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Cordycepin (3´-deoxyadenosine) Has an Anti-platelet Effect by Regulating the cGMP-Associated Pathway of Human Platelet Activation

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Abstract

Cordycepin (3´-deoxyadenosine), which comes from *Cordyceps militaris*, the Chinese medicinal fungal genus *Cordyceps*, is used in the treatment of various diseases such as cancer and chronic inflammation. We recently reported that cordycepin has a novel antiplatelet effect through the down regulation of $[Ca^{2+}]_i$ and the elevation of cGMP/cAMP production. In this study, we further investigated the effect of cordycepin on collagen-induced platelet aggregation in the presence of cGMP-dependent protein kinase (PKG)- or cAMP-dependent protein kinase (PKA)-inhibitor. PKG inhibitor Rp-8-pCPT-cGMPS potentiated the collagen-induced platelet aggregation, but PKA inhibitor Rp-8-Br-cAMPS did not. However, both Rp-8-pCPT-cGMPS and Rp-8-Br-cAMPS reduced inhibition by cordycepin of collagen-induced platelet aggregation. Moreover, cordycepin inhibited Ca^{2+}-dependent phosphorylation of both 47 kDa- and 20 kDa-protein in the presence of both PKG inhibitor and PKA inhibitor. Taken altogether, these results suggest that the inhibitory effect of cordycepin on collagen-induced platelet aggregation is associated with cGMP/PKG- and cAMP/PKA-pathways, and thus cordycepin may be an efficacious intervention against platelet aggregation-mediated thrombotic disease.

Key words: cordycepin, PKG, PKA, antiplatelet activity

INTRODUCTION

Platelet aggregation is absolutely essential to the formation of a hemostatic plug when normal blood vessels are injured. However, the interaction between platelets and collagen can also cause circulatory disorders, such as thrombosis, atherosclerosis, and myocardial infraction (1). Inhibition of the platelet-collagen interaction could, therefore, be a promising approach in preventing thrombosis. An important role in the mechanism by which collagen induces platelet aggregation is mediated by thromboxane A_2 (TXA_2) formation (2), which also contributes to an increase in cytosolic free Ca^{2+} level ($[Ca^{2+}]_i$) in collagen-activated platelets. It is known that collagen and its-related peptide stimulation of platelets activate tyrosine kinase-dependent mechanisms that involve the tyrosine phosphorylation of Syk and phospholipase C-γ2 (PLC-γ2) (3). PLC-γ2 hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). Moreover, IP_3 causes Ca^{2+} mobilization from the endoplasmic reticulum and DG activates DG-dependent-protein kinase C (4). An increase in $[Ca^{2+}]_i$ activates both the Ca^{2+}/calmodulin-dependent phosphorylation of myosin light chain (20 kDa) and the DG-dependent phosphorylation of cytosolic protein (40 or 47 kDa) to induce platelet aggregation (5,6). In addition, DG also can be hydrolyzed by DG lipase to produce arachidonic acid (20:4), a precursor of TXA_2, which is a potent platelet aggregation agent generated from 20:4 liberated when PIP_2 is broken down by collagen, thrombin and ADP (5-7). Verapamil and theophylline have antiplatelet functions through modulation of adenosine 3´,5´-cyclic monophosphate (cAMP) levels, and then decreasing $[Ca^{2+}]_i$, an essential factor for platelet aggregation. Vasodilators (such as molsidomine and nitroprusside) and guanosin 3´,5´-cyclic monophosphate (cGMP) phosphodiesterase (PDE) inhibitors (such as zaprinast and erythro-9-[2-hydroxy-3-nonyl]adenine) elevate cGMP levels in platelets (7). It is believed that cGMP is produced *via* the activation of guanylate cyclase in the presence or absence of nitric oxide (NO). NO, synthesized in the platelets, decreases ago-

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nist-elevated $[Ca^{2+}]_i$ (8) and has a role in inhibiting the platelet activation (9).

Cordycepin (3′-deoxyadenosine) is a nucleoside derivative isolated from *Cordyceps militaris*, a species of the fungal genus *Cordyceps*, which is an ingredient of traditional Chinese medicine and is prescribed for inflammatory diseases and cancer (10,11). Cordycepin is indeed known to have anti-tumor effects on cancers of bladder, colon, lung, and fibrosarcoma (12) and to inhibit the production of inflammatory mediators (13). Although it has been recently reported that cordycepin has other biological functions (11,14-16), there are few reports on its anti-platelet effects. A cordycepin analogue, 2′,5′-dideoxyadenosine, does not inhibit platelet aggregation, and the production of cGMP or cAMP is not altered by this analogue during collagen-induced platelet aggregation (17). Cordycepin has also been reported to inhibit cAMP-dependent protein kinase (PKA) in bovine heart as well as cGMP-dependent protein kinase (PKG) in fetal guinea pig *in vitro* (18). In general, the inhibition of PKA or PKG is known to be closely related to the stimulation of platelet aggregation.

Therefore, in the present study we report that cordycepin participates in the regulation of PKG and PKA activity in collagen-induced human platelet aggregation.

MATERIALS AND METHODS

Materials

Cordycepin from *Cordyceps militaris* was purchased from the Sigma Chemical Co. (St. Louis, USA), and collagen was obtained from the Chrono-Log Corporation (Havertown, PA). Protein molecular weight standards were obtained from Bio-Rad Laboratories (Richmond, CA). Rp-isomer-8-(4-chlorophenylthio)-guanosine 3′, 5′-cyclic monophosphorothioate (Rp-8-pCPT-cGMPS), and Rp-isomer-8-bromo-adenosine 3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS) were purchased from Calbiochem (La Jolla, CA). Carrier-free phosphorus-32 for protein phosphorylation was obtained from Amersham Bioscience (Buckinghamshire, UK).

Preparation of washed human platelets

Blood was drawn from the antecubital vein of normal healthy human volunteers and anticoagulated with ACD solution (0.8% citric acid, 2.2% sodium citrate, 2.45% glucose). Platelet-rich plasma was centrifuged at 125× *g* for 10 min to remove red blood cells, and the platelets were washed twice with washing buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 5.5 mM glucose, and 1 mM EDTA, pH 6.5). The washed platelets were then suspended in suspension buffer (138 mM NaCl, 2.7 mM KCl, 12 mM $NaHCO_3$, 0.36 mM NaH_2PO_4, 0.49 mM $MgCl_2$, 5.5 mM glucose, 0.25% gelatin, pH 6.9) to a final concentration of 5×10^8/mL. All of the procedures above were carried out at 25°C to avoid platelet aggregation on cooling.

Measurement of platelet aggregation

Washed platelets (10^8/mL) were preincubated for 3 min at 37°C in the presence of $CaCl_2$, Rp-8-pCPT-cGMPS (PKG inhibitor), and Rp-8-Br-cAMPS (PKA inhibitor) with or without cordycepin, and then stimulated with 10 μg of collagen/mL for 5 min. Aggregation was monitored using an aggregometer (Chrono-Log, Corp., Havertown, PA) at a constant stirring of 1000 rpm. Each aggregation rate was evaluated as an increase in light transmission. The suspension buffer was used as reference (transmission 0%). Cordycepin was dissolved in platelet suspension buffer (pH 6.9).

Determination of protein phosphorylation

Protein phosphorylations were carried out according to the method of Laemmli (19). Washed platelets (10^8/mL) were suspended in Tris buffer (10 mM Tris-hydroxymethyl-aminomethane, 129 mM sodium chloride, 10.9 mM sodium citrate, tribasic, 8.9 mM sodium bicarbonate, 1 mg/mL dextrose, 2.8 mM potassium chloride, pH 6.5), and then incubated for 60 min at 37°C with phosphorus-32 (0.5 mCi/mL). [^{32}P]-labeled platelets (10^8/mL) were preincubated with or without cordycepin in the presence of 2 mM $CaCl_2$, PKG- and PKA-inhibitor at 37°C for 3 min, and then collagen (10 μg/mL) was added for 5 min to trigger protein kinase activation. Activation was terminated by the addition of an equal volume of sodium-dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) buffer (0.125 M Tris, 4% SDS, 20% glycerol, 5% mercaptoethanol, 0.01% bromophenol blue, pH 6.8). Samples were boiled to completely denature the proteins for 5 min, 50 μg proteins were taken from each reaction tube and subjected to SDS-PAGE (11%, 1.0 mm gel). The gels were then dried, and the relative intensities of the phosphoproteins were analyzed using a Storage Phospho System (Cyclone, A Packard Bioscience Company, USA).

Statistical analysis

All data are shown as means±SD. Student's *t*-test was used for data analysis and paired or unpaired comparisons were used as appropriate.

RESULTS AND DISCUSSION

Effects of cordycepin on collagen-induced platelet aggregation in the presence of cGMP-dependent protein

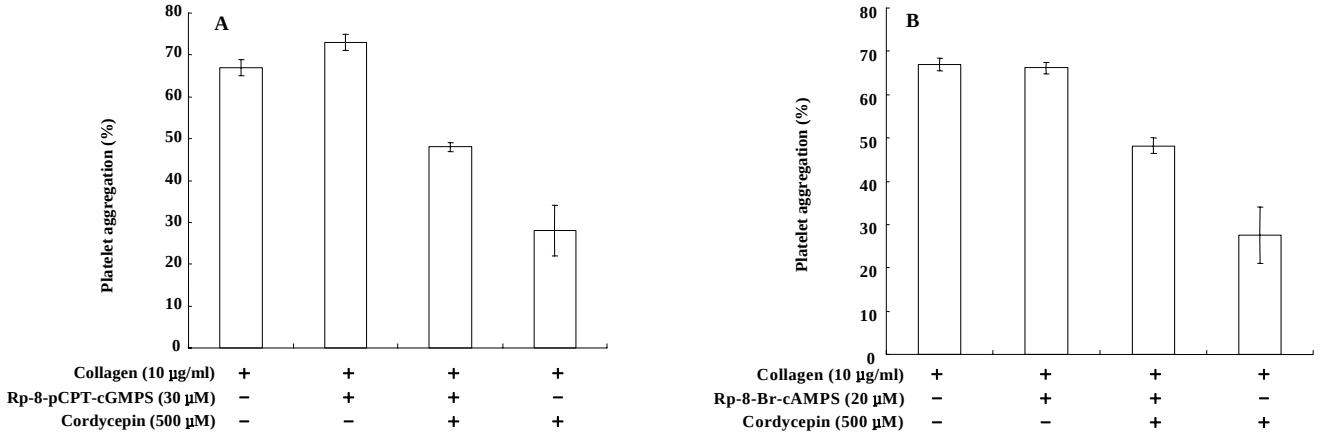

Fig. 1. Effect of cordycepin on collagen-induced platelet aggregation in the presence of PKG inhibitor Rp-8-pCPT-cGMPS and PKA inhibitor Rp-8-Br-cAMPS.
A. Effects of cordycepin on collagen-induced platelet aggregation in the presence of PKG inhibitor.
B. Effects of cordycepin on collagen-induced platelet aggregation in the presence of PKA inhibitor. Washed platelets (10^8/mL) were preincubated with or without cordycepin in the presence of PKG- or PKA-inhibitor, and $CaCl_2$ (2 mM) for 3 min at 37°C, and thus stimulated with collagen for 5 min. Platelet aggregation was recorded as increase in light transmission. Inhibition by cordycepin was recorded as percentage of collagen-induced platelet aggregation with or without PKG- or PKA-inhibitor. Rp-8-pCPTS-cGMPS and Rp-8-Br-cAMPS are dissolved in a platelet suspending buffer (pH 6.9). Data are expressed as means±SD (n=4).

Table 1. Inhibitory effects of cordycepin on collagen-induced platelet aggregation

	Aggregation (%)	Inhibition (%)
Collagen (10 μg/mL)	67±2	0
Cordycepin (500 μM) + collagen (10 μg/mL)	28±6	58

Table 2. Effect of cordycepin on collagen-induced platelet aggregation with Rp-8-pCPT-cGMPS, cGMP-dependent protein kinase inhibitor

	Aggregation (%)	Inhibition (%)
Rp-8-pCPT-cGMPS (30 μM) + collagen (10 μg/mL)	73±2	0
Rp-8-pCPT-cGMPS (30 μM) + cordycepin (500 μM) + collagen (10 μg/mL)	48±1	34

kinase (PKG) inhibitor Rp-8-pCPT-cGMPS or cAMP-dependent protein kinase (PKA) inhibitor Rp-8-Br-cAMPS

The concentration of collagen that induced maximal platelet aggregation was approximately 10 μg/mL (20). Therefore 10 μg of collagen/mL was used as a platelet agonist in this study. The half-maximal inhibitory concentration (IC_{50}) of cordycepin was 500 μM on collagen-induced platelet aggregation (21). We previously reported that the inhibitory effect of cordycepin on platelet aggregation is associated with the elevation of cGMP- and cAMP-production (21,22). Activation of cGMP-dependent protein kinase (PKG) and cAMP-dependent protein kinase (PKA) by cAMP and cGMP are regarded as negative regulatory events in platelet aggregation. Because the inhibition of PKG or PKA induces the platelet aggregation, we investigated the effect of cordycepin in the presence of PKG- or PKA-inhibitor on collagen-induced platelet aggregation. As shown in Fig. 1A and Table 1, collagen induced platelet aggregation up to 67±2% (Fig. 1A). However, this aggregation was increased by 73±2% in the presence of Rp-8-pCPT-cGMPS (30 μM) (Fig. 1A and Table 2), a PKG inhibitor. Considering that Rp-8-pCPT-cGMPS antagonized the activation of PKG (23), this result suggests that Rp-8-pCPT-cGMPS could be involved in either the decrease of cGMP level or the inhibition of cGMP/PKG action in collagen-induced platelet aggregation, and as a result, collagen-induced platelet aggregation (67±2%) was increased by 73±2% in the presence of Rp-8-pCPT-cGMPS, a PKG inhibitor (Fig. 1A, Table 1 and 2). Cordycepin (500 μM) inhibited collagen (10 μg/mL) -induced platelet aggregation by 58% (Fig. 1A and Table 1) (21), while its inhibition rate was decreased by 34% in the presence of Rp-8-pCPT-cGMPS (Fig. 1A and Table 2). These results suggested that cordycepin might participate in the activation of the cGMP/PKG pathway since it is reported that the inhibitory mode of platelet aggregation by cGMP is mediated *via* PKG activation (21). Unlike Rp-8-pCPT-cGMPS, a PKA inhibitor Rp-8-Br-cAMPS did not increase collagen-induced platelet ag-

Table 3. Effect of cordycepin on collagen-induced platelet aggregation with Rp-8-Br-cAMPS, cAMP-dependent protein kinase inhibitor

	Aggregation (%)	Inhibition (%)
Rp-8-Br-cAMPS (20 μM) + collagen (10 μg/mL)	66±1	0
Rp-8-Br-cAMPS (20 μM) + cordycepin (500 μM) + collagen (10 μg/mL)	48±2	27

gregation (Fig. 1B and Table 3). However, cordycepin (500 μM) inhibited collagen-induced platelet aggregation by 27% in the presence of Rp-8-Br-cAMPS (Fig. 1B and Table 3). The inhibitory degree (27%) is lower than that (58%) by cordycepin alone (Table 1). These results suggest that Rp-8-Br-cAMPS-induced inhibition of PKA may be involved in modulating the pharmacology of cordycepin such as elevating cAMP level and consequent activation of PKA as reported previously (21,24).

Effects of cordycepin on collagen-phosphorylated 20 kDa and 47 kDa in the presence of cGMP-dependent protein kinase (PKG) inhibitor Rp-8-pCPT-cGMPS or cAMP-dependent protein kinase (PKA) inhibitor Rp-8-Br-cAMPS

It is reported that the platelet PKG-I isoform of PKG phosphorylates the TXA_2 receptor, preventing the coupling of the receptor to its cognate GTP-binding protein G_q, and consequently inhibiting activation of the effector, phospholipase C and prevention of $[Ca^{2+}]_i$-mobilization (25). Indeed, lack of PKG-I enhances the aggregation of platelets (26). In addition, these previous reports suggest that PKG-I as intracellular receptor of cGMP might participate in the inhibition of 20 kDa (myosin light chain) and 47 kDa (pleckstrin)-phosphorylation. We have reported that the inhibitory effect of cordycepin on platelet collagen-induced platelet aggregation is associated with a decrease in $[Ca^{2+}]_i$, the elevation of cGMP/cAMP production, and the inhibition of 47 kDa- or 20 kDa-phosphorylation (21). However, it is unknown whether the inhibition of Ca^{2+}-dependent phosphoproteins (47 kDa and 20 kDa) by cordycepin is mediated by the activation of PKG or PKA by cGMP/cAMP. Therefore we investigated the effect of cordycepin on these phosphoproteins by using PKG- and PKA-inhibitors.

When [^{32}Pi]-labeled platelets (10^8/mL) were incubated with collagen (10 μg/mL) for 5 min at 37°C with or without PKG inhibitor, Rp-8-pCPT-cGMPS (30 μM), the 47 kDa- and 20 kDa-polypeptides, were markedly found to be phosphorylated (Fig. 2A, lanes 2 and 4), up to 40～60%, as compared to intact level (Fig. 2A, lane 1, Fig. 2B and 2C). However, cordycepin (500 μM) inhibited collagen-induced phosphorylations of 47 kDa- and 20 kDa-polypeptides with or without Rp-8-pCPT-cGMPS (Fig. 2A, lanes 3 and 5), and then reversed up to control level (100%) (Fig. 2B and 2C). And also, 47 kDa- and 20 kDa-polypeptides were markedly phosphorylated by collagen in the presence of PKA inhibitor Rp-8-Br-cAMPS (Fig. 3A, lanes 2 and 4), which were increased up to 160～200% as compared with (100%) intact platelets (Fig. 3A, lane 1, Fig. 3B and 3C). However, cordycepin potently inhibited collagen-induced phosphorylations of 47 kDa- and 20 kDa-polypeptide with or without Rp-8-Br-cAMPS (Fig. 3A, lanes 3 and 5), and then returned up to control level (100%) (Fig. 3B and 3C). It is especially important that cordycepin inhibited these phosphoproteins in the presence of PKG- and PKA-inhibitor, which is in accordance with the cordycepin inhibition of collagen-induced platelet aggregation in the presence of PKG- and PKA-inhibitor (Fig. 1A and 1B). PKG and PKA are known to inhibit phospholipase C, involved in generating DG and IP_3 from PIP_2 in various agonist-induced platelet activations (27). These previous reports mean that PKG and PKA inhibit the activity of DG receptor C-kinase, and thus could inhibit the phosphorylation of 47 kDa by C-kinase. In particular, PKA is known to inhibit the phosphorylation of 20 kDa by suppressing the activity of a Ca^{2+} receptor, CaM-PK (27,28). Accordingly the inhibitory effect of cordycepin on the phosphorylation of 20 kDa- and 47 kDa- by PKG/PKA-inhibitor (Fig. 2A, lanes 3 and 5, Fig. 3A, lanes 3 and 5) seems to suggest that cordycepin could augment the inhibitory effect of C-kinase or CaM-PK by PKG and PKA. Cordycepin inhibited the phosphorylation of 20 kDa- and 47 kDa-polypeptides by suppressing $[Ca^{2+}]_i$ levels which were elevated in TXA_2 analogue U46619-activated platelets (29). If PKG-I phosphorylates TXA_2 receptor to inhibit TXA_2-induced platelet aggregation (11), it is believed that cordycepin would inhibit these phosphoproteins *via* the inhibitory action of $[Ca^{2+}]_i$ (29) by cGMP/PKG-I (Fig. 2A, lanes 3 and 5). It is known that the occurrence of thrombosis is mainly due to the irreversible aggregations, which is intimately related with serotonin release by 20 kDa phosphorylation (30). The release of serotonin by platelets is inhibited by cGMP-elevation during platelet aggregation (30). Cordycepin potently inhibited collagen-induced 20 kDa phosphorylation (Fig. 2, lanes 3 and 5, Fig. 3, lanes 3 and 5, Fig. 2C, Fig. 3C). Accordingly, these results also suggest that cordycepin might inhibit serotonin release from platelets, and thus might have an-

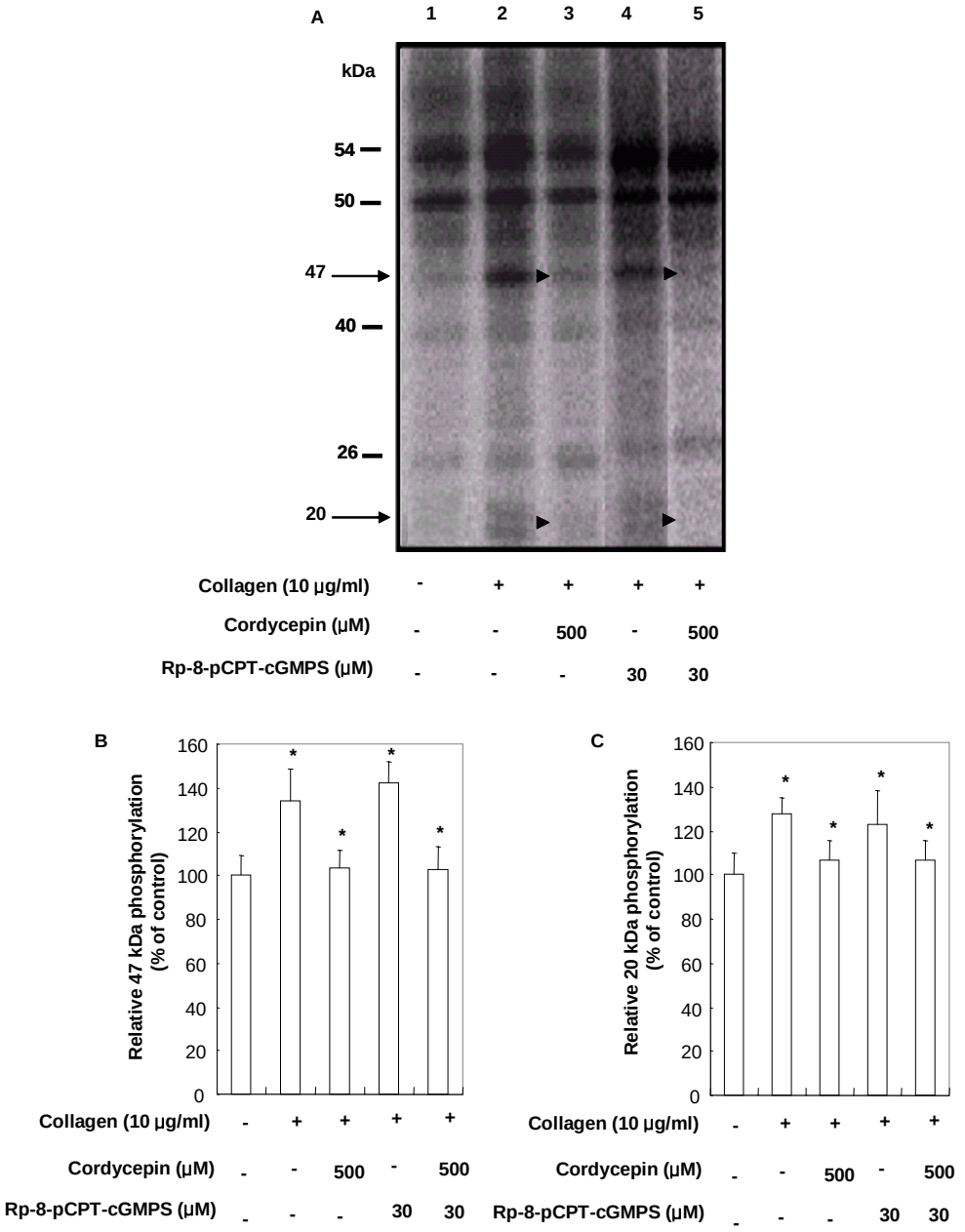

Fig. 2. Effects of cordycepin on protein phosphorylation in the presence of PKG inhibitor Rp-8-pCPT-cGMPS.
A. The pattern of protein phosphorylation. B and C. The inhibition of 20 kDa- and 40 kDa-phosphorylation by cordycepin. Protein phosphorylations were performed as described in Materials and Methods. [^{32}P]-labeled platelets (10^8/mL) were pre-incubated with or without cordycepin (500 μM) for 3 min in the presence of $CaCl_2$ (2 mM) or Rp-8-pCPT-cGMPS (30 μM), and then the platelets were stimulated with collagen (10 μg/mL). Lane 1, intact platelets as control; lane 2, collagen (10 μg/mL); lane 3, cordycepin (500 μM) + collagen (10 μg/mL); lane 4, Rp-8-pCPT-cGMPS (30 μM) + collagen (10 μg/mL); lane 5, Rp-8-pCPT-cGMPS (30 μM) + cordycepin (500 μM) + collagen (10 μg/mL). Data are expressed as means±SD (n = 4). $^*p<0.05$ vs collagen or collagen + Rp-8-pCPT-cGMPS.

tithrombotic effect. In another experiments, we reported that hypha-water extracts (HWE) and fruit body-water extracts (FEW) from *Cordyceps militaris* contain 166 μg and 78 μg of cordycepin/mg-powder, respectively (20). HWE and FWE inhibited the release of [H^3]-serotonin from human platelets activated by thrombin or collagen in a does-dependent manner (20). In conclusion, we found that cordycepin inhibits collagen-induced platelet aggregation *via* the cGMP/PKG pathway. In addition, it is thought that cordycepin also has an important functionality in the inhibition of platelet aggregation in addition to its other biological activities such as antitumor and anti inflammatory effects (10,11).

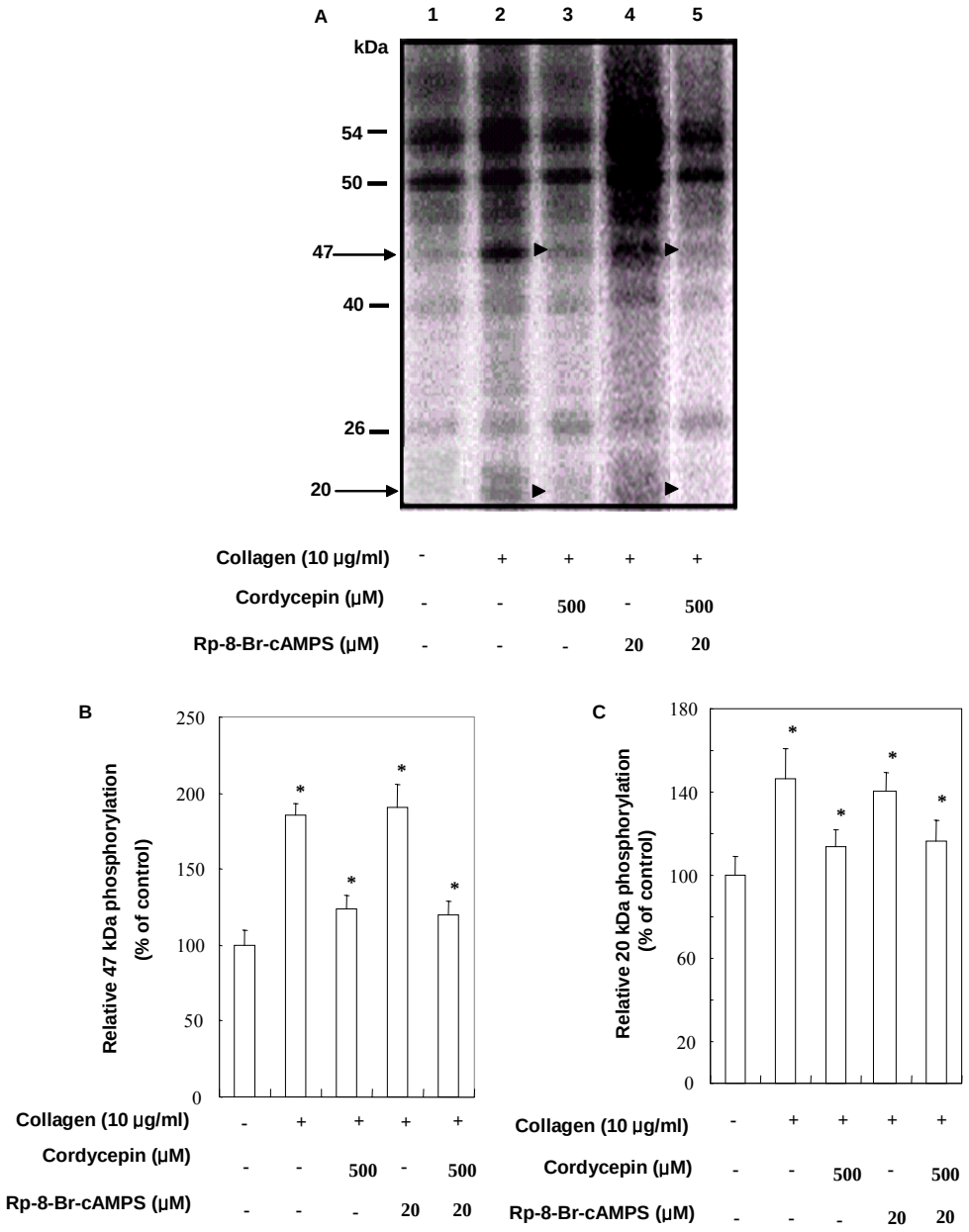

Fig. 3. Effects of cordycepin on protein phosphorylation in the presence of PKA inhibitor Rp-8-Br-cAMPS.
A. The pattern of protein phosphorylation. B and C. The inhibition of 20 kDa- and 40 kDa-phosphorylation by cordycepin. Protein phosphorylation processes were performed as described in Materials and Methods. [^{32}P]-labeled platelets (10^8/mL) were preincubated with or without cordycepin (500 μM) for 3 min in the presence of $CaCl_2$ (2 mM) or Rp-8-Br-cAMPS (30 μM), and then the platelets were stimulated with collagen (10 μg/mL). Lane 1, intact platelets as control; lane 2, collagen (10 μg/mL); lane 3, cordycepin (500 μM) + collagen (10 μg/mL); lane 4, Rp-8-Br-cAMPS (30 μM) + collagen (10 μg/mL); lane 5, Rp-8-Br-cAMPS (30 μM) + cordycepin (500 μM) + collagen (10 μg/mL). Data are expressed as means±SD (n = 4). $^*p<0.05$ vs collagen or collagen + Rp-8-Br-cAMPS.

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REFERENCES

1. Schwartz SM, Heinmark RL, Majesky MW. 1990. Developmental mechanisms underlying pathology of arteries. *Physiol Rev* 70: 1177-1209.
2. Cattaneo M, Tenconi PM, Lecchi A, Mannucci PM. 1991. In vitro effects of picotamide on human platelet ag-

gregation, the release reaction and thromboxane B_2 production. *Thromb Res* 62: 717-724.
3. Wonerow P, Obergfell A, Wilde JI, Bobe R, Asazuma N, Brdicka T, Leo A, Schraven B, Horejsi V, Shattil SJ, Watson SP. 2002. Differential role of glycolipid-enriched membrane domains in glycoprotein VI- and integrin-mediated phospholipase C gamma 2 regulation in platelets. *Biochem J* 364: 755-765.
4. Berridge MJ, Irvine RF. 1989. Inositol phosphates and cell signalling. *Nature* 341: 197-205.
5. Kaibuchi K, Sano K, Hoshijima M, Takai Y, Nishizuka Y. 1982. Phosphatidylinositol turnover in platelet activation; calcium mobilization and protein phosphorylation. *Cell Calcium* 3: 323-335.
6. Nishikawa M, Tanaka T, Hidaka H. 1980. Ca^{2+}-calmodulin-dependent phosphorylation and platelet secretion. *Nature* 287: 863-865.
7. Menshikov MYu, Ivanova K, Schaefer M, Drummer C, Gerzer R. 1993. Influence of the cGMP analog 8-PCPT-cGMP on agonist-induced increases in cytosolic ionized Ca^{2+} and on aggregation of human platelets. *Eur J Pharmacol* 245: 281-284.
8. Pasqui AL, Capecci PL, Ceccatelli L, Mazza S, Gistri A, Laghi Pasini F, Di Perry T. 1991. Nitroprusside in vitro inhibits platelet aggregation and intracellular calcium translocation. Effect of haemoglobin. *Thromb Res* 61: 113-122.
9. Rodomski MW, Palmer RMJ, Monacade S. 1990. An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* 87: 5193-5197.
10. Cuningham KG, Hutchinson SA, Manson W, Spring FS. 1951. Cordycepin, a metabolic product from culture of ordyceps militaris (Linn) Link. Part Ⅰ. Isolation and characterization. *J Chem Soc* 2: 2299-2302.
11. Ng TB, Wang HX. 2005. Pharmacological actions of *Cordyceps,* a prized folk medicine. *J Pharm Pharmacol* 57: 1509-1519.
12. Hubbell HR, Pequignot EC, Wllis DH, Lee C, Suhadolnik RJ. 1985. Differential antiproliferative actons of 2', 5'-oligo A trimer core and its cordycepin analogue on human tumor cells. *Int J Cancer* 36: 389-394.
13. Won SY, Park EH. 2005. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of *Cordyceps militaris*. *J Ethnopharmacol* 96: 555-561.
14. Son DJ, Kim YM, Chung NH, Lee HS. 2005. Antiplatelet activity of *Thujopsis dolabrata* var. hondai-derived component against platelet aggregation. *J Microbiol Biotechnol* 15: 425-427.
15. Yang BK, Ha JY, Jong SC, Das S, Yun JW, Lee YS, Choi JW, Song CH. 2000. Production of exopolymers by submerged mycelial culture of *Cordyceps militaris* and its hypolipidemic effect. *J Microbiol Biotechnol* 10: 784-788.
16. Yu KW, Suh HJ, Bae SH, Lee CS, Kim SH, Yoon CS. 2001. Chemical properties and physiological activities of stromata of *Cordyceps militaris*. *J Microbiol Biotechnol* 11: 266-274.
17. Jang EK, Azzam JE, Dickinson NT, Davidson MM, Haslam RJ. 2002. Roles for both cyclic GMP and cyclic AMP in the inhibition of collagen-induced platelet aggregation by nitroprusside. *Br J Haematol* 117: 664-675.
18. Glazer RI, Kuo JF. 1977. Inhibitory effects of cordycepin on cyclic nucleotide-dependent and cyclic nucleotide-independent protein kinase. *Biochem Pharmacol* 26: 1287-1290.
19. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
20. Cho HJ, Ham HS, Lee TK, Jung YJ, Choi SA, Kang HC, Park HJ. 2004. Inhibitory effect of cordycepin on human platelet aggregation. *J Biomed Lab Sci* 10: 1-8.
21. Cho HJ, Cho JY, Rhee MH, Park HJ. 2007a. Cordycepin (3'-deoxyadenosine) inhibits human platelets aggregation in a cyclic AMP- and cyclic GMP-dependent manner. *Eur J Pharmacol* 558: 43-51.
22. Cho HJ, Cho JY, Rhee MH, Kim HS, Lee HS, Park HJ. 2007b. Inhibitory effects of platelet aggregation indyced by thapsigargin. *J Microbiol Biotechnol* 17: 1334-1138.
23. Butt E, Eigenthaler M, Genieser HG. 1994. Rp-8-pCPT, a novel cGMP-dependent protein kinase inhibitor. *Eur J pharmacol* 269: 265-268.
24. Li Z, Ajdic J, Eigenthaler M, Du X. 2003. A predominant role for cAMP-dependent protein kinase in the cGMP-induced phosphorylation of vasodilator-stimulated phosphoprotein and platelet inhibition in humans. *Blood* 101: 4423-4429.
25. Yuasa K, Michibata H, Omori K, Yanaka N. 2000. Identification of a conserved residue responsible for the autoinhibition of cGMP-dependent protein kinase Iα and β. *FEBS Letters* 466: 175-178.
26. Massberg S, Sausbier M, Klatt P, Bauer M, Pfeifer A, Siess W, Fässler R, Ruth P, Krombach F, Hofmann F. 1999. Increased adhension and aggregation of platelets lacking cyclic guanosine 3',5'-monophosphate kinase I. *J Exp Med* 189: 1255-1263.
27. Schwarz UR, Walter U, Eigenthaler M. 2001. Taming platelets with cyclic nucleotides. *Biochem Pharmacy* 62: 1153-1161.
28. Münzel T, Feil R, Mülsch A, Lohmann SM, Hofmann F, Walter U. 2003. Physiology and pathophysiology of vascular signaling controlled by cyclic guanosine 3',5'-cyclic monophosphate-dependent protein kinase. *Circulation* 108: 2172-2183.
29. Cho HJ, Cho JY, Rhee MH, Lim CR, Park HJ. 2006. Cordycepin (3'-deoxyadenosine) inhibits human platelet aggregation induced by U46619, a TXA_2 analogue. *J Pharm Pharmacol* 58: 1677-1682.
30. Charo IF, Feinman RD, Detwiler TC. 1977. Interrelations of platelet aggregation and secretion. *J Clin Invest* 60: 866-873.

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