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Review Article

Research Progress in Pharmacokinetics of Phosphocreatine a Cardioprotective Agent with a Dual Antiplatelet Activity

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Abstract

Phosphocreatine (PCr), a natural physiological active high energy phosphate compound, plays a pivotal role in maintaining energy homeostasis via acting as a temporal and spatial buffers of bodily energy. Exogenous PCr has been developed as a cardioprotective agent with a dual antiplatelet activityand has found wide range of clinical application. Since PCr is known to have many beneficial effects , it is important to elucidate pharmacokinetic(PK) properties of PCr to get deep insight to action mechanism and clinical rational uses. Thus, here we review its PK profiles in animals and humans as well as possible transmembrane transport mechanism based on the present writer's years of devoted research and published reports. Accruing evidence shows that PCr belongs to a two-compartment model drug. Regardless of animal species and humans, PCr exhibits rapid elimination from body. IP-HPLC and HPLC-MS/MS bioanalysis have demonstrated that most of PCr entering systemic circulation is converted to creatine (Cr). Iv administration of PCr resulted in elevated ATP level in heart and RBC. Cr as an active metabolite has proved to partially mediate PCr hemorheological improvement. PCr can be taken up by myocardiocytes, in spite of slow transcellular rate, via some special and hypothetical transport mechanisms.

INTRODUCTON

Phosphocreatine, also known as creatine phosphate, is a N P bonded guanidino high energy phosphate compound founded in mammalian animals and human beings. It plays a pivotal role in the homeostasis of cell energy by acting as a form of energy reservation (temporal energy buffer) and an intracellular energy transport carrier known as CK/PCr energy shuttle or circuit(spatial energy buffer) [1,2].

Exogenous PCr has been synthesized as creatine phosphate disodium tetrahydrate and made into sterile powder for injection for clinical application. It has been developed as a high efficacious and low toxicity cardioprotective drugwith a dual antiplatelet activity [3,4] Its therapeutic effects rank the first among drugs of the same kind [5]. Recent studies have shown that it is a multi-target drug, which has found wide range of clinical application, especially in the treatment of cardiovascular diseases [6,7]. It has been published in the Martindale: The Complete Drug Reference [8] and the China Pharmacopoeia [9].

Although the pharmacodynamics (PD) of PCr has been well studied, its pharmacokinetic (PK) study is relatively insufficient and lag. In the early years, several scholars utilized non-specific spectrophotometric analysis methods for determination of concentration of PCr in plasma, and the reliability of the resultant PK was poor, and only 3 articles were reported [10-12]. Since the 21st century, the emergence of highly specific and sensitive bioanalytical methods has led to a rapid development of PK research of PCr. The PD study of PCr has been extensively reviewed by several scholars of note [4-7], however, to date, there is no article reviewing PK of PCr. In light of the above fact, the present paper is undertaken to specifically review the advances in PK research of PCr to provide insight to how to rationally use PCr in clinical settings and elucidate its action mechanisms.

NON-CLINICAL PK STUDY

PK Study of Intravenous(iv) PCr

Over the past 2 decades, the authors developed and validated a specific and sensitive ion-pair HPLC (IPHPLC) method for simultaneous determination of PCr and its active metabolite creatine(Cr) as well as related ATP in the plasma, red blood cell(RBC) and myocardium ,and thereby to study PK of PCr [13-17]. After iv dosing 1000 mg / kg to mice, 500 ~ 1000 mg / kg to rats and 500 mg / kg to rabbits, the plasma concentrations of PCr showed a bi-exponential decay, which could be best fitted by

the two-compartment model assuming the first order kinetics (Figure 1). PCr was found to be eliminated rapidly from blood circulation system ($t_{1/2}\beta$ 22-38 min) and rapidly degraded to creatine (Cr). The plasma concentration (C) -time (T) curve of Cr manifested as an initial rapidly ascending formation phase followed by a slowly descending elimination phase , which could be best simulated by means of the one-compartment model of extravascular administration. Cr concentration quickly reached its maximum (t_{max} < 30min), but elimination was slow, $t_{1/2}$ being about 2 times that of the parent drug. Therefore, the metabolite PK shows the elimination rate-limiting (ERL) rather than the formation rate-limiting (FRL) *in vivo* disposition characteristics, that is, the elimination of Cr is carried out at its own intrinsic rate constant, independent of the degradation of the parent PCr [16,17]. The formation fraction of Cr(f_m) is 71-76% , meaning that about three fourths of PCr is converted to Cr, which constitutes the main elimination mode of PCr in the body [14-16]. After iv administration, PCr was not detected in the myocardium, but Cr and related ATP are detected at about 2 times higher than the baseline value and maintained for 480 min (Figure 1), with $t_{1/2}$ of 350 min and 680 min respectively, which was much longer than the $t_{1/2}$ of PCr and Cr in plasma [14].

In mice with ischemic myocardium , iv dosed PCr resulted in an increased plasma $t_{1/2}\beta$ and AUC of PCr by 52% and 55%, respectively, and a similar increase in that of Cr compared with healthy mice, It may be related to the decrease of cardiac output, which eventually led to the decrease of clearance ability of clearance organs. In addition, the levels of Cr and ATP in ischemic myocardium were significantly increased over normal myocardium (375 and 175 vs133 and 126 μg / g, $p < 0.01$), and lasted for a long time (500 min) [14]. After iv PCr in rats and rabbits, PCr was not detected in RBC. However, Cr and related ATP appeared in RBC almost immediately, and showed a first rising and then falling in concentration, with the main PK parameters of Cr/ATP as follows: $t_{1/2}$ of about 70 / 50 min, t_{max} of 120 / 67-82 min, C_{max} being 3-5 times the basic value, and they were maintained for a long time (200-300 min, respectively) [15,16].

More importantly, during the PK study of PCr authors were the first to advance a new concept concerning the apparent activity ratio(R_{app}) and real activity ratio(R_{real}) of metabolite to parent drug as an essential part of metabolism disposition research of drugs [16]. The R_{app} is defined as $R_{app} = AUC_{E',met} / AUC_{E,.parent,}$ where $AUC_{E,met}$ is area under effect(E)-time(T) curve of preformed metabolite administered at equimolar dose to parent drug , $AUC_{E,parent}$ refers to that of parent drug. The R_{real} is defined as $R_{real}=R_{app}xf_{m}$, namely corrected R_{app} by f_m [16]. The R_{app} and R_{real} of the Cr has been found to be 0.53-0.68 and 0.38-0.48, respectively [16]. Meanwhile, It has also been found that the $f_{ATP,Cr/PCr}$ (ratio of the increase of ATP induced by metabolite Cr to that induced by the parent PCr) , which is used to express the extent of Cr-made contribution to PCr-caused rise in ATP level, was 40-43%, meaning that about 40% of PCr-caused ATP increase derives from its metabolite Cr [14-16]. The $f_{ATP,Cr/PCr}$ is

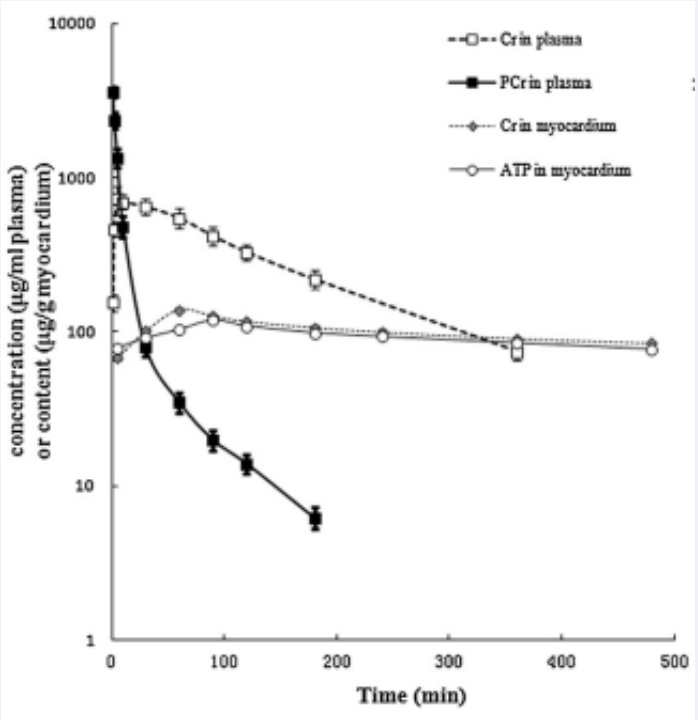

Figure 1 Mean plasma and myocardium concentration–time.

shown to be consistent with R_{real} of Cr, this coincidence further supports that Cr partially mediates the myocardial protection and hemorheological improvement of PCr by increasing ATP levels [16].

PCr is mainly distributed in high energy consumption tissues such as myocardial and skeletal muscle, followed by brain and kidney, and least in lung and liver. This specificity may be directly related to the structure and organization of cell phospholipid membrane and its combination [4]. The main metabolic pathway of PCr is dephosphorylation to Cr, which in turn produces creatinine(Cre) through non-enzymatic cyclization, and finally excreted by the kidney [18]; PCr can also be excreted by forming an intermediate product, phosphocreatinine, which is hydrolyzed into Cre, which accounts for only 20% ~ 25% of the total amount of Cre produced by PCr [19] .

The Pk Study Of Oral Pcr

After oral administration of PCr, the parent drug was not detected in the plasma, but significant level of metabolite Cr was detected. This can be explained by (1) strong polarity of PCr intact molecules, leading to its difficulty to be absorbed through gastrointestinal tract; (2) acid instability of PCr , leading to its degradation to Cr in the stomach by gastric acid, and subsequent absorption of Cr into the blood by means of specific Cr transporter (CrT) in the gastrointestinal tract [15]. The C-T course of the above-mentioned Cr absorbed can be best described by a one-compartment model of extravascular administration, with $t_{1/2}$ of 56 min, t_{max}90-95min, $F_{(m)}$(metabolite bioavailability) 55-62% [15], indicating that over half of the Cr produced by PCr is absorbed. In addition, the oral administration of PCr results in more than half of PCr being converted into Cr. The above can explain why PCr is not recommended to be administered orally and must be dosed by injection [15].

CLINICAL PK STUDY

In the last century, there were three reports on the PK of PCr in humans, which also showed that iv dosing PCr was followed by a biphasic rapid clearance like that in animals [10-12]. Lorenzi E et al. [10], administered a single iv therapeutic dose of PCr to 10 healthy subjects. Blood concentrations were measured by bioluminescence, The $t_{1/2}\beta$ of PCr was as short as 20 min, V_d 50.32 L, CL 1.63L / min. When the plasma concentration of PCr attenuated, a progressive increase of whole blood ATP concentration was evident, which was about twice the baseline value and lasted for up to 5 hours [10]. Sharov VG et al., administered a single iv dose of 14 mg / kg PCr to patients with HDD. Blood concentrations were measured by spectrophotometry. Its distribution and elimination was rapid, as reflected by very short $t_{1/2}\alpha$ of 5.7 min and $t_{1/2}\beta$ of 27 min. Plasma PCr was maintained at 0.2 mM level when iv injected at 60 mg / kg / h. The plasma concentration of PCr decreased rapidly in biphasic manner after discontinuation of the drug, with $t_{1/2\alpha}$ 4.6 min, $t_{1/2}\beta$ 50 min. [11]. Afonskaia NL et al., obtained similar results in human studies [12]. Many studies have shown that the increase of myocardial ATP level after the administration of PCr results from a direct phosphorylation of ADP by PCr via the CK reaction and the role of the metabolite Cr as a mitochondrial oxidative phosphorylation (OXPHOS) stimulant [20].

In recent years, Sun N et al developed a specific and sensitive HPLC-MS / MS method for the simultaneous determination of PCr and its metabolites Cr and Cre in children's plasma, and successfully applied this method to study the PK and metabolic disposition in 6 Chinese children with viral myocarditis after iv infusion of 2g PCr [21]. It has been found that iv PCr to the above children is followed by a rapid clearance of PCr from blood and a almost immediate appearance of Cr, and subsequent rapid rise in Cr concentration, with C_{max} exceeding the parent drug as well as slow Cr clearance. A slight change in the plasma concentration of Cre as the final metabolite of PCr is evident ,as reflected by a narrow fluctuation range of 3.40–5.62 μg/mL. The concentration of Cre is returned to baseline after 2 hours. It is inferred that the human body has sufficient capacity to clear the excess Cre in plasma introduced by exogenous PCr. The PK parameters of PCr / Cr are as follows: $t_{1/2}$ 0.24 / 0.83 h, T_{max} 0.49 / 0.55 h, C_{max} 47.34 / 59.29μg / ml, AUC 17.16 / 79.01 h ▪ ug / ml and MRT 0.29 / 0.67h [21]. From the above parameters, it is also found that in the abovementioned children, most of iv PCr is converted to Cr, and elimination of Cr as metabolite is conducted in much slower rate than the parent drug.

More recently, a parent-metabolite joint population PK (PPK) model has been successfully developed for the first time by He H et al. to characterize the PK profile for PCr and its metabolite Cr in children suffered from myocarditis [22]. A total of 947 plasma samples from 100 pediatric patients with myocarditis were determined by HPLC-MS / MS assay with LLOQs of 1.96 μmol/L and 30.53 μmol/L for PCr and Cr, respectively. A NONMEM approach was used to build the PPK model. Allometric scaling based on body weight is applied to PK parameters. The results have demonstrated that the in vivo process of PCr and Cr after iv infusion of PCr can be adequately described by a four-compartment chain model (central and peripheral compartments for both PCr and Cr) with the first-order elimination. The covariate analysis identified that the glomerular filtration rate (GFR) was strongly associated with Cr clearance (CL_{cr}) as follows: CL_{cr} (L/min) = $0.0825\times(BW/20)^{0.75}\times(GFR/127.78)^{0.311}\times e^{\eta CLcr}$. The simulation results showed that there was no accumulation phenomenon in vivo for PCr. Bootstrapping and visual predictive checks suggested that a robust and reliable PPK model had been developed. With the infusion of PCr, the concentration of Cr increased rapidly [22]. The joint PPK simulation fully describes the PK characteristics of both the parent and metabolite, and the individual predicted values are close to the measured values. This model is not only helpful for the rational application of PCr in pediatric clinical practice, but also provides an example for the future study of the parent - metabolite joint PPK of other drugs.

Cellulae Uptake and Possible Transmembrane Transport Mechanisms of PCr

Cellular Uptake

In view of the indisputable cardioprotective effects of PCr, the

question to be addressed is whether this extremely polar molecule can be taken up across cell membrane by myocardiocytes ? The following typical studies almost give a positive answer.

(1) The in vitro incubation experiment of rabbit heart slices with ^{14}C-^{32}P double-labeled PCr and in vivo experiment with iv PCr in rabbit carried out by Breaccia A et al. [23], revealed significant myocardial uptake of the double-labeled marker and showed that the ^{14}C: ^{32}P ratio (40: 60) remained constant during cellular uptake. Moreover, TLC analysis of myocardial homogenate and supernatant showed that the double markers moved parallel to the intact PCr, which confirmed that PCr could pass through the myocardial cell membrane, even through the mitochondrial membrane. The study also found that the uptake process can be inhibited by hypoxia, suggesting that 1) the uptake process is energy-dependent; 2) this inhibition is secondary to energy depletion; 3)the binding of PCr to mitochondria requires the integrity of certain specific binding sites, such as CK [23].

(2) A study by Down et al. [24], who gave rats iv^{32}P-PCr 50 mg / kg, and detected the myocardial extract by HPLC, revealed that the levels of ATP and PCr in myocardium at 120 min after administration were significantly higher than those in the control group (3.5 vs 2.5 μmol / g and 2.5 vs 1.7 μmol / g), Furthermore, there was a significant incorporated radioactivity in ATP molecules, amounting to 61.1-95.3 nmol 32P-ATP/g tissue. The increase of in ATP myocardial content may be caused by the phosphorylation of ADP catalyzed by mitochondrial CK after intake of PCr [24].

(3) Preobrazhenskii et al found that 32P-PCr could be taken up by isolated perfused rat heart [25]. When the myocardial ischemia lasted for 35min, the uptake rate was twice as fast as that of the control group. As the concentration of ^{32}P-PCr in perfusate was 10mM, the uptake rate reached 182 nmol/min/g dry weight [25].

(4) In a study, carried out by Rosenshetraukh et al. [26], with isolated frog ventricular muscle model perfused by Ringer solution, it was found that the increase of PCr concentration in myocardiocytes was linearly correlated with the concentration of PCr in perfusate, and the increase of cardiac contractility and action potential duration (APD) was parallel to the concentration of PCr, suggesting that myocardiocytes was permeable to PCr [26]. It was also found that the cardiac contractility increased rapidly and significantly when PCr was added to 10mM and 20mM [26], which was consistent with the optimal concentration of PCr in surgical cardioplegic solution and in anoxic and glucose deficient cell incubation model [4,27]. However, when the concentration increased to 70mM, cardiac contractility and APD changed inversely. Presumably, this might be due to the decrease of Cr rephosphorylation caused by excessive concentration of PCr competing with Cr for the active center of CK in mitochondria [26]. Moreover, high Na^+ concentration was found to inhibit the PCr-induced excitatory effect on myocardial contractile force, which may be a result of the influence of high concentration of Na^+ on the ion transport of cell membrane [26]. Since PCr is administered in the form of sodium salts, the correction of the salt composition in Ringer's solution is of special significance [26].

(5) Soball et al., found that PCr can be taken up by the mitochondria of isolated rat heart and also by the liposomes [28].

The above experimental studies show that PCr can be taken up by myocardiocytes, but the literature also points out that this rate of uptake is slow, much lower than the rate of ATP metabolic turnover in the working heart [4,6]. However, this low rate is important in maintaining the high-energy phosphate pool under the sarcolemma. In this local pool, PCr plays an important role in adenine nucleotide metabolism through inhibiting nucleotide catabolism or by activating adenylate pool de novo synthesis and salvage pathway [4,6]. Since the above necessary related enzymes are confined to the sarcolemma, the rapid uptake of PCr may be unnecessary [4,6].

It is worth emphasizing that the cellular uptake of exogenous PCr, which leads to increased levels of high-energy phosphate pools and adenine nucleotides, only is a part of its roles, and it has increasingly been recognized as a multi-target action mechanism drug, including not only energy-related but also non-energy-related mechanisms, not only intracellular but also extracellular mechanisms [7]; in addition, not only the parent drug plays a role, but also its metabolites partly mediate its myocardial protective effect. In fact, many of the pharmacological activities of PCr are the result of the synergistic effect of its prototype molecule and its metabolite, and the literature also indicates that the effect of PCr is likely to be more dependent on its pleiotropic effect [7,15,16,29].

Possible Transmembrane Transport Mechanisms

According to classical theory, PCr, a polar compound, should be difficult to penetrate cell membrane by means of passive transport. To date, it has not been confirmed that PCr has a specific transmembrane transport system. Of concern is how to explain its complex transmembrane transport mechanism? To one's joy, there have now been several hypotheses that suggest possibility of PCr to be taken up by cells in the following special ways:

(1) PCr can be combined with divalent metal ions as activators or inhibitors of transphosphoryl reactions to form a complex, thereby weakening its polarity, so that it can pass through the cell membrane, or modify the spatial arrangement of membrane proteins to affect membrane permeability [23].

(2) PCr may, like another important high-energy phosphate compound ATP, interact with some specific sites on the cell surface to induce the flow of ions across the membrane: Na^+ inflow and K^+ outflow, resulting in a decrease in membrane potential ($\Delta\psi$) and depolarization of the membrane. The dissipation of $\Delta\psi$ induces conformational changes in membrane components, leading to the formation of aqueous channels, or by affecting the topology of the membrane, such as the polymerization of membrane proteins to form aqueous channels [20,30].

(3) PCr cell uptake can be inhibited by hypoxia, suggesting that this is an energy-dependent active uptake process [23].

(4) PCr may enter the cell through non-specific mechanisms, such as receptor-mediated endocytosis, and it is even hypothesized that PCr may enter the cell through voltage-dependent anion channels on the serosa [31,32].

(5) Cr as metabolite is likely to mediate the transmembrane transport of PCr, It have been confirmed that PCr is mostly metabolized into Cr in vivo [14-16]. The latter enters the cell by means of CrT on effector organ cells and acts as an energy messenger and energy precursor as well as OXPHOS stimulant [18,20,26,33] to promote mitochondrial ATP synthesis and subsequent regeneration of PCr via the CK reaction [18,20,26,33]. In fact, the aforementioned notion has gained further support from a study on cellular PK of Cr performed by Speer et al., who found that once Cr entered the cell, Cer would be taken up by mitochondria via CrT, and finally, about 2/3 of Cr in cytosol was phosphorylated to PCr [18,34].

8. Conclusion and Outlook

This review summarizes for the first time the PK research progress of PCr. The accumulated data show that the PK of single iv dose of PCr is biphasic, with rapid elimination of PCr from systemic circulation and most conversion to Cr in plasma, accompanied by an increase in RBC and myocardial ATP levels. Cr partially mediates the hemorheological improvement of PCr. Many evidences reveal that Cr is a pleiotropic substance with various pharmacological activities. Iv Cr can also increase the level of ATP in RBC and myocardium. It is reasonable to believe that Cr may also partially mediate the myocardial protection of PCr. Limited data suggest that PCr can enter cells, albeit at a low rate. A PCr (parent drug)-Cr (metabolite) joint model in Chinese children with myocarditis has been established for the first time, using NONMEM program to characterize the PPK properties of the PCr and Cr. This pioneering study provides a PPK basis for the rational use of PCr in pediatric clinical practice, and also affords a reference for similar studies of other drugs.

Despite achieved significant progress in PK studies of PCr, there are still some aspects that require further research. For example, (1) Cellular PK studies of PCr: Current studies of PCr mainly focus on in vivo animals and human subjects, and there is an urgent need to deepen the PK studies of PCr from conventional “macroscopic” blood drug concentration to “microscopic” cellular/subcellular PK research to understand how the drug molecules are transported across cell membrane and how intracellular drug molecules are transported to action target sites such as mitochondria, etc, as well as how much the drug concentration of action target sites is , and thus to resolve PK/PD inconsistency and other related problems; (2) Relative contribution of Cr as a active metabolite to cardioprotective effect induced by PCr as parent drug. Albeit demonstrated Cr-partially mediated improvement of hemorheology induced by PCr, whether or not Cr also similarly mediates PCr-induced cardioprotection remains urgently to be elucidated. In early years, a study once deemed that PCr –induced cardioprotection derived from intact PCr molecule only, but not Cr plus P_i [35]. However, author’s studies have shown that iv Cr also can elevate cardiomyocye ATP level, It seems to indicate the possibility of Cr also partially to mediate PCr-induced cardioprotection [4-16]. This discrepancy should be clarified. Cr effect has not to be neglected or underestimated; (3) Clinical PK (CPK) studies of PCr: In relation to non-CPK study, its CPK study is insufficient and thus should be intensified to optimize clinical dosage regimen; (4) PK-PD combination model studies of PCr: Use of this model to simultaneous explore blood drug concentration(C)- time(T)-effect (E) interrelationship has major significance for elucidating action mechanisms and optimizing dosage regimen. This is especially important for PCr. It has been demonstrated that the effects of PCr are the results of synergistic action of both PCr and Cr, moreover, there exists a constraint-dependency relationship between the PCr disappearance and subsequent Cr appearance. Advancing this model is a new challenge, requiring innovative idea to resolve the complicated and difficult problem.

Overall, further exploration of the PK research of PCr is eagerly in need. We firmly believe that with rapid advances in modern molecule biology and more specific and sensitive bioanalysis technology, the PK of PCr will surely be fully characterized to make much more contribution to its clinical rational application and elucidation of action mechanisms.

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