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Pharmacokinetics of phosphocreatine and its active metabolite creatine in the mouse plasma and myocardium

Lei Xu [a], Chang-Yuan Wang [b], Li Lv [b], Ke-Xin Liu [b], Hui-Jun Sun [b,*], Guo-Zhu Han [b,*]

[a] Department of Medical Affairs, The First Affiliated Hospital, Dalian Medical University, Dalian, China
[b] Department of Clinical Pharmacology, College of Pharmacy, Dalian Medical University, Dalian, China

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ABSTRACT

Background: The pharmacokinetic (PK) studies of phosphocreatine (PCr) and its active metabolite creatine (Cr) are considerably lacking. This study is to comparatively investigate the PK profiles of PCr and Cr in mice plasma and myocardium as well as the ATP level.
Methods: After *iv* administration of equimolar PCr and preformed Cr to healthy and Pit-induced myocardial ischemic mice, plasma and myocardium samples were analyzed for exogenous PCr, Cr and related ATP concentrations using a specific ion-pair reversed-phase HPLC–UV assay.
Results: The plasma *C–T* data of iv PCr and Cr were well fitted to two-compartment model. Following iv PCr, Cr appeared in plasma as early as 1.0 min postdose with a longer $t_{1/2}$ than PCr and had a f_m of 72%. The mice dosed iv PCr preceded 5 min by ip Pit 30 U/kg showed longer $t_{1/2\beta\ PCr}$ and $t_{1/2\ Cr}$ in plasma and elevated $C_{max,\ Cr}$ and $C_{max,\ ATP}$ in myocardium compared with mice dosed iv PCr alone, and it was estimated that about 40% ATP produced by *iv* PCr was from Cr.
Conclusion: The PCr in plasma is converted to Cr rapidly and mostly, and shows an elimination rate limited (ERL) metabolite disposition. *Iv* PCr caused a significantly elevated and long-lasing myocardial ATP and Cr levels. The Pit-induced myocardial ischemia brings slower elimination of PCr and Cr and higher peak concentrations of Cr and ATP in myocardium. The metabolite Cr at least partially mediates PCr-caused rise in myocardial ATP level and also possibly the cardio-protective effects of PCr.

Introduction

Phosphocreatine (PCr), an important high-energy phosphate compound naturally occurring in the body of mammalian animals and humans, plays key roles in cellular energy metabolism by serving as an immediately available temporal energy buffer and a spatial energy buffer or intracellular energy transport system (the CK/PCr energy shuttle or circuit) [1,2]. Now PCr can be obtained by artificial synthesis. Exogenous PCr has currently become the most extensively prescribed cardio-protective drug due to its high efficacy in protection of myocardium against ischemic injury and its excellent safety [3–8], and has been recorded in Martindale: The Extra Pharmacopoeia [9] and Martindale: The Complete Drug Reference [10]. More interestingly, it has been also shown by recent numerous studies to be a potential neuro-protective agent [11], and thus exhibits very attractive broad developing prospect.

Notwithstanding extensive research on pharmacological effects of PCr and creatine (Cr), the study on their pharmacokinetics (PK) is considerably lacking [4,5,12]. The extensive studies of their PK, especially the metabolic disposition in heart are inevitable for their further development and rational application as well as clarification of action mechanisms. In recent years, a growing body of attention has been paid to PK and pharmacodynamics (PD) of the metabolic products of drugs. Most importantly, Cr as a metabolic product of PCr has increasingly been shown also to have extensive pharmacological activities and is under development as one of ergogens that may provide a useful therapeutic strategy to patients with neurological diseases [13,14]. Unfortunately, to date, no information is available regarding the comparison in PK between PCr and Cr. Therefore, a comparative study between parent drug and metabolites of PCr becomes very necessary. On the other hand, it has been well documented that PCr can donate phosphoryl group

Abbreviations: Cr, creatine; ERL, elimination rate limited; f_m, metabolite formation fraction; ip, intraperitoneal; IP-HPLC, ion-pair high performance liquid chromatography; PCA, perchloric acid; PCr, phosphocreatine; PD, pharmacodynamics; Pit, Posterior Pituitary Injection; PK, pharmacokinetics; QC, quality control.

* Corresponding authors.
E-mail addresses: sunhuijun@hotmail.com (H.-J. Sun), hgzhx2236@sina.com (G.-Z. Han).

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to ADP to re-synthesize ATP so as to maintain high-energy phosphate pool at constant level, and Cr as an energy precursor can promote PCr production [15]; but, little is known about the influence of *iv* administration of PCr and Cr on ATP level in myocardium and if the Cr as metabolite mediates PCr-caused elevated ATP level.

In light of the above facts, the present study aims: (1) to comparatively study PK properties of PCr and its active metabolite Cr after intravenous (*iv*) administration of both PCr and preformed metabolite Cr to mice with particular focus on the metabolic disposition in plasma and myocardium by means of an improved ion-pair reversed-phase HPLC assay; (2) to investigate influence of *iv* administration of PCr and Cr on related ATP level in attempt to get deep understanding of possible action mechanism of PCr in cardio-protection.

Materials and methods

Animal experiment

Male Kunming mice weighing 18–22 g were obtained from Animal Center of Dalian Medical University (Dalian, China) and housed in standard laboratory conditions and allowed access to water and commercial mouse chow ad libitum. The animals were maintained to adapt to the new environment for one week before experimental procedures. All animal care and experiments were carried out in accordance with the institutional guidelines and ethics.

Mice, which were randomly grouped according to sampling time points (5 animals per time point), were administered *iv via* tail vein with PCr or preformed metabolite Cr at equimolar dose of 1000 mg/kg or 456 mg/kg, respectively. The blood samples (400 μl per mouse) were drawn prior to dosage and at 1, 2, 5, 10, 30, 60, 90, 120, 180 and 360 min post dosing, from orbital sinus of mice grouped as per sampling time points, respectively, into heparinized eppendorff tubes and immediately centrifuged at 3000 r/min for 10 min at 4 °C to yield supernatants as plasma samples, which were immediately stored at −20 °C until assayed. After blood sampling, the myocardium tissues were collected predose and 5, 30, 60, 90, 120, 180, 240, 360 and 480 min postdose *via* decapitation of mice to die and dissection, and immediately stored at −20 °C until preparation of myocardium homogenate and pretreatment of samples for HPLC analysis.

For investigation of the PK properties in mice with myocardial ischemia, the drug administration was preceded 5 min by intraperitoneal (ip) injection of Pit 30 U/kg.

Chemicals and reagents

Test drug PCr, as creatine phosphate disodium tetrahydrate (*Mr* 327, >97% purity) formulated as sterile powder for injection, was a generous gift from Bolai Pharm (Harbin, China) and Cr, as Cr monohydrate (*Mr* 149, 98% purity), was purchased from Bailinwei Chemicals (Shanghai, China). Reference standards of PCr, Cr and ATP (>99% purity, respectively) were obtained from Merck (Germany and USA, respectively); Posterior Pituitary Injection (pituitrin, Pit; 6 U/ml by bioassay) was supplied by Tianjin Biopharm (Tianjin, China); trimethoprim (TMP) as an internal standard (IS) (99% purity) came from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); tetrabutyl ammonium hydrogen sulphate (TBA) was used as ion-pairing reagent (JK Co., Ltd., USA); MeOH (Tedia, USA) was of HPLC grade; and other chemicals including perchloric acid (PCA), KH_2PO_4, NaOH, K_2CO_3 and heparin were all of analytical grade. The bidistilled water was used for preparation of mobile phase and solutions.

Sample analysis

Samples of plasma and myocardium collected were analyzed using an Agilent 1100 series LC system, as described by Lv et al. [16], and by means of ion-pair reversed-phase HPLC–UV assay modified from that of Teerlink, Sanduja, Cordis, Ally and Juengling [17–21], which permits the simultaneous assay of PCr, Cr, ATP and TMP as internal standard (IS).

Briefly, a 100 μl aliquot of plasma spiked with 20 μl of TMP solution (5 mg/ml) was deproteinized with equal volume of pre-cooled 6% PCA and subsequently neutralized with 2 mol/L K_2CO_3 to pH 7.0, then 10 μl of supernatant obtained after centrifugation was chromatographed on a Kromacil C_{18} column (250 mm × 4.6 mm, 5 μm) (Ilite, Dalian, China) coupled with a Kromasil C_{18} guard column (20 mm × 4.6 mm, 10 μm) (Ilite, Dalian, China) using a tertiary gradient mobile phase consisting of 0.2% KH_2PO_4 containing 0.08% TBA (pH 3.0) as solvent A, above mentioned solvent A adjusted to pH 7.5 with 1 mol/L NaOH as solvent B and MeOH as solvent C, delivered at a flow rate changed from 1.0 to 1.3 ml/min; the following gradients were used: 0 min (100% A, 1.0 ml/min) → 10 min (100% A, 1.0 ml/min) → 11 min (80% B/20% C, 1.2 ml/min) → 19 min (75% B/25% C, 1.2 ml/min) → 20 min (75% B/25% C, 1.3 ml/min) → 33 min (75% B/25% C, 1.3 ml/min) → 34 min (100% A, 1.0 ml/min). The effluent was monitored at detection wavelength of 210 nm for PCr and Cr and 260 nm for ATP and TMP. The system control and data processing was carried out by a Chemstation 32 software (Agilent, USA). Also, a 200 μl aliquot of myocardium homogenate prepared with ice-cold 6% PCA at a ratio of 1 g:5 ml was analyzed by the same procedures as the above plasma except without additional deproteinization with PCA.

The samples were quantified by reference to accompanying calibration curves constructed by plotting peak area ratio of analyte to IS against known analyte concentrations in samples. For quantification of exogenous compounds, the blank samples of naive mice were initially run for baseline subtraction.

PK analysis

Drug concentration–time data were analyzed based on compartmental technique for parent drug and non-compartmental technique for metabolite using a computer program 3P97 version 1.1 developed by China Math Pharmacology Society. The C_{max} and corresponding time t_{max} were obtained directly from *C–T* profile as actual value measured. The $t_{1/2}$ was calculated as ln 2 divided by the corresponding rate constant. The terminal rate constant (λ) was estimated from the last ln-linear phase slope. The AUC and AUMC of the compounds of interest were calculated by the trapezoidal rule and extrapolated to infinity using λ. The CL was estimated from the dose divided by the AUC. The V_d was estimated from CL/λ. The MRT was calculated as AUMC/AUC. The metabolite formation fraction (f_m) was estimated from:

$$f_m\,(\%) = \frac{AUC_{(m)}}{AUC'_{(m)}} \times 100 \quad (1)$$

where $AUC_{(m)}$ and $AUC'_{(m)}$ were AUC of Cr produced after iv administration of PCr and chemically synthesized Cr, respectively, at equimolar doses. The fraction of PCr-derived ATP accountable for by Cr-derived ATP ($f_{ATP,\ Cr/PCr}$), which served to express the extent of contribution made by Cr to PCr-caused rise in ATP level in myocardium, was estimated from:

$$f_{ATP,Cr/PCr}\,(\%) = \left(\frac{AUC_{ATP,Cr}}{AUC_{ATP,PCr}}\right) \times f_m \times 100 \quad (2)$$

where $AUC_{ATP,\ Cr}$ and $AUC_{ATP,\ PCr}$ were AUC of ATP in myocardium after iv equimolar doses of Cr and PCr, respectively. The analyte