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ARTICLE

Pharmacokinetics of Adenosine and Cordycepin, a Bioactive Constituent of *Cordyceps sinensis* in Rat

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Cordycepin is a bioactive constituent of *Cordyceps sinensis* that has been shown to regulate homeostatic function. As an adenosine analogue, it is possible cordycepin goes through a similar metabolic pathway to that of adenosine. To investigate this hypothesis, a sensitive liquid chromatography with photodiode-array detector (HPLC−PDA) coupled to a microdialysis sampling system was developed to monitor cordycepin and adenosine in rat blood and liver. Other endogenous nucleosides were simultaneously measured to further understand the downstream metabolic pathway. The experiments were divided into six parallel groups for drug administration: (1) normal saline vehicle, (2) adenosine, (3) cordycepin, (4) normal saline + erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA; a potent adenosine deaminase inhibitor), (5) adenosine + EHNA, and (6) cordycepin + EHNA. The pharmacokinetic results suggest that the levels of both adenosine and cordycepin decreased rapidly in blood around 30 min after drug administration. When adenosine was given, the concentrations of adenosine metabolites, hypoxanthinosine and hypoxanthine, increased in rat blood. This phenomenon was inhibited by EHNA pretreatment. An unidentified peak was observed in the blood and liver samples after cordycepin administration. The decline of this unidentified peak paralleled the decreased of the concentration of cordycepin, and it was not observed in the presence of the adenosine deaminase inhibitor. It is concluded that adenosine and cordycepin had short elimination half-lives and high rates of clearance and their biotransformation was suppressed by EHNA.

KEYWORDS: Adenosine; cordycepin; *Cordyceps militaris*; microdialysis; pharmacokinetics

INTRODUCTION

Cordycepin (3′-deoxyadenosine; **Figure 1**) is a naturally occurring adenosine analogue, and one of the bioactive constituents of *Cordyceps sinensis* (*1*). *C. sinesis* has been used for hundreds of years as a traditional medicine in treating disorders of the lung and kidney through mechanisms of immunomodulation (*2*). Cordycepin results from a 3′-deoxypentose (cordycepose) with a branched carbon chain replacing the ribose of adenosine (*3*), and has been shown to have many intracellular activities, such as selective interruption of nucleolar RNA synthesis (*4*), enhanced cell differentiation (*5*), cytoskeleton restructuring (*6*), and inhibition of protein kinase activity (*7*). Medicinal properties range from antibacterial and antifungal activity (*8*), to *in vitro* antitumor activity in bladder, colon, leukemia, and lung carcinoma cells (*9*−*11*).

Several assay methods have been established for the qualitative and quantitative analysis of adenosine, cordycepin, nucleosides, and nucleobases that are found in *Cordyceps*. These methods include capillary electrophoresis (*12*, *13*), HPLC with UV detection (*14*), and LC with electrospray ionization tandem mass spectrometry (LC−ESI-MS/MS) (*15*). Most research has focused on the pharmacological effects of cordycepin and its drug−drug interaction with cyclosporine (*16*). No reports discuss the pharmacokinetic profile of cordycepin in its protein unbounded forms, and there has not been any comparison of cordycepin and adenosine pharmacokinetics or their physiological effect on the other endogenous nucleosides in rats.

According to the report of Manfredi and Sparks (*17*), during physiological homeostasis the normal level of endogenous adenosine in plasma is about 0.1−1 μM. Adenosine is usually used for the treatment of supraventricular tachycardia (SVT) and paroxysmal supraventricular tachycardia (PSVT). When adenosine is administered into a mammal, the extracellular adenosine will be rapidly deaminated into hypoxanthinosine by adenosine deaminase in the plasma (*18*). Adenosine is otherwise transported into cells by several nucleoside permeases (*17*, *18*), and then the adenosine will be phosphorylated to form ATP by adenosine kinase. If the cell does not utilize the adenosine, it will be deaminated to hypoxanthinosine by adenosine deaminase inside the cell (**Figure 2**) (*18*).

Cordycepin is quickly deaminated by adenosine deaminase, and rapidly metabolized to an inactive metabolite, 3′-deoxyhypoxanthinosine, *in vivo* (*19*). When cordycepin is given by

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continuous intravenous injection in combination with 2′-deoxycoformycin (dCF), there is an increase of adverse effects from such combination treatment compared to cordycepin alone (*20*), although this combination can cure *Trypanosoma brucei* infection in mice when given by intraperitoneal injection (*21*, *22*).

According to the basic principles of pharmacology, only the protein unbound form of a drug in biological fluid is available to produce pharmacological effects. Microdialysis sampling is a well established technique for collecting protein unbound drug and endogenous fractions simultaneously from plasma and other tissues of interest (*23*−*27*).

To our knowledge no reports have investigated the *in vivo* pharmacokinetics of protein unbound adenosine and cordycepin. The aim of this study is to determine the pharmacokinetics of adenosine and cordycepin in order to determine the plausibility of a shared metabolic pathway. Therefore, in this study, we developed an analytical method to monitor protein unbound adenosine, cordycepin, adenosine metabolites, and endogenous nucleosides after adenosine or cordycepin administration. In addition, to evaluate its biotransformation mechanism, EHNA, is used to block metabolism of adenosine and cordycepin *in vivo* and thereby to affect intracellular levels of adenosine, cordycepin, other endogenous nucleosides and bases.

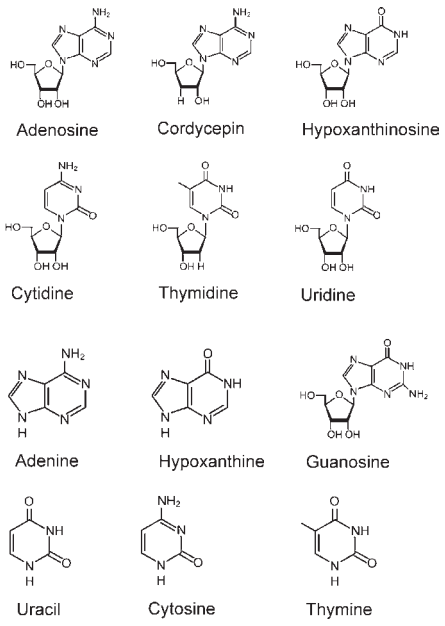

Figure 1. Chemical structures of adenosine, cordycepin, nucleosides, and bases.

MATERIALS AND METHODS

Chemicals. Adenosine, cordycepin, cytidine, guanosine, hypoxanthinosine, thymidine, uridine, and their bases, adenine, cytosine, hypoxanthine, thymine, and uracil (**Figure 1**) were obtained from Sigma-Aldrich (St. Louis, MO) and used as authentic standards of nucleosides and some related bases. EHNA, urethane and α-chloralose were also purchased from Sigma-Aldrich. Methanol of HPLC-grade was provided from Tedia Company Inc. (Fairfield, OH). Triply deionized water (Millipore, Bedford, MA) was used in all the experiments presented. Potassium dihydrogen phosphate used in the preparation of the mobile phase was supplied by Merck (Darmstadt, Germany).

Animals. Specific pathogen-free adult Sprague−Dawley male rats weighing between 210 to 250 g obtained from the Laboratory Animal Center of the National Yang-Ming University were used in the experiment. All animals were housed in temperature-controlled quarters (24 ± 1 °C) with a 12 h/12 h light/dark cycle. Food (Laboratory Rodent Diet 5001, PMI Feeds Inc., Richmond, IN) and water were available *ad libitum*. The animal protocols were reviewed and approved by the Institutional Animal Experimentation Committee of National Yang-Ming University. For each experimental group, six Sprague−Dawley male rats were randomly chosen and anesthetized by anesthetic (1 mL/kg, ip) composed of urethane (1 g/mL) and chloralose (0.1 g/mL) at the beginning of all experiments, and remained anesthetized throughout the experimental period. During the experiment, the body temperature of the experimental rats was maintained with a heating pad set to 37 °C.

Chromatography. Liquid chromatography analysis was performed on a Shimadzu system (Shimadzu, Kyoto, Japan) consisting of a LC-20AT pump, a SIL-20AC autosampler, and a SPD-M20A photodiode array detector. Separation was achieved in a LiChroCART precolumn (4 mm × 4 mm I.D., particle size 5 μm, Merck), and an analytical column (LiChroCART, 250 mm × 4.6 mm i.d., RP-18e, particle size 5 μm, Merck).

The mobile phases for chromatographic delivery were 10 mM potassium dihydrogen phosphate (KH_2PO_4) in water, adjusted to pH 4.5 by phosphoric acid (solvent A), and methanol (solvent B). The mobile phase gradient program applied was 99:1 (A:B) at 0 min and held for 5 min; 98:2 (A:B) at 10 min; 95:5 (A:B) at 15 min; 80:20 (A:B) at 30 min; 70:30 (A:B) at 40 min; 99:1 (A:B) at 45 min and held 10 min for chromatographic balance. The flow rate of the mobile phase was set at 1 mL/min. Both the buffer solution and methanol were filtered (Millipore 0.45 μm) and degassed prior to use. All samples were placed in a temperature controlled autosampler at 6 °C before analyzing. The optimal detection UV wavelength was set at 260 nm. Output signals from the HPLC−PDA were integrated via a Class-VP 7.0 Client/Server Chromatography Data System (Shimadzu, Kyoto, Japan).

Method Validation. For all authentic standards, a stock solution of each standard (1 mg/mL) was prepared in 5 mM ammonium acetate solution and stored at −80 °C. For the daily preparation of a standard mixture, portions of these stock solutions were thawed at 4 °C and diluted to the appropriate concentration with triply deionized water. All calibration curves of adenosine, cordycepin, nucleosides, and bases were quantified in six individual runs, and they were all required to have coefficient of determination (r^2) values of at least 0.995. The intra- and interbench

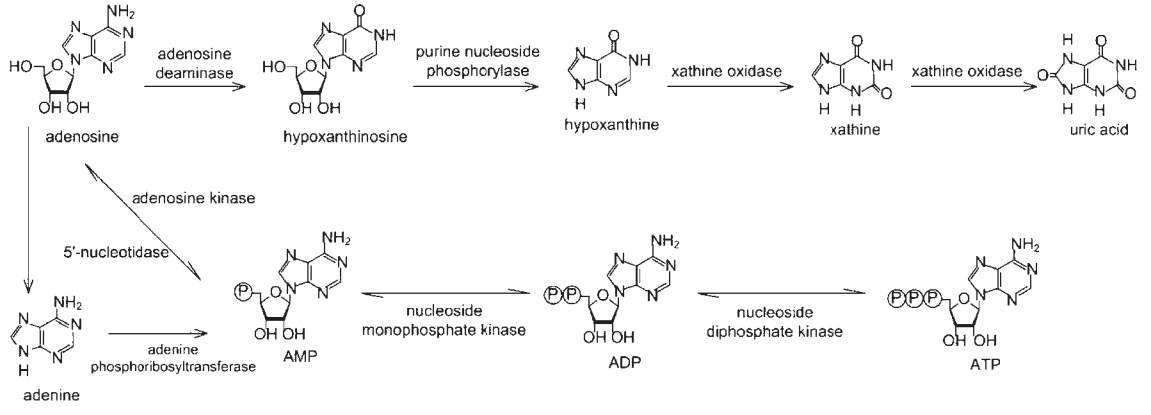

Figure 2. Metabolic pathway of adenosine in mammalian.

variabilities for all analytes were assayed at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, and 50 μg/mL on the same day and on six consecutive days, respectively. The limit of quantification (LOQ) was defined as the lowest concentration of the linear range, and the limit of detection (LOD) was defined as the concentration of analyte giving a signal-to-noise ratio (S/N) of 3.

The accuracy (% Bias) was calculated by the following equation: Bias (%) = $[(C_{obs} - C_{nom})/C_{obs}] \times 100$, where C_{nom} is the nominal concentration and C_{obs} is the mean value of observed concentration. The relative standard deviation (RSD) was calculated from the observed concentrations by the equation RSD (%) = [standard deviation (SD)/C_{obs}] × 100.

Microdialysis Experiments. All surgical procedures followed the guidelines detailed in the Guide for the Care and Use of Laboratory Animals. Both blood and liver microdialysis systems consisted of a microinjection pump (CMA/100, CMA, Stockholm, Sweden), appropriate microdialysis probes and a fraction collector (CMA/140). The flexible and rigid microdialysis probes for blood and liver (10 mm in length) were made of silica glass capillary tubing arranged in a concentric design and used to sample the unbound endogenous and exogenous small molecules in rats (*23*, *24*). All microdialysis probes for blood and liver sampling were covered by a dialyzing membrane (150 μm diameter, with nominal molecular mass cutoff 13000 Da, Spectrum Co, Laguna Hills, CA).

The blood microdialysis probe was positioned within the right jugular vein/superior vena cava/right atrium (*25*, *26*), and the liver microdialysis probe was placed in the median lobe of the liver (*23*, *24*). Then, the anticoagulant dextrose solution (3.5 mM citric acid; 13.6 mM dextrose;7.5 mM sodium citrate) was perfused at a constant flow rate (2.5 μL/min) using the microinjection pump. Perfusate (C_{perf}) and dialysate (C_{dial}) concentrations were assayed using the HPLC−PDA system. The *in vivo* relative recovery (R_{invivo}) of cordycepin was calculated by the equation of: $R_{invivo} = 100(C_{perf} - C_{dial})/C_{perf}$ (*27*, *28*). The endogenous analytes (C_{endo}) across the microdialysis probe in rat blood and liver were calculated by the equation $R_{invivo} = 100[C_{perf} - (C_{dial} - C_{endo})]/C_{perf}$.

Drug Administration. The dialysates from the rat blood and liver were collected over a 2 h stabilization period after probe implantation. Then, the intravenous dose of normal saline (1 mL/kg), adenosine (10 mg/kg dissolved in normal saline), cordycepin (10 mg/kg dissolved in normal saline) or EHNA (10 mg/kg dissolved in normal saline) was slowly injected into individual rats from the femoral vein at a volume of 1 mL/kg. All the dialysates from the blood and liver were collected into a fraction collector every 10 min and then analyzed by a validated HPLC system on the same experimental day.

Pharmacokinetics and Statistics. The areas under concentration vs time curves (AUC) were calculated according to the trapezoidal method. The elimination half-life ($t_{1/2}$) was considered as $t_{1/2} = 0.693/K$, where K is the first order rate constant of the elimination phase. The clearances (Cl) of the drug were expressed as follows: Cl = dose/AUC. The mean residence time (MRT) was estimated as MRT = AUMC/AUC, where AUMC is the area under the first moment curve (*29*). Pharmacokinetic calculations were optimized by the compartmental and noncompartmental models with the software WinNonlin Standard Edition Version 1.1 (Scientific Consulting Inc., Apex, NC). The results were analyzed by Student's t-test to detect significant differences between the pharmacokinetic data of adenosine and cordycepin administration. A p value of less than 0.05 was considered significant. The results are expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Under the validated HPLC system coupled to microdialysis system described above, optimal separation and determination of adenosine and cordycepin were achieved in both blood and liver dialysates following drug administration. Identification of all analytes was accomplished by comparing their retention times and individual UV spectrum over the wavelength range between 190 to 400 nm with authentic standards. Typical chromatograms of all analytes are shown in **Figures 3** and **4**.

Figures 3A and **4A** show the chromatograms of the authentic standard solutions of adenosine, cordycepin, other nucleosides and bases, each with a concentration of 0.5 μg/mL. **Figures 3B** and **4B** show the chromatograms of blank blood and liver dialysates, respectively. Exogenous cordycepin was separated from endogenous substances in both the dialysates of blood and liver. No significant interference peaks were observed at the same retention time of the analyte peaks. **Figures 3C** and **4C** show the chromatograms of the blood (containing 0.78 μg/mL adenosine) and liver dialysate samples respectively collected at 20 to 30 min after adenosine administration (10 mg/kg). **Figures 3D** and **4D** show the chromatograms of blood (cordycepin concentration 1.76 μg/mL) and liver dialysates respectively collected at 20 to 30 min after cordycepin administration (10 mg/kg) by microdialysis. Although the concentration of adenosine and cordycepin in the liver dialysate was undetectable, the detection system could monitor changes in the other endogenous nucleoside levels during the experimental times. There was an unidentifiable peak in the blood and liver dialysates at the retention time of 24.97 min (**Figures 3D** and **4D**). This unidentified peak appeared only after cordycepin administration, and its UV spectrum was similar to the UV spectra of other known nucleosides in the wavelength range between 190 to 400 nm. This unidentified peak was a possible the metabolite of cordycepin.

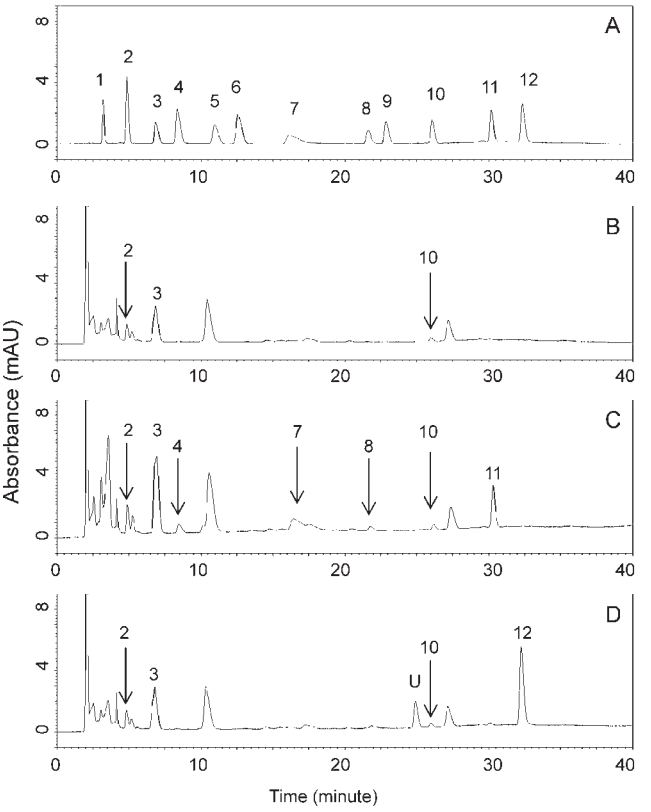

Figure 3. Typical chromatograms of (**A**) standards of cordycepin, nucleosides, and bases (0.5 μg/mL), (**B**) blank blood dialysate from the microdialysis probe before drug administration, (**C**) blood dialysate sample containing adenosine (0.78 μg/mL) collected at 20−30 min after adenosine administration (10 mg/kg), and (**D**) blood dialysate sample containing cordycepin (1.76 μg/mL) collected at 20−30 min after cordycepin administration (10 mg/kg, iv); (1) cytosine, (2) uracil, (3) cytidine, (4) hypoxanthine, (5) uridine, (6) thymine, (7) adenine, (8) hypoxanthinosine, (9) guanosine, (10) thymidine, (11) adenosine, (12) cordycepin, (U) cordycepin-induced unidentified peak.

Linear regression analysis was performed on six individual runs, and the linearity was evaluated between the peak response and nominal concentration of all authentic standards over the concentration range of 0.01−50 μg/mL. The results showed that

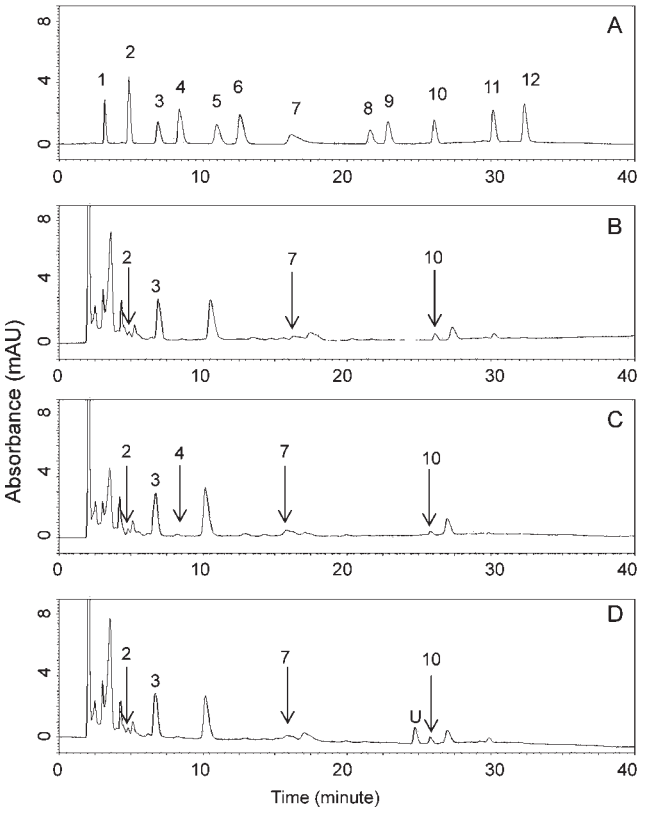

Figure 4. Typical chromatograms of (**A**) standard of cordycepin, nucleosides, and bases (0.5 μg/mL), (**B**) blank liver dialysate from the microdialysis probe before drug administration, (**C**) liver dialysate sample collected 20−30 min after adenosine administration (10 mg/kg, iv), and (**D**) liver dialysate sample collected at 20−30 min after cordycepin administration (10 mg/kg, iv); (1) cytosine, (2) uracil, (3) cytidine, (4) hypoxanthine, (5) uridine, (6) thymine, (7) adenine, (8) hypoxanthinosine, (9) guanosine, (10) thymidine, (11) adenosine, (12) cordycepin, (U) unidentified peak.

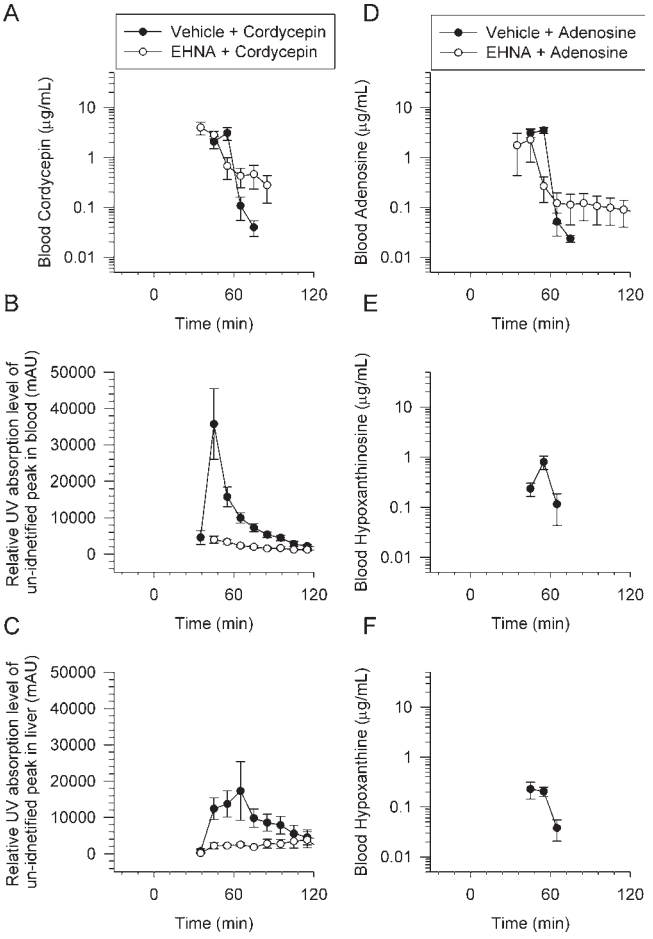

Figure 5. Concentration−time profiles for the treatment of cordycepin (10 mg/kg, iv), adenonsine (10 mg/kg, iv) and with/without EHNA (10 mg/kg, iv), (**A**) cordycepin level in rat blood after cordycepin administration and EHNA pretreated 30 min before cordycepin administration, (**B**) and (**C**) represent the UV absorption level of unidentified compound in rat blood and liver dialysate, respectively after cordycepin administration and EHNA pretreated 30 min before cordycepin administration, (**D**) adenosine, (**E**) hypoxanthinosine, and (**F**) hypoxanthine in rat blood dialysate after adenosine administration and EHNA pretreated 30 min before adenosine administration. The data represent mean ± SEM from six individual rats.

the coefficient of determination (r^2) for each analyte was greater than 0.999. The LOD and LOQ for all analytes were 0.01 and 0.05 μg/mL, respectively. This method was shown to be highly sensitive when compared with former analytic methods such as detection by electrophoresis with UV detection (*12*), HPLC with UV detection (*14*), and LC−ESI-MS/MS (*15*), where the LOQ of adenosine was 48.0, 0.07, and 0.5 μg/mL, respectively.

The reproducibility of the presented method was examined by the intra- and interbench variabilities. The intra- and interbench precision and accuracy (bias) of adenosine, cordycepin, nucleosides and bases were all within the predefined limits of acceptability (< 15%). This method is sufficiently sensitive and stable for the measurement of adenosine, cordycepin, and other endogenous nucleosides in rat blood and liver for pharmacokinetic study.

The average *in vivo* microdialysis recovery (n = 6) of adenosine in blood (1, 5, and 10 μg/mL) and liver (1, 5, and 10 μg/mL) were 33.7 ± 2.3 and 42.6 ± 2.9%, and cordycepin were 37.7 ± 1.6 and 48.3 ± 2.0%, respectively. For other nucleosides and nucleobases, their *in vivo* microdialysis recoveries in blood and liver were about 35−50%. The actual concentrations of adenosine, cordycepin, and other nucleosides in the rat blood and liver were corrected by their respective recoveries.

The concentration versus time profiles of cordycepin, adenosine, adenosine metabolites (hypoxanthine and hypoxanthinosine), and the AUC of the unidentified peak in blood and liver presented after cordycepin (10 mg/kg, iv) or adenosine administration (10 mg/kg, iv) with/without EHNA pretreatment are shown in **Figure 5**. The results indicate that the endogenous levels of nucleosides and bases in rat blood and liver are not altered by the vehicle. The measurable protein unbound concentration of adenosine and cordycepin in rat blood disappears in the liver within 30 min.

The levels of hypoxanthinosine and hypoxanthine, the two major metabolites of adenosine, could be detected in rat blood and liver dialysate after adenosine administration (10 mg/kg, iv) (**Figures 5E** and **5F**). These two adenosine metabolites were metabolized by adenosine deamination in plasma, erythrocytes, and endothelial cells (*30*, *31*). The results showed that the concentration of these two metabolites decreased rapidly.

Deamination of adenosine to hypoxanthinosine, and then purine nucleoside phosphorylase, further converts it to hypoxanthine rapidly in the blood (**Figure 2**). This short elimination half-life for hypoxanthinosine, and its conversion to hypoxanthine, is consistent with the previous report by Farthing (*30*). Microdialysis excludes adenosine's metabolic enzymes, and the enzymatic process is terminated. Thus, an accurate simultaneous

Table 1. Pharmacokinetic Data of Adenosine and Cordycepin at the Dose of 10 mg/kg, Iv in Rat Blood[a]

parameters	adenosine	cordycepin	adenosine + EHNA	cordycepin + EHNA
AUC (min μg/mL)	79.6 ± 7.6	38.5 ± 10.3	89.5 ± 16.3*	73.9 ± 11.3#
$t_{1/2}$ (min)	10.4 ± 0.9	1.6 ± 0.0	16.6 ± 5.2 *	23.3 ± 0.8 #
C_{max} (μg/mL)	4.8 ± 0.4	3.1 ± 0.9	1.0 ± 0.4 *	2.3 ± 0.4 #
Cl (L/min per kg)	0.1 ± 0.0	2.1 ± 1.2	0.25 ± 0.05 *	0.02 ± 0.00 #
MRT (min)	14.9 ± 1.2	4.8 ± 1.5	23.9 ± 7.5 *	33.6 ± 1.2 #

[a] Data expressed as mean ± standard error of the mean (n = 6). *P < 0.05 significantly different from adenosine + with/without EHNA in rat blood. #P < 0.05 significantly different from cordycepin + with/without EHNA in rat blood.

Table 2. Pharmacokinetic Data of Cordycepin-Induced Unidentified Peak in Rat Blood and Liver after Cordycepin Administration (10 mg/kg, Iv)[a]

	cordycepin-induced unidentified peak			
	cordycepin		cordycepin + EHNA	
parameters	blood	liver	blood	liver
AUC × 10^6	1.09 ± 0.15	0.92 ± 0.15	0.16 ± 8.71*	0.10 ± 0.03#
$Peak_{max}$ × 10^4	3.29 ± 0.37	1.21 ± 0.19	0.25 ± 0.03*	0.24 ± 0.03 #

[a] Data expressed as mean ± standard error of the mean (n = 6). AUC: area under the curve of the peak area of unidentified peak versus time. $Peak_{max}$: maximum peak area of the cordycepin-induced unidentified peak. *P < 0.05 significantly different from cordycepin + with/without EHNA in rat blood. #P < 0.05 significantly different from cordycepin + with/without EHNA in rat liver.

measurement of the concentration of these molecules and their metabolites in blood and liver at the given time point can be achieved.

The pharmacokinetic data indicated that the elimination half-lives ($t_{1/2}$) of adenosine and cordycepin in rat blood were 10.4 ± 0.9 and 1.6 ± 0.0 min after administrations of adenosine and cordycepin. When pretreated with EHNA, the elimination half-lives of adenosine and cordycepin were increased up to 16.6 ± 5.2 and 23.3 ± 0.8 min. The AUC of adenosine and cordycepin were 79.6 ± 7.6 and 38.5 ± 10.3 min μg/mL. After EHNA pretreatment, the AUC of adenosine and cordycepin increased to 89.5 ± 16.3 and 73.9 ± 11.3 min μg/mL, respectively (**Table 1**).

The AUC of the cordycepin induced unidentified peak in rat plasma and liver (**Figures 5B** and **5C** and **Table 2**) showed the pharmacokinetic data of the unidentified peak in rat blood and liver after cordycepin administration with or without EHNA pretreatment. The maximum peak area ($Peak_{max}$) of this unidentified peak in rat blood and liver was 3.29 ± 0.37 × 10^4 and 1.21 ± 0.19 × 10^4. In the EHNA pretreatment groups, the $Peak_{max}$ of the unidentified peak in rat blood and liver decreased to 0.25 ± 0.03 × 10^4 and 0.24 ± 0.03 × 10^4. These results suggested that although adenosine and cordycepin are rapidly eliminated and metabolized in a short period of time, a cordycepin induced unidentified compound appears in blood and liver for over 2 h after cordycepin administration. The cordycepin induced compound could be restrained by pretreatment of EHNA, suggesting that it could be a metabolite of cordycepin.

In 1989, Möser et al. used radiolabled adenosine to determine the elimination half-life and turnover of adenosine in plasma, demonstrating that, in the physiological concentration range, adenosine half-life in human plasma is 0.6−1.5 s (*32*). To overcome the problem of rapid elimination, a high dosage must be administered or the concentration of adenosine and cordycepin in plasma would be below detection limitations. Hence, this study selected the dosage of adenosine and cordycepin as 10 mg/kg, which was sufficient for detection and did not cause acute toxicity.

Previous studies noted that the extracellular metabolic enzymes could be collected by total blood drawing, and these enzymes would continue their biological activity in *ex vivo*. To estimate these endogenous nucleosides during the experiment, the whole blood sample must be drawn quickly, kept cold, and inhibited by a stopping solution containing dipyridamole and EHNA (*30*). A benefit of the microdialysis sampling system is that it collects the protein unbound drug and excludes large molecules and metabolic enzymes to prevent further enzymatic reaction (*24*, *33*, *34*).

The enzymatic adenosine pathway is highly specific and widespread in the body organs and tissues. The metabolic pathway for an adenosine analogue may be similar to adenosine. Other adenosine analogues and deoxyadenosine analogues, such as 8-chloro-adenosine, may be deaminated by adenosine deaminase to 8-chloro-inosine or phosphorylated by adenosine kinase to 8-chloro-inosine monophosphate (*35*). These complex metabolic reactions may be extensively carried out in the whole body system (*36*, *37*). The adenosine structural analogue, cordycepin, is likely to become readily involved in the adenosine metabolic and uptake pathways. Cordycepin may react with adenosine deaminase to form 3′-deoxyhypoxanthinosine, or cordycepin may undergo phosphorylation by adenosine kinase to become 3′-deoxyadenosine mono-, di-, and triphosphate (*20*). The levels of endogenous nucleosides and bases were not significantly affected by normal saline or pretreatment with EHNA. However, the adenosine metabolites, hypoxanthinosine and hypoxanthine, were inhibited by pretreatment with EHNA. These results suggest that adenosine deamination was inhibited by EHNA pretreatment and this phenomenon could be reproduced in cordycepin experiments.

The signal strength of the cordycepin-induced unidentified peak was diminished by EHNA pretreatment. This result suggests that the formation of this compound may be correlated to the pharmacological effect of EHNA inhibition of adenosine deaminase. In contrast with metabolic reactions of adenosine, the cordycepin-induced unidentified compound may result from the reaction of cordycepin with adenosine deaminase to form 3′-deoxyhypoxanthinosine.

In summary, the present study developed a microdialysis technique coupled to an HPLC system to measure the protein unbound concentrations of adenosine, cordycepin and endogenous nucleosides in rat blood and liver after adenosine or cordycepin administration. The pharmacokinetic profiles demonstrated that adenosine and cordycepin had short elimination half-lives and high rates of clearance. Adenosine and cordycepin were rapidly metabolized in plasma. Both hypoxanthinosine (adenosine deamination product) and hypoxanthine (hypoxanthinosine phosphorylated product) were eliminated rapidly after adenosine administration. The results suggested that rapid biotransformation of exogenous adenosine and cordycepin may both be metabolized by adenosine deaminase in the endogenous nucleoside metabolic pathway. Investigation of this unidentified peak isolation, purification, identification and measurement using mass spectrometry and nuclear magnetic resonance techniques is a topic for another project.

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