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Determination of 8-chloroadenosine 3′,5′-monophosphate in dog plasma by capillary electrophoresis

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Abstract

A method for the quantitative determination of 8-chloroadenosine 3′,5′-monophosphate (8-Cl-cAMP) in dog plasma by capillary zone electrophoresis (CE) has been developed and validated. Samples of plasma (with 2′-*O*-monobutyryladenosine-3′,5′-monophosphate as internal standard) were deproteinized with two volumes of acetonitrile. The supernatant was evaporated and reconstituted in water. A BioFocus 2000 system (Bio-Rad, Hercules, CA, USA) was used. The UV detector was set at 261 nm. The samples were loaded into uncoated fused silica capillary (40 cm × 50 μm) by pressure injection. A running electrolyte contained 30 mM SDS, 100 mM Tris, pH 7.55, with 20% of methanol added. The typical analytical conditions were: voltage, 18 kV; injection, 12 psi × s; capillary and carousel temperature were 20°C. The linear relationship was observed between 0.063–2.00 μM using the time-corrected peak area ratio of 8-Cl-cAMP to internal standard with correlation coefficient greater than 0.99. The intra-day and inter-day coefficients of variation (CV's) were less than 12%. The developed method was used for the analysis of plasma samples from beagle dogs ($n = 12$) to examine the toxicity of the anticancer drug, 8-Cl-cAMP, following two, 5-day cycles of continuous intravenous infusion at various doses of 8-Cl-cAMP as the sodium salt.

Keywords: 8-Chloroadenosine 3′,5′-monophosphate; 8-Chloroadenosine; Capillary electrophoresis

1. Introduction

8-Cl-cAMP (Fig. 1A) has progressed through clinical evaluation as an anticancer drug where it is administered as a continuous intravenous infusion. However, there is debate as to whether 8-Cl-cAMP or its cytotoxic metabolite 8-Cl-adenosine (Fig. 1B) [1] is the active principal. There is very limited information available in the literature about the detection of 8-Cl-cAMP and 8-Cl-adenosine in plasma. HPLC methods preceded by solid-phase extraction have been developed for the analysis of 8-Cl-cAMP and 8-Cl-adenosine in human plasma, but it was nec-

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essary to develop separate sample preparation methods for the 8-Cl-cAMP and 8-Cl-adenosine [1]. G. Tortora et al. [2] described another HPLC method using fluorescence detection for 8-Cl-cAMP, where the retention time of the 8-Cl-cAMP peak was 41.8 min. None of these methods were able to detect both the parent and its metabolite in a single run. We developed a CE based method for the simultaneous determination of 8-Cl-cAMP and 8-Cl-adenosine in dog plasma. However, once it was established that 8-Cl-adenosine was not detected in plasma after continuous infusion of 8-Cl-cAMP at the maximum dose in our study, we developed and validated the CE based method for quantitative determination of only 8-Cl-cAMP in dog plasma. The method was applied to the analysis of plasma samples from beagle dogs in order to examine the toxicity of 8-Cl-cAMP during two, 5-day cycles of continuous intravenous infusion at various doses of 8-Cl-cAMP as the sodium salt.

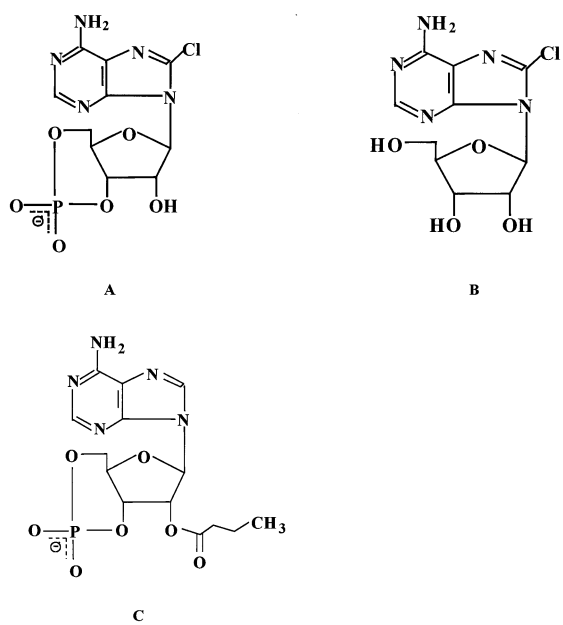

Fig. 1. Structure of 8-Cl-cAMP: (A), 8-Cl-adenosine; (B), and 2′-*O*- monobutyryladenosine-3′,5′-monophosphate.

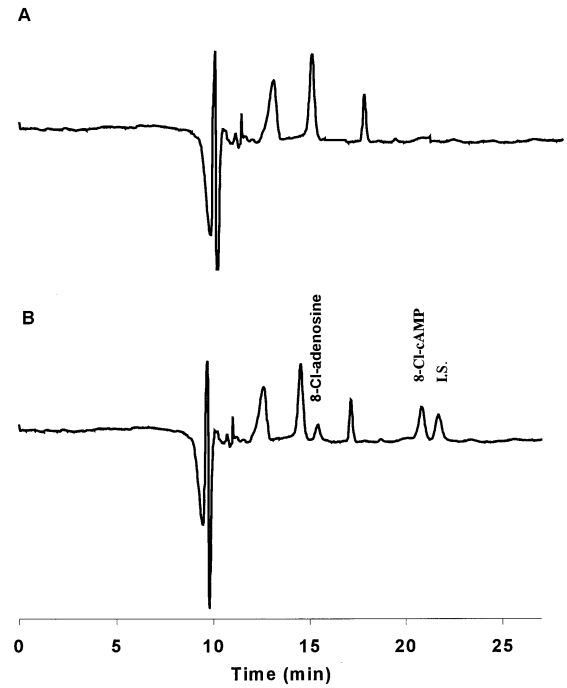

Fig. 2. Electropherograms of plasma samples: (A) blank plasma; (B) plasma spiked with 8-Cl-cAMP, 8-Cl-adenosine, and 2′-*O*- monobutyryladenosine-3′,5′-monophosphate (I.S.).

2. Experimental

2.1. Reagents

8-Chloroadenosine 3′,5′-monophosphate sodium salt (8-Cl-cAMP, Lot No: 4278), and 8-chloroadenosine (Lot No.: Na 553598) were received from ICN Hungary Co. Ltd., (Hungary), 2′-*O*-monobutyryladenosine-3′,5′-monophosphate (cat. # M-1505) and TRIZMA® BAZE ($C_4H_{11}NO_3$, cat. # T-6791) were obtained from Sigma (St. Louis, MO), boric acid (H_3BO_3, cat. # A73-500), sodium dodecyl sulfate (SDS, cat. # BP166-100), acetonitrile (MeCN, HPLC grade, cat. # A998-4) were manufactured by Fisher Scientific (Fair Lawn, NJ), methanol (MeOH, HPLC grade, cat. # MX0475-1) was from EM Science (Darmstadt, Germany).

2.2. Apparatus and electrophoretic conditions

A BioFocus 2000 system (Bio-Rad, Hercules, CA) was used for method development with UV detection at 261 nm. The samples were loaded into uncoated fused silica capillary (40 cm × 50 μm) by pressure injection.

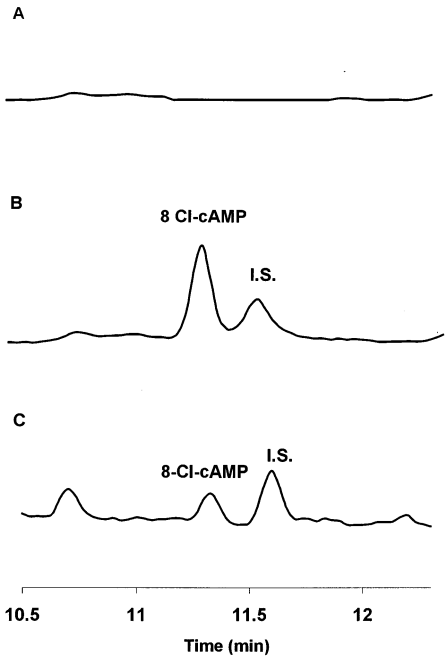

Fig. 3. The fragments of electropherograms of plasma samples: (A) blank plasma; (B) plasma spiked with 8-Cl-cAMP (2 μM); (C) plasma from dog at day 5 after continuous administration of 8 Cl-cAMP at a dose of 3.0 mg/kg per day.

Table 1
Intra-day and inter-day precision and accuracy of 8-Cl-cAMP in dog plasma

8-Cl-cAMP	LoQC	MeQC	HiQC
Concentrated (μM)	0.10	0.50	1.50
Intra-day			
N	5	5	5
Mean	0.11	0.54	1.58
S.D.	0.01	0.03	0.12
C.V.%	6.83	5.77	7.37
Accuracy	109.54	108.88	105.64
Inter-day			
N	13	15	15
Mean	0.11	0.54	1.56
S.D.	0.01	0.05	0.10
C.V.%	10.58	9.12	6.51
Accuracy	110.04	109.01	104.64

For simultaneous determination of 8-Cl-cAMP and 8-chloroadenosine in dog plasma, the running electrolyte contained 30 mM SDS, 100 mM Tris, pH 7.55 (pH adjusted with saturated boric acid solution), with 30% of methanol added. The typical analytical conditions were: voltage, 15 kV; injection, 10 psi × s; capillary and carousel temperature were 20°C. The capillary was rinsed with 0.1 M NaOH (100 s), with distilled water (120 s), and running buffer (120 s) between each injection.

For determination of 8-Cl-cAMP in dog plasma alone, the running electrolyte contained 30 mM SDS, 100 mM Tris, pH 7.55 (pH adjusted with saturated boric acid solution), with 20% of methanol added. The typical analytical conditions were: voltage, 18 kV; injection, 12 psi × s; capillary and carousel temperature were 20°C. The capillary was rinsed with 0.1 M NaOH (120 s), with distilled water (60 s), and running buffer (180 s) between each injection.

2.3. Preparation of stock solutions

Standard stock solutions of 8-Cl-cAMP (1 mM), 8-Cl-adenosine (1 mM), and an internal standard solution of 2′-*O*-monobutyryladenosine-3′,5′-monophosphate (0.5 mM) were prepared in distilled deionized (DD) water. Working standards from the concentrated stock solutions were prepared by serial dilution with DD water to yield concentrations of 40.000, 20.000, 10.000, 5.000, 2.500, 1.250, 0.625 μM. The concentrated internal standard solution was diluted with DD water to make working stock solution with the final concentration of 10 μM. All stocks were stored in capped glass vials at 4°C.

2.4. Calibration standard preparation

For simultaneous determination of 8-Cl-cAMP and 8-chloroadenosine, aliquots of 900 μl beagle plasma were spiked with 50 μl each of 8-Cl-cAMP and 8-Cl-adenosine working standard to yield

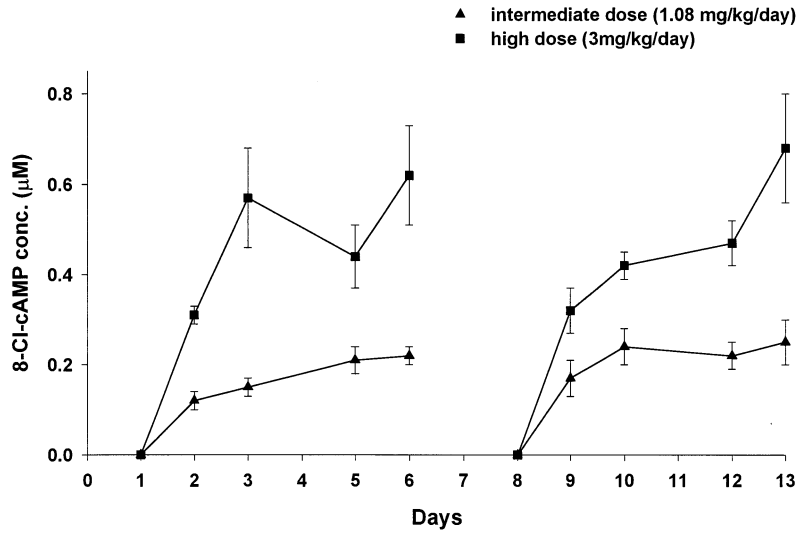

Fig. 4. Mean concentration of 8-Cl-cAMP and standard errors during two, 5-day cycles of continuous intravenous infusion in dogs ($n = 4$ for each dose).

concentrations of 2.000, 1.000, 0.500, 0.250, 0.125, 0.063 μM for each. For determination of 8-Cl-cAMP alone, aliquots of 950 μl beagle plasma was spiked with 50 μl each of 8-Cl-cAMP working standard to yield concentrations of 2.000, 1.000, 0.500, 0.250, 0.125, 0.063 μM.

2.5. Sample preparation

One ml of plasma was mixed with 100 μl of internal standard (2′-*O*-monobutyryladenosine-3′,5′-monophosphate, 10 μM) and 2 ml of ice cold acetonitrile were added into each plasma sample to precipitate the plasma proteins. The samples were vortexed briefly and then centrifuged at $2015 \times g$ for 20 min. The clear supernatant was transferred to other labeled tubes and evaporated to dryness under nitrogen (maximum temperature during evaporation was 35°C). At the time of analysis the residue was dissolved in 100 μl DD water. The solution was transferred into microporous centrifugal filter (NANOSEP MF with 0.2 μm pore size, Pall Filtron Co.) and centrifuged at $14\,000 \times g$ for 2 min. The filtrate was transferred into labeled 500 μl evaporation control vials (Bio-Rad, cat. # 148-6091) and used for CE analysis.

2.6. Intra-day and inter-day validation.

Intra-day and inter-day validation studies for precision and accuracy were performed on five replicates, each of low-, medium-, and high concentration quality control standards in plasma. Quality control samples with the following 8-Cl-cAMP concentration were prepared: 0.1 (LQC), 0.5 (MQC), 1.5 μM (HQC). The analyses were repeated on three separate days.

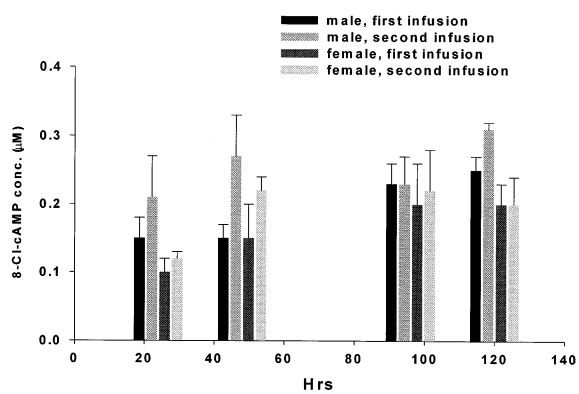

Fig. 5. Comparison of mean ($\pm$SE) concentrations of 8-Cl-cAMP in male ($n = 2$) and female ($n = 2$) beagle dogs during two, 5-day cycles of continuous i.v. infusion of 8-Cl-cAMP at dose 1.08 mg/kg per day.

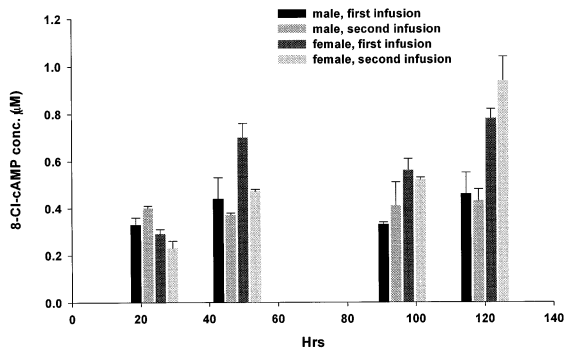

Fig. 6. Comparison of mean (± SE) concentrations of 8-Cl-cAMP in male ($n = 2$) and female ($n = 2$) beagle dogs during two, 5-day cycles of continuous i.v. infusion of 8-Cl-cAMP at dose 3.00 mg/kg per day.

2.7. Subjects

Sixteen beagle dogs (eight male, eight female), aged 5–8 months with body weights between 4–10 kg were used for this study. The animals were separated into four groups (two male and two female in each group). The first group received no drug. The second group received 8-Cl-cAMP at a dose of 0.015 mg/kg per h, the third group received a dose of 0.0454 mg/kg per h, and the fourth one received 0.125 mg/kg per h of 8-Cl-cAMP. 8-Cl-cAMP was administered as a sodium salt by continuous intravenous infusion (0.5 ml/kg per h) 5 days/week for 2 weeks using a catheter implanted into the jugular vein. The animals were maintained on continuous infusion (1 ml/h per animal) with physiological saline between the two 5-days infusion periods. The animals were necropsied at the end of the second infusion (day 13).

For toxicokinetics 3 ml venous blood samples were taken during the 8-Cl-cAMP infusion. The timing of these samples were at days 2 (after 24 h of infusion), 3, 5, 6 during the first period of infusion, and at day 8 (prior to the second cycle of the infusion), days 9 (after 24 h of infusion), 10, 12, 13. For each sampling time, 3 ml of blood were collected into tubes containing heparin as an anticoagulant. Samples were centrifuged, and the plasma was collected before being stored frozen at − 20°C.

3. Results and discussion

3.1. Electropherograms

3.1.1. Simultaneous determination of 8-Cl-cAMP and 8-Cl-adenosine in dog plasma

Electropherograms from typical blank dog plasma, spiked with 8-Cl-cAMP, 8-Cl-adenosine and internal standard are shown in Fig. 2. 8-Cl-adenosine was not detected in dog plasma during two, 5-day cycles of continuous intravenous infusion of 8-Cl-cAMP at the doses of 1.08 mg/kg per day and 3.00 mg/kg per day. The pharmacokinetics, metabolism and tumor disposition in previously run studies of 8-Cl-cAMP [3] had shown that 8-Cl-adenosine was not detected in human plasma after continuous infusion (28 day) of 8-Cl-cAMP at doses of 0.54 or 1.08 mg/kg per day. The results were the same in mouse plasma after continuous infusion (7 day) of active drug dose 50 mg/kg per day. Based on our results and previous information, we modified our electrophoretic conditions to reduce the total analysis time and increase the sensitivity for 8-Cl-cAMP.

3.1.2. Determination of 8-Cl-cAMP in dog plasma

Electropherograms of typical blank dog plasma, blank plasma spiked with 8-Cl-cAMP and 2′-*O*-monobutyryladenosine-3′,5′-monophosphate, and a typical electro-pherogram of plasma from dogs during intravenous infusion of 8-Cl-cAMP are shown in Fig. 3. As was shown by J. Cummings et al. [3], the fate of 8-Cl-cAMP in human tumors is characterized by extensive metabolism to products, which are not generally observed in plasma. Therefore, under the present analytical conditions, interference from possible metabolites was not taken into consideration.

3.2. Linearity

Calibration curve was created at 6 different concentrations (0.063–2.000 μM) in beagle plasma by plotting the corrected peak area ratio of 8-Cl-cAMP to 2′-*O*-monobutyryladenosine-3′,5′-monophosphate versus the nominal 8-Cl-cAMP concentration. The correlation coefficient

was 0.9941 or better. The equation for the relationship of 8-Cl-cAMP in plasma was $y = 1.406$ (± 0.006) $x - 0.0508$ (± 0.008), where y represents the peak area ratio of 8-Cl-cAMP and internal standard, and x is the analyte concentration in μM.

3.3. Stability

8-Cl-cAMP and 2′-*O*-monobutyryladenosine-3′,5′-monophosphate were found to be stable in the final reconstituted solution for the period of 10–12 h during which samples were in the instrument, and for at least 4 weeks, when stored at −20°C.

3.4. Validation

The results of intra-day and inter-day precision and accuracy for LQC, MQC, and HQC of 8-Cl-cAMP in dog plasma are reported in Table 1. All coefficients of variation (CV) were less than 11%.

3.5. Dog study

Fig. 4 shows the mean plasma concentration profiles for intermediate and high doses of 8-Cl-cAMP during first and second infusion cycles. The comparisons of mean plasma concentrations for male and female groups during first and second infusions are presented in Figs. 5 and 6.

The plasma concentration–time points for the first and the second infusion period within each same gender group (male or female) were compared using two-sited paired Student's t test, and the plasma concentration-time points for male vs. female groups were compared using two-sited unpaired 't' test with 95% confidence intervals. The difference between the first and the second infusion for males or for females, and difference between male and female groups, as well, were found to be not statistically significant ($P > 0.05$).

4. Conclusion

The CE method developed and validated in this work was found to be accurate for estimating 8-Cl-cAMP concentration in dog plasma after two, 5-day cycles of continuous intravenous infusion at the doses of 1.08 and 3.00 mg/kg per day.

Acknowledgements

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