Inhibitory Effects of Cordycepin on Platelet Activation via Regulation of Cyclic Adenosine Monophosphate-downstream Pathway

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Platelet activation is essential at the sites of vascular injury, which leads to hemostasis through adhesion, aggregation, and secretion process. However, potent and continuous platelet activation may be an important reason of circulatory disorders. Therefore, proper regulation of platelet activation may be an effective treatment for vascular diseases. In this research, inhibitory effects of cordycepin (3’-deoxyadenosine) on platelet activation were determined. As the results, cordycepin increased cAMP and cGMP, which are intracellular Ca\(^{2+}\)-antagonists. In addition, cordycepin reduced collagen-elevated [Ca\(^{2+}\)]\(_i\) mobilization, which was increased by a cAMP-dependent protein kinase (PKA) inhibitor (Rp-8-Br-cAMPS), but not a cGMP-protein kinase (PKG) inhibitor (Rp-8-Br-cGMPS). Furthermore, cordycepin increased IP\(_{3}\)RI (Ser\(^{1756}\)) phosphorylation, indicating inhibition of IP\(_3\)-mediated Ca\(^{2+}\) release from internal store via the IP\(_3\)RI, which was strongly inhibited by Rp-8-Br-cAMPS, but was not so much inhibited by Rp-8-Br-cGMPS. These results suggest that the reduction of [Ca\(^{2+}\)]\(_i\) mobilization is caused by the cAMP/A-kinase-dependent IP\(_3\)RI (Ser\(^{1756}\)) phosphorylation. In addition, cordycepin increased the phosphorylation of VASP (Ser\(^{157}\)) known as PKA substrate, but not VASP (Ser\(^{239}\)) known as PKG substrate. Cordycepin-induced VASP (Ser\(^{157}\)) phosphorylation was inhibited by Rp-8-Br-cAMPS, but was not inhibited by Rp-8-Br-cGMPS, and cordycepin inhibited collagen-induced fibrinogen binding to αIIbβ\(_3\), which was increased by Rp-8-Br-cAMPS, but was not inhibited by Rp-8-Br-cGMPS. These results suggest that the inhibition of αIIbβ\(_3\) activation is caused by the cAMP/A-kinase-dependent VASP (Ser\(^{157}\)) phosphorylation. In conclusion, these results demonstrate that inhibitory effects of cordycepin on platelet activation were due to inhibition of [Ca\(^{2+}\)]\(_i\) mobilization through cAMP-dependent IP\(_3\)RI (Ser\(^{1756}\)) phosphorylation and suppression of αIIbβ\(_3\) activation through cAMP-dependent VASP (Ser\(^{157}\)) phosphorylation. These results strongly indicated that cordycepin might have therapeutic or preventive potential for platelet activation-mediated disorders including thrombosis, atherosclerosis, myocardial infarction, or cardiovascular disease.

Key Words: Cordycepin, Ca\(^{2+}\) mobilization, cAMP, Inositol trisphosphate receptor, VASP, Fibrinogen binding

INTRODUCTION

Platelets are activated by several molecules (i.e., collagen, ADP and thrombin) at the sites of vascular injury, which is essential hemostatic process. However, it can also lead to circulatory disorders, including thrombosis, atherosclerosis, or myocardial infarction (Schwartz et al., 1990). Therefore, inhibition of platelet activation might be an effective approach to prevent vascular diseases. When collagen is released from the vascular endothelial cell in vascular injury, it binds to the receptor glycoprotein VI (GP VI), and then
platelets activate by tyrosine kinase-dependent mechanisms, which is related to phospholipase C-γ2 (PLC-γ2) phosphorylation (Wonerow et al., 2002). The phosphorylated PLC-γ2 produces diacylglycerol (DG) and inositol 1, 4, 5-trisphosphate (IP$_3$) hydrolyzing phosphatidylinositol 4, 5-bisphosphate (PIP$_2$). IP$_3$ mobilizes cytosol free Ca$^{2+}$ ([Ca$^{2+}$]$_i$) from dense tubular system through IP$_3$ receptor type I (IP$_3$RI), which works as calcium ion channel (Quinton et al., 1992). The increased [Ca$^{2+}$], phosphorylates myosin light chain (MLC) via activation of Ca$^{2+}$/calmodulin-dependent protein kinase, which induces platelet aggregation and granule secretion (Nishikawa et al., 1980; Kaibuchi et al., 1982). On the other hand, cyclic adenosine monophosphate (cAMP) or cyclic guanosine monophosphate (cGMP) has antiplatelet effects by decreasing the [Ca$^{2+}$]$_i$ mobilization (Menshikov et al., 1993; Schwarz et al., 2001). The cAMP or cGMP activate cAMP- or cGMP-dependent protein kinases (PKA orPKG), which phosphorylate substrate proteins, IP$_3$RI and vasodilatator-stimulated phosphoprotein (VASP) (Halbrügge et al., 1990; Butt et al., 1994). IP$_3$RI (Ser$^{1796}$) phosphorylation results in inhibition of [Ca$^{2+}$]$_i$ mobilization (Quinton et al., 1992; Schwarz et al., 2001), and VASP (Ser$^{157}$ and Ser$^{239}$) phosphorylation leads to inhibition of fibrinogen binding to glycoprotein IIb/IIIa (αIIb/β$_3$), so that they consequentially inhibit platelet activation (Laurent et al., 1999; Sudo et al., 2003). Therefore, regulation of these cascades is good factor for evaluating antiplatelet effects of any compound or substance.

A species of Cordyceps known as ingredient of Chinese traditional medicine is prescribed for inflammatory and cancer diseases, and its major component is cordycepin (3'-deoxyadenosine). In previous report, cordycepin (3'-deoxyadenosine, Fig. 1) from Cordyceps militaris has antiplatelet effects in cAMP- and cGMP-dependent manner, which is involved in down-regulation of [Ca$^{2+}$]$_i$ and TXA$_2$ (Cho et al., 2007). However, its pathway remains uncertain and it is unknown how cordycepin regulates cAMP- or cGMP downstream pathway (i.e. phosphorylation of IP$_3$RI and VASP). In this study, we investigated how cordycepin regulates cAMP- or cGMP downstream pathway to inhibit platelets activation. These provide novel information and possibility of cordycepin as an antiplatelet drug.

**MATERIALS AND METHODS**

**Materials**

Cordycepin, Fura 2-AM, and other reagents were purchased from Sigma Chemical Corporation (St. Louis, MO., USA). Collagen was purchased from Chrono-Log Co. (Havertown, PA., USA). Lysis buffer and Antibodies for western blotting were purchased from Cell Signaling Co. (Beverly, MA., USA). Polyvinylidene difluoride (PVDF) membrane and enhanced chemiluminescence (ECL) solution were obtained from GE Healthcare (Chalfont St., Giles, Buckinghamshire, UK), and Fibrinogen Alexa Fluor 488 conjugate was purchased from Invitrogen Molecular Probes (Eugene, OR., USA).

**Preparation of washed platelets**

Human platelet-rich plasma (PRP) treated by ACD anticoagulant (2.2% sodium citrate, 0.8% citric acid, 2.45% dextrose) was offered from Korean Red-Cross Blood Center (Changwon, Korea). PRP was centrifuged at 1,300 g for 10 min to obtain platelets removing a little red blood cell, and the platelets were washed twice with washing buffer (138 mM NaCl, 12 mM NaHCO$_3$, 5.5 mM glucose, 2.7 mM KCl, 1 mM EDTA, and 0.36 mM Na$_2$PO$_4$, pH 6.5). The washed
platelets were suspended in suspending buffer (138 mM NaCl, 12 mM NaHCO₃, 5.5 mM glucose, 2.7 mM KCl, 0.49 mM MgCl₂, 0.36 mM NaH₂PO₄, and 0.25% gelatin, pH 6.9) to 5×10⁸/mL of final concentration. All these procedures were carried out avoiding platelet aggregation at 25°C.

Measurement of cAMP and cGMP level

Washed platelets (10⁹/mL) were pre-incubated with the compound in 2 mM CaCl₂ at 37°C for 3 min, and stimulated by 10 μg/mL collagen at 37°C for 5 min. The reaction was terminated with 80% ice-cold ethanol, and cAMP and cGMP were determined by cAMP and cGMP EIA kits using synergy HT multi-model microplate reader.

Measurement of cytosolic-free Ca²⁺ mobilization

Fura 2-loaded platelets were prepared by incubating PRP in the presence of 5 μM Fura 2-AM at 37°C for 60 min, and were pre-incubated with the compound in 2 mM CaCl₂ at 37°C for 3 min, and stimulated with 10 μg/mL collagen at 37°C for 5 min to determine cytosolic-free Ca²⁺ ([Ca²⁺]ᵢ) mobilization. Fura 2 fluorescence was determined by a spectrofluorometer (SFM 25; Bio-Teck Instrument, Italy) in the excitation wavelength changing each 0.5 second from 340 to 380 nm and the emission wavelength at 510 nm. The [Ca²⁺]ᵢ were calculated by the method of Schaeffer (Schaeffer et al., 1989).

Western blot for analysis of IP₃RI-, VASP-phosphorylations

Washed platelets (10⁹/mL) were pre-incubated with the compound in 2 mM CaCl₂ for 3 min, and stimulated with 10 μg/mL collagen at 37°C for 5 min. The reactions were terminated with equal volume (250 μL) of lysis buffer (Cayman Chemical Co., MI., USA). Platelet lysates containing 15 μg protein were used for analysis. The effects of cordycepin on IP₃RI-, VASP-phosphorylation were analyzed by western blotting. 6~8% SDS-PAGE were used for electrophoresis, and PVDF membrane was used for gel transfer. The membranes were visualized with ECL, which was analyzed by the Quantity One, Ver. 4.5 (Hercules, CA., USA).

Measurement of fibrinogen binding to platelets

Washed platelets (10⁹/mL) were pre-incubated with the compound in 2 mM CaCl₂ for 3 min, and stimulated with 10 μg/mL collagen in the presence of 30 μg/mL Alexa Flour 488-human fibrinogen at room temperature for 5 min. The reaction was terminated with 0.5% paraformaldehyde in phosphate-buffered saline, which were placed in the dark. Alexa Fluor 488-fibrinogen binding to platelets was measured using flow cytometry (San Jose, CA., USA) and the data are analyzed by cellQuest software.

Statistical analyses

These data were expressed as the mean ± SEM accompanied with the number of observations. These data were assessed through analysis of variance (ANOVA). If the analysis indicated significant differences among the control group means, the each group was compared through the Newman-Keuls method. P<0.05 was considered to have statistically significant mean.

RESULTS & DISCUSSION

We examined whether cordycepin was concerned with elevation of cAMP or cGMP in collagen-stimulated platelet activation. As the result, collagen reduced intracellular cAMP production to 46.2% as compared with basal level (Table 1, Fig. 2A). However, when platelets were incubated in the presence of both cordycepin and collagen, the level of cAMP was increased to 8.3 ± 0.7 pmoL/10⁹ platelets (Table 1, Fig. 2A), which increased collagen-decreased cAMP level to 196.4% (Table 1, Fig. 2A). On the contrary, collagen reduced intracellular cGMP production to 25.0% as compared with basal level (Table 1, Fig. 2B). However, when platelets were incubated in the presence of both cordycepin and collagen, the level of cGMP was increased to 8.3 ± 0.7 pmoL/10⁹ platelets (Table 1, Fig. 2A), which increased collagen-decreased cGMP level to 196.4% (Table 1, Fig. 2A). On the contrary, collagen reduced intracellular cGMP production to 25.0% as compared with basal level (Table 1, Fig. 2B). However, when platelets were incubated in the presence of both cordycepin and collagen, the cGMP level was increased to 46.7% as compared with the result (3.0 ± 0.4 pmoL/10⁹ platelets) by collagen only (Table 1, Fig. 2B). The intracellular cAMP and cGMP levels are regulated by balance between cyclic nucleotide-producing enzymes such as adenylate/guanylate cyclase, and hydrolyzing enzymes such as cAMP/cGMP phosphodiesterases (PDEs). It is known that platelets have PDE₂, PDE₃, and
PDE₄ (Schwarz et al., 2001; Walter et al., 2009). PDE₂ hydrolyzes both cAMP and cGMP as cGMP-stimulated PDE, PDE₃ hydrolyzes cAMP rather than cGMP as cGMP-inhibited PDE, and PDE₅ hydrolyzes cGMP only as cGMP-binding-cGMP specific PDE. Because cordycepin increased both cAMP and cGMP in collagen-induced platelet aggregation, although it is thought that cordycepin might involve inhibition of PDE₂ to increase both cAMP and cGMP, we cannot exclude that cordycepin might involve activation of adenylate cyclase to produce cAMP from ATP, because cordycepin-elevated cAMP level was decreased by adenylate cyclase inhibitor SQ22536 (Cho et al., 2007). Nieswandt group provided an evidence for cross-talk between collagen receptor GPVI and Gᵢ-coupled receptor during collagen-induced platelet aggregation (Nieswandt et al., 2001). If so, there is a possibility that collagen-reduced cAMP was medi-

|                  | cAMP | cGMP | cAMP/cGMP |
|------------------|------|------|-----------|
|                  | pmol/10⁶ platelets | Change (%) | pmol/10⁶ platelets | Change (%) | Ratio | Change (%) |
| Base             | 5.2 ± 0.4         | -     | 4.0 ± 0.3   | -         | 1.3   | -          |
| Collagen (10 μg/mL) | 2.8 ± 0.5³   | - 46.2¹) | 3.0 ± 0.4³  | - 25.0²)  | 0.9   | - 30.7³)   |
| Collagen (10 μg/mL) + cordycepin (500 μM) | 8.3 ± 0.7*³ | + 196.4⁴) | 4.4 ± 0.6*³ | + 46.7⁵) | 1.9   | + 111.1⁵) |

Data are from Fig. 2A, B. 1) to 3) Changes to base; 4) to 6) Changes to collagen 1) and 2) of inhibition (%) = (Base - Collagen)/Base × 100 4) and 5) of inhibition (%) = [(Collagen + cordycepin) - Collagen]/Collagen × 100 3) ratio decrease (%) = (Base - Collagen)/Base × 100 6) ratio increase (%) = [(Collagen + cordycepin) - Collagen]/Collagen × 100 These data were expressed as the mean ± SEM (n = 4). *P<0.05 compared with base, **P<0.001 compared with the collagen-stimulated platelets.

Fig. 2. Effect of cordycepin on cAMP and cGMP production. (A) Effect of cordycepin on cAMP production in collagen-induced platelets. (B) Effect of cordycepin on cGMP production in collagen-induced platelets. The level of cAMP and cGMP was examined as described in "Methods". These data were expressed as the mean ± S.E.M. (n = 4). *P<0.05 compared with the non-stimulated platelets, **P<0.05, ***P<0.001 compared with the collagen-stimulated platelets.
activated via Gs-coupled receptor, and by contrast, the elevation of cAMP level achieved by cordycepin was induced by Gs-coupled receptor through adenylate cyclase activation. This is supported from report (Johnston-Cox et al., 2011) that adenosine and the analogues increase cAMP level via adenosine receptor A2A, which are coupled to Gs-coupled receptor. Cordycepin is an analogue of adenosine as 3’-deoxyadenosine. cGMP is known to be produced via the activation of guanylate cyclase in the presence or absence of nitric oxide (NO) synthesized in platelets (Pasqui et al., 1991). Because cordycepin did not produce NO in platelets (Cho et al., 2007), the elevation of cGMP achieved by cordycepin is independent on NO/guanylate cyclase pathway.

In addition, collagen-elevated [Ca2+]i was potently decreased by 500 μM of cordycepin, and PKA activator pCPT-cAMP or PKG activator 8-Br-cGMP reduced collagen-elevated [Ca2+]i (Fig. 3A). Because [Ca2+]i level could be reduced through cAMP/PKA or cGMP/PKG downstream pathway, next, we investigated whether cordycepin is concerned with cAMP/PKA or cGMP/PKG downstream pathway to decrease [Ca2+]i level. As shown in Fig. 3B, [Ca2+]i level in the presence of both collagen and cordycepin was 217.2 ± 3.6 nM, which was significantly elevated by PKA inhibitor Rp-8-Br-cAMPS to 283.9 ± 8.2 nM (Fig. 3B). On the other hand, the [Ca2+]i level in the presence of both collagen and cordycepin was not elevated by PKG inhibitor Rp-8-Br-cGMPS (Fig. 3B). Because [Ca2+]i reduction could be resulted from cAMP/PKA-, or cGMP/PKG-phosphorylated IP3RI (Ser1756), next we checked whether cordycepin induces IP3RI (Ser1756) phosphorylation. As shown in Fig. 4 lane 3, and the ratio of phospho-IP3RI (Ser1756) [p-IP3RI] to β-actin strongly increased in the presence of collagen and cordycepin. However, Rp-8-Br-cAMPS potently decreased the ratio of p-IP3RI increased by both collagen and cordycepin (Fig. 4 lane 4), but not Rp-8-Br-cGMPS (Fig. 4 lane 5). In addition, the ratio of p-IP3RI were increased in the presence of both collagen and 1 mM pCPT-cAMP as PKA activator, or 1 mM 8-Br-cGMP as PKG activator (Fig. 4 lane 6 and 7). Accordingly, cAMP/PKA-dependent IP3RI (Ser1756) phosphorylation exclusively contributed to the inhibition of [Ca2+]i mobilization by cordycepin in collagen-activated platelets.

Another downstream pathway of cAMP/PKA or cGMP/PKG is VASP phosphorylation, which is concerned with
\( \alpha IIb/\beta 3 \) inactivation inhibiting platelet aggregation. The cAMP/PKA pathway phosphorylates Ser\(^{157} \) of VASP, whereas the cGMP/PKG pathway phosphorylates Ser\(^ {239} \) of VASP (Horstrup et al., 1994; Smolenski et al., 1998). Therefore, examination of VASP phosphorylation is a useful indicator for monitoring cAMP/PKA- or cGMP/PKG-pathway. As the results, collagen increased weakly phosphorylation of Ser\( ^{157} \) at 50 kDa of VASP [p-VASP (Ser\(^{157} \))] (Fig. 5 lane 2). It is known that thrombin or collagen is concerned with a feedback inhibition by increasing p-VASP (Ser\(^{157} \)) (Gambaryan et al., 2010). A PKA activator pCPT-cAMP increased p-VASP (Ser\(^{157} \)) (Fig. 5 lane 6), which means that cAMP/PKA pathway results in phosphorylation of VASP (Ser\(^{157} \)). In addition, cordycepin increased the ratio of p-VASP (Ser\(^{157} \)) in collagen-induced platelet activation (Fig. 5 lane 3), and Rp-8-Br-cAMPS decreased cordycepin-elevated p-VASP (Ser\(^{157} \)) (Fig. 5 lane 4). On the other hand, even if PKG activator 8-Br-cGMP increased p-VASP (Ser\(^{239} \)) indicating the activation of PKG via cGMP (Fig. 5 lane 7), the p-VASP (Ser\(^{239} \)) was not observed in the presence of both collagen and cordycepin (Fig. 5 lane 3). In addition, p-VASP (Ser\(^{239} \)) was not changed by Rp-8-Br-cGMPS in the presence of cordycepin (Fig. 5 lane 5). Accordingly, the elevation of p-VASP (Ser\(^{157} \)) by cordycepin was obviously resulted from cAMP/PKA pathway in collagen-activated platelets. As the result, cordycepin phosphorylated VASP (Ser\(^{157} \)), but VASP (Ser\(^{239} \)) is not. These can be explained by the previous report that a small elevation in cAMP is enough to activate most PKA potently, whereas even several fold elevation of the cGMP level may stimulate only a small fraction of total

![Fig. 4. Effects of cordycepin on inositol 1,4,5-trisphosphate receptor type 1 (IP\(_3\)RI) (Ser\(^{1756} \)) phosphorylation. The phosphorylation was determined as described in "Materials and Methods". These data were expressed as the mean ± SEM (n = 4), and *\( P < 0.05 \) compared with the non-stimulated platelets, **\( P < 0.001 \) compared with the collagen-stimulated platelets, ††\( P < 0.001 \) compared with the collagen-stimulated platelets in the presence of 500 μM cordycepin.](image)
PKG (Eigenthaler et al., 1992). This report reflects that a little cAMP level is enough to phosphorylate VASP (Ser\textsuperscript{157}), but more cGMP level is required to phosphorylate VASP (Ser\textsuperscript{239}). In this study, cordycepin increased cAMP level 1.9 fold more than cGMP level (Table 1). Accordingly, it is thought that cordycepin-elevated cGMP level is not enough to phosphorylate VASP (Ser\textsuperscript{239}), which is similar that natriuretic peptides weakly induce cGMP-dependent phosphorylation of VASP (Ser\textsuperscript{239}) (Borgogone et al., 2013), and PKA plays predominately to inhibit platelet aggregation (Li et al., 2003).

Phosphorylated VASP is known to inhibit fibrinogen binding to αIIb/β\textsubscript{3} complex, which are concerned with inhibition of platelet activation (Horstrup et al., 1994; Barragan et al., 2003). Therefore, we examined whether the phosphorylation of VASP by cordycepin are associated with inhibition of fibrinogen binding to αIIb/β\textsubscript{3}. As shown in Fig. 6, collagen activated fibrinogen binding to αIIb/β\textsubscript{3} (Fig. 6A-b) compared with intact cell, basal (Fig. 6A-a), and increased fibrinogen binding to αIIb/β\textsubscript{3} by 83.2 ± 7.3% as compared with intact platelets, basal (Fig. 6B). However, cordycepin inhibited collagen-activated fibrinogen binding to αIIb/β\textsubscript{3}.
by 14.5 ± 1.2% (Fig. 6B). The inhibition of αIIb/β3 is resulted from cAMP/PKA- or cGMP/PKG-mediated VASP phosphorylation, and it is known that cAMP- or cGMP-increasing compounds is associated with inhibition of αIIb/β3 (Horstrup et al., 1994; Hauser et al., 1999; Barragan et al., 2003), so that we examined whether the inhibition of fibrinogen binding to αIIb/β3 by cordycepin was contributed to cyclic-nucleotide of cAMP or cGMP. PKA activator pCPT-cAMP or PKG activator 8-Br-cGMP inhibited collagen-stimulated fibrinogen binding to αIIb/β3 (Fig. 6A-f, g). It was confirmed that cAMP/PKA and cGMP/PKG pathway involve the inhibition of fibrinogen binding to αIIb/β3 in collagen-induced platelet activation. Cordycepin-inhibited fibrinogen binding to αIIb/β3 (Fig. 6A-c) was elevated by PKA inhibitor Rp-8-Br-cAMPS (Fig. 6A-d). However, cordycepin-inhibited fibrinogen binding to αIIb/β3 was very

**Fig. 6.** Effect of cordycepin on fibrinogen binding. (A) The flow cytometry histograms on collagen-induced fibrinogen binding. a, Intact platelets; b, Collagen (10 μg/mL); c, Collagen (10 μg/mL) + cordycepin (500 μM); d, Collagen (10 μg/mL) + cordycepin (500 μM) + Rp-8-Br-cAMPS (250 μM); e, Collagen (10 μg/mL) + cordycepin (500 μM) + Rp-8-Br-cGMPS (250 μM); f, Collagen (10 μg/mL) + pCPT-cAMP (1 mM); g, Collagen (10 μg/mL) + 8-Br-cGMP (1 mM). (B) Effect of cordycepin on collagen-induced fibrinogen binding (%). The reactions were determined as described in "Methods". These data were expressed as the mean ± SEM (n = 4), and **P<0.001 compared with non-stimulated platelets, †P<0.05, ††P<0.001 compared with the collagen-stimulated platelets, ††P<0.001 compared with the collagen-stimulated platelets in the presence of 500 μM cordycepin.
slightly elevated by PKG inhibitor Rp-8-Br-cGMPS than Rp-8-Br-cAMPS (Fig. 6A-e). These results are in accord with that the inhibition of p-V ASP (Ser$^{157}$) by Rp-8-Br-cAMPS was stronger than Rp-8-Br-cGMPS (Fig. 5). Therefore, it is inferred that cordycepin-mediated αIIb/β₃ inhibition was resulted from cAMP dependent-V ASP (Ser$^{157}$) phosphorylation.

In conclusion, cordycepin contributed to cAMP-dependent phosphorylation of IP$₃$RI (Ser$^{1756}$) and V ASP (Ser$^{157}$) to inhibit both [Ca$^{2+}$]$_i$ mobilization and fibrinogen binding to αIIb/β₃, which accompanied the inhibition of platelet activation. Antiplatelet agents such as thienopyridine derivatives (i.e. clopidogrel, ticlopidine) have characteristics that inhibit [Ca$^{2+}$]$_i$ mobilization, phosphorylate V ASP and inhibit αIIb/β₃ activation, which is mediated by cAMP- or cGMP-dependent manner (Barragan et al., 2003). Therefore, we suggest that cordycepin might represent a useful agent in prevention and therapy of vascular diseases (i.e., thrombosis, myocardial infarction, atherosclerosis, and ischemic cerebrovascular disease) associated with platelet activation.

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CONFLICT OF INTEREST
The author declares no conflict of interest.

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