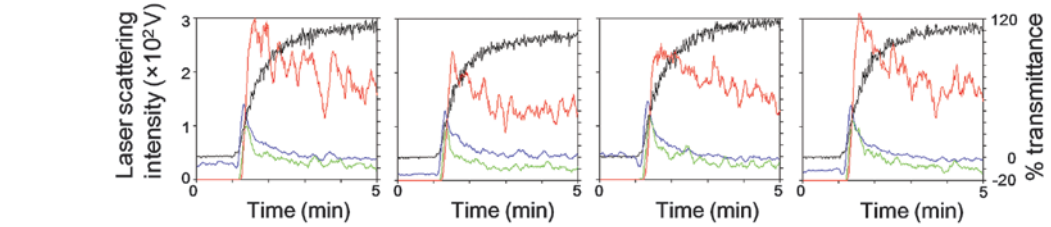


Figure 2. Effect of αB-crystallin on ristocetin-induced TXB_2 production in human platelets. Platelet-rich plasma was pretreated with various doses of αB-crystallin at 37˚C for 15 min, and then stimulated by 1.5 mg/ml ristocetin for 30 min. The reaction was terminated by the addition of 10 mM ice-cold EDTA solution. The mixture was centrifuged at 10,000 x g at 4˚C and the supernatants were then subjected to enzyme-linked immunosorbent assay for TXB_2. The net increase in levels of ristocetin alone were presented as 100%. Representative results from three individuals are shown. Each value is represented as the mean ± standard error of the mean. *P<0.05, compared with the value of control. TXB_2, thromboxane B_2.



Panel	1	2	3	4
αB-crystallin (μg/ml)	0	0.6	1.8	6
U46619 (3 μM)	+	+	+	+
Ratio (%)				
Small	21	26	24	20
Medium	12	13	14	13
Large	67	61	62	67
Total	100	100	100	100

Figure 3. Effect of αB-crystallin on U46619-induced platelet aggregation. Platelet-rich plasma was pretreated with various doses of αB-crystallin at 37˚C for 15 min, and then stimulated by 3 μM U46619 for 5 min. The reaction was terminated by the addition of 10 mM ice-cold EDTA solution. The black line indicates the percentage of transmittance of each sample (the isolated platelets were recorded as 0%, and platelet-free plasma was recorded as 100%). The blue line indicates small aggregates (9-25 μm); the green line indicates medium aggregates (25-50 μm) and the red line indicates large aggregates (50-70 μm). The distribution (%) of aggregated particle size was measured by the laser scattering method. Representative results obtained from five healthy donors are indicated.

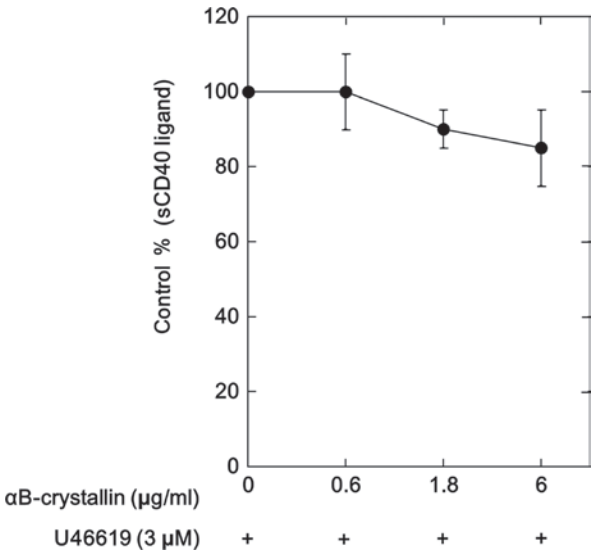
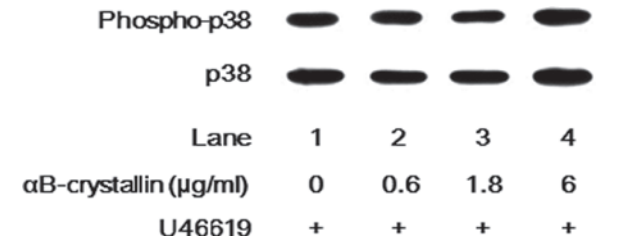
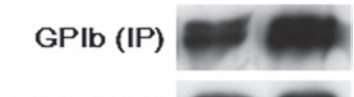


Figure 4. Effect of αB-crystallin on U46619-induced sCD40 ligand release in human platelets. Platelet plasma were pretreated with various doses of αB-crystallin at 37˚C for 15 min, and then stimulated by 3 μM U46619 for 30 min. The reaction was terminated by the addition of ice-cold EDTA (10 mM) solution. The mixture was centrifuged at 10,000 x g at 4˚C and the supernatants were then subjected to ELISA for sCD40 ligand. The net increase in the level of ristocetin alone were represented as 100%. Representative results from three individuals are shown. Each value is represented as the mean ± standard error of the mean.



Lane	1	2	3	4
αB-crystallin (μg/ml)	0	0.6	1.8	6
U46619	+	+	+	+

Figure 5. Effect of αB-crystallin on U46619-induced phosphorylation of p38 MAP kinase in human platelets. Platelet-rich plasma were pretreated with the indicated doses of αB-crystallin at 37˚C for 15 min, and then stimulated by 3 μM U46619 for 5 min. The reaction was terminated by the addition of 10 mM ice-cold EDTA solution. Lysed platelets were subjected to western blot analysis using antibodies against phospho-specific p38 MAP kinase or p38 MAP kinase. Representative results from five individuals are shown.



Lane	1	2
αB-crystallin	-	+
Ristocetin	+	+

Figure 6. Effect of αB-crystallin on the binding of SZ2 to the ristocetin-stimulated human platelets. Platelet-rich plasma was pretreated with 6.0 μg/ml αB-crystallin or vehicle at 37˚C for 15 min, and then stimulated by 1.5 mg/ml ristocetin for 5 min. The reaction was terminated by the addition of ice-cold EDTA (10 mM) solution. The mixture was centrifuged at 10,000 x g at 4˚C. The pellets were washed twice with phosphate-buffered saline and lysed by suspension in ice-cold TNE lysis buffer and then the lysates were centrifuged at 10,000 x g at 4˚C for 30 min. Immunoprecipitation was performed with the supernatants using a monoclonal antibody against SZ2. Immunoprecipitated lysates (IP) and preimmunoprecipitated lysates (total) were subjected to western blot analysis using another monoclonal antibody against SZ2.

of sCD40 ligand (Fig. 4). Furthermore, the U46619-induced phosphorylation levels of p38 MAP kinase were not affected by αB-crystallin (Fig. 5).

Effect of αB-crystallin on the binding of SZ2 to ristocetin-stimulated human platelets. It is recognized that von

Willebrand factor binds glycoprotein Ib/IX/V on the platelet membrane and initiates signals leading to platelet activation under shear stress or in the presence of modulators, such as ristocetin. Thus, the effect of αB-crystallin on the binding of SZ2, a monoclonal antibody to the sulfated tyrosine/anionic glycoprotein Iba residues Tyr-276-Glu-282 (14), to the ristocetin-stimulated human platelets was further examined. However, αB-crystallin failed to suppress SZ2-binding to the ristocetin-stimulated platelets (Fig. 6).

Discussion

αB-crystallin, a small HSP, is ubiquitously expressed in a variety of types of tissues and cells, including cardiac, smooth and skeletal muscle (1,2). It is firmly established that HSPs act intracellularly as molecular chaperones (1). In our previous study (11), it was demonstrated that the αB-crystallin levels in injured arteries are markedly lower than those in non-injured arteries *in vivo* and that αB-crystallin levels in the plasma of hamsters with cardiomyopathy are markedly higher than those of control hamsters. Recently, it has been shown that αB-crystallin is secreted from epithelial cells (20). Our group has previously reported that the specific binding sites of αB-crystallin exist on human platelets and that αB-crystallin affects human platelets extracellularly and inhibits the platelet aggregation induced by ristocetin (11,12). In addition, it was demonstrated that glycoprotein Ib/IX/V activation induces the release of sCD40 ligand, an inflammatory mediator, via TXA_2 production in human platelets (10). On the basis of these findings, in the present study, the extracellular effect of αB-crystallin on the ristocetin-induced release of sCD40 ligand in human platelets and the underlying mechanism were investigated. It was observed that αB-crystallin significantly suppressed the release of sCD40 ligand from platelets stimulated by ristocetin. In addition, αB-crystallin failed to affect the platelet aggregation induced by U46619, an agonist of TP (TXA_2 receptor). It is currently recognized that the MAP kinases, such as p38 MAP kinase, are activated downstream of TP-mediated responses (21). In our previous study (10), it was shown that TP-induced activation of MAP kinases is involved in the ristocetin-stimulated sCD40 ligand release from human platelets. Additionally, the present study demonstrated that the U46619-induced phosphorylation of p38 MAP kinase or the release of sCD40 ligand was not significantly affected by αB-crystallin in human platelets. Based on these findings, it is unlikely that αB-crystallin suppresses ristocetin-induced sCD40 ligand release at a point downstream of TP.

In the present study, αB-crystallin significantly inhibited the ristocetin-induced the production of TXB_2, a stable metabolite of TXA_2. It has been reported that ristocetin-activated glycoprotein Ib results in activation of cytosolic phospholipase A_2, which stimulates the release of arachidonic acid and leads to the production of TXA_2 in human platelets (8). In our previous study (10), ristocetin induced TXA_2 generation via cyclooxygenase, which lead to the release of sCD40 ligand from human platelets through TP. Thus, it is most likely that αB-crystallin reduces ristocetin-stimulated sCD40 ligand release via inhibiting TXA_2 generation in human platelets.

Furthermore, it was demonstrated that αB-crystallin failed to reduce the SZ2-binding to the ristocetin-stimulated platelets. SZ2 is known to be a monoclonal antibody against the heparin-like, sulfated tyrosine/anionic glycoprotein Iba residues Tyr-276-Glu-282, recognized as a binding site for von Willebrand factor (14). Therefore, it is unlikely that αB-crystallin suppresses the ristocetin-dependent binding of von Willebrabd factor to glycoprotein Ib. Our group have recently reported that antithrombin III inhibits ristocetin-induced release of sCD40 ligand via inhibition of TXA_2 production accompanied by the reduction of SZ2 binding in human platelets (22). Thus, the mechanism of αB-crystallin affecting GPIb/IX/V signaling may be different from that of antithrombin III in human platelets. Considering all findings, it is most likely that αB-crystallin suppresses the TXA_2 production induced by ristocetin, resulting in the inhibition of sCD40 ligand release from human platelets.

When exposed to various stimuli, human platelets rapidly respond and release inflammatory mediators causing atherosclerosis, such as sCD40 ligand in addition to granule secretion of PDGF-AB and serotonin (5-HT) (23). CD40 ligand, which is a member of the tumor necrosis factor-α family, exists in the cytoplasm of resting platelets and is immediately translocated to the surface following platelet activation (24). sCD40 ligand is subsequently released from the platelet membrane as a functional soluble fragment into the circulation. It is recognized that the platelet-derived sCD40 ligand induces inflammatory responses via CD40 expressed on vascular endothelial cells that produce inflammatory mediators, such as reactive oxygen species and chemokines (23,25). Platelet-derived sCD40 ligand becomes mobilized in patients with acute coronary syndrome (26). Reportedly, the elevation of plasma sCD40 ligand is associated with an increased risk of cardiovascular events in patients with acute coronary syndrome (27). In the present study, it was demonstrated that αB-crystallin obviously suppressed the glycoprotein Ib/IX/V activation-induced release of sCD40 ligand from human platelets. Therefore, our findings indicate that αB-crystallin may be an anti-inflammatory agent for patients under high shear stress conditions. Further investigation is required to clarify the exact mechanism underlying the effects of αB-crystallin on human platelets.

In conclusion, the results suggest that αB-crystallin extracellularly suppresses the ristocetin-induced release of sCD40 ligand by inhibiting TXA_2 production in human platelets.

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References

1. Mymrikov EV, Seit-Nebi AS and Gusev NB: Large potentials of small heat shock proteins. Physiol Rev 91: 1123-1159, 2011.
2. Taylor RP and Benjamin IJ: Small heat shock proteins: a new classification scheme in mammals. J Mol Cell Cardiol 38: 433-444, 2005.

3. Kato K, Shinohara H, Goto S, Inaguma Y, Morishita R and Asano T: Copurification of small heat shock protein with αB crystallin from human skeletal muscle. J Biol Chem 267: 7718-7725, 1992.
4. Kato K, Hasegawa K, Goto S and Inaguma Y: Dissociation as a result of phosphorylation of an aggregated form of the small stress protein, hsp27. J Biol Chem 269: 11274-11278, 1994.
5. Kato K, Goto S, Inaguma Y, Hasegawa K, Morishita R and Asano T: Purification and characterization of a 20-kDa protein that is highly homologous to a B crystallin. J Biol Chem 269: 15302-15309, 1994.
6. Vos MJ, Hageman J, Carra S and Kampinga HH: Structural and functional diversities between members of the human HSPB, HSPH, HSPA and DNAJ chaperone families. Biochemistry 47: 7001-7011, 2008.
7. Berndt MC, Shen Y, Dopheide SM, Gardiner EE and Andrews RK: The vascular biology of the glycoprotein Ib-IX-V complex. Thromb Haemost 86: 178-188, 2001.
8. Garcia A, Quinton TM, Dorsam RT and Kunapuli SP: Src family kinase-mediated and Erk-mediated thromboxane A2 generation are essential for vWF/GPIb-induced fibrinogen receptor activation in human platelets. Blood 106: 3410-3414, 2005.
9. Dong JF, Berndt MC, Schade A, McIntire LV, Andrews RK and Lopez JA: Ristocetin-dependent, but not botrocetin-dependent, binding of von Willebrand factor to the platelet glycoprotein Ib-IX-V complex correlates with shear-dependent interactions. Blood 97: 162-168, 2001.
10. Enomoto Y, Adachi S, Matsushima-Nishiwaki R, Doi T, Niwa M, Akamatsu S, *et al*: Thromboxane A2 promotes soluble CD40 ligand release from human platelets. Atherosclerosis 209: 415-421, 2010.
11. Kozawa O, Matsuno H, Niwa M, Hatakeyama D, Kato K and Uematsu T: αB-crystallin, a low-molecular-weight heat shock protein, acts as a regulator of platelet function. Cell Stress Chaperones 6: 21-28, 2001.
12. Matsuno HI, Ishisaki A, Nakajima K, Kato K and Kozawa O: A peptide isolated from αB-crystallin is a novel and potent inhibitor of platelet aggregation via dual prevention of PAR-1 and GPIb/V/IX. J Thromb Haemost 12: 2636-2642, 2003.
13. Enomoto Y, Adachi S, Matsushima-Nishiwaki R, Niwa M, Tokuda H, Akamatsu S, *et al*: Alpha B-crystallin extracellularly suppresses ADP-induced granule secretion from human platelets. FEBS Lett 583: 2464-2468, 2009.
14. Ward CM, Andrews RK, Smith AI and Berndt MC: Mocarhagin, a novel cobra venom metalloproteinase, cleaves the platelet von Willebrand factor receptor glycoprotein Iba. Identification of the sulfated tyrosine/anionic sequence Tyr-276-Glu-282 of glycoprotein Iba as a binding site for von Willebrand factor and a-thrombin. Biochemistry 35: 4929-4938, 1996.
15. Fabre JE, Nguyen M, Latour A, Keifer JA, Audoly LP, Coffman TM and Koller BH: Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice, Nature Med 5: 1199-1202, 1999.
16. Kato K, Ito H, Hasegawa K, Inaguma Y, Kozawa O and Asano T: Modulation of the stress-induced synthesis of hsp27 and α B-crystallin by cyclic AMP in C6 rat glioma cells. J Neurochem 66: 946-950, 1996.
17. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685, 1970.
18. Catella F, Healy D, Lawson JA and FitzGerald GA: 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. Proc Natl Acad Sci USA 83: 5861-5865, 1986.
19. Bertele V, Di Minno G and de Gaetano G: U-46619, a stable analogue of prostaglandin H2, induces retraction of human platelet-rich plasma clots. Thromb Res 18: 543-545, 1980.
20. Gangalum RK, Atanasov IC, Zhou ZH and Bhat SP: αB-crystallin is found in detergent-resistant membrane microdomains and is secreted via exosomes from human retinal pigment epithelial cells. J Biol Chem 286: 3261-3269, 2011.
21. Nakahata N: Thromboxane A2: physiology/pathophysiology, cellular signal transduction and pharmacology. Pharmacol Ther 118: 18-35, 2008.
22. Doi T, Tokud H, Matsushima-Nishiwaki R, Cuong NT, Kageyama Y, Iida Y, Kono A, Akamatsu S, Otsuka T, Iida H, Kozawa O and Ogura S: Effect of antithrombin III on glycoprotein Ib/IX/V activation in human platelets: suppression of thromboxane A2 generation. Prosta Leukot Essent Fatty Acids 87: 57-62, 2012.
23. Davi G and Patrono C: Platelet activation and atherothrombosis. New Engl J Med 357: 2482-2494, 2007.
24. Hermann A, Rauch BH, Braun M, Schror K and Weber AA: Platelet CD40 ligand (CD40 L)-subcellular localization, regulation of expression and inhibition by clopidogrel. Platelets 12: 74-82, 2001.
25. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, *et al*: CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 391: 591-594, 1998.
26. Heeschen C, Dimmeler S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, *et al*: Soluble CD40 ligand in acute coronary syndromes. New Engl J Med 348: 1104-1111, 2003.
27. Varo N, de Lemos JA, Libby P, Morrow DA, Murphy SA, Nuzzo R, *et al*: Soluble CD40L: risk prediction after acute coronary syndromes. Circulation 108: 1049-1052, 2003.