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REVIEW

Creatine Phosphate: Pharmacological and Clinical Perspectives

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ABSTRACT

Since the 1970s, extensive experimental and clinical research has demonstrated that relevant reductions of creatine phosphate (CrP) or phosphocreatine availability occur in a wide spectrum of pathophysiological situations. A decrease in intracellular concentrations of creatine (Cr) and CrP results in a hypodynamic state of cardiac and skeletal muscle pathology. Many experimental and clinical studies have evaluated the possibility to improve cardiac and skeletal muscle performance by exogenous administration of CrP. Furthermore, many experimental studies have shown that CrP may play two important roles in the regulation of muscle energetics and work. First, CrP maintains local adenosine triphosphate pools and stabilizes cellular membranes due to electrostatic interactions with phospholipids. The second mechanism decreases the production of lysophosphoglycerides in hypoxic hearts, protects the sarcolemma of cardiac cells against ischemic damage, decreases the frequency of arrhythmias, and increases post-ischemic recovery of contractile function. Recent research on CrP has demonstrated positive therapeutic results in various clinical applications. These benefits have been applied in several pathological conditions, such as heart failure, acute myocardial ischemia, chronic ischemic heart disease, cardiac surgery, skeletal muscle hypotonotrophy, and cerebral ischemia. This review describes the CrP shuttle, pathophysiological basis of the supplementation of CrP, and its therapeutic effects in multiple clinical conditions. The major aim is to summarize results of the intense research carried out over 40 years to provide evidence to support the adjunctive use of CrP in

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many pathological conditions that may target cellular energy impairment; thus, increasing energy metabolism.

Keywords: cardioplegia; cardiac surgery; congestive heart failure; creatine phosphate or phosphocreatine; CrP; myocardial ischemia; skeletal muscle rehabilitation and performance

INTRODUCTION

Creatine (Cr), or methylguanidine-acetic acid, is a naturally occurring compound synthesized from arginine, glycine, and methionine. It is found in meat and fish and is endogenously produced by humans in the liver and pancreas, both of which are capable of de novo Cr synthesis [1]. Cr exists in the cell as both free Cr and creatine phosphate (CrP) or phosphocreatine, which together comprises the total Cr pool. According to Ennor and Morrison [2], this compound should be named "phosphorylcreatine" as it includes a -N-P- bond typical of the phosphorylated guanidines, instead of a covalent binding via -O-, which is characteristic of the phosphate compounds. However, taking into account the long period of clinical use of the names "creatine phosphate" or "phosphocreatine," in the present review these names, or the acronym "CrP," will be used.

The aim of this review article is to describe the role of CrP treatment in cardiological indications, such as cardioprotection in cardioplegia and in myocardiopathies of various etiopathogenesis, as well as in other clinical indications such as skeletal muscle rehabilitation and neurological conditions.

METHODS

To select studies, a Medline search was performed up to October 2011 using the reported key words. Previous review articles on Cr and CrP were screened for relevant information and references. The authors also had access to the CrP database of Alfa Wassermann, Inc. (Bologna, Italy). Results from selected studies were reviewed and classified according to the topics in this review article.

THE CrP SHUTTLE

In tissues with high-energy requirements, such as the heart, skeletal muscle, and brain, CrP plays an essential role. Although the ultimate energy compound used for skeletal and cardiac muscle contraction is adenosine triphosphate (ATP), the primary transport medium of energy in striated muscle is CrP [3,4].

Phosphoryl high-energy bonds transfer from sites of production, the mitochondria, to sites of utilization by means of metabolic relays; namely, the phosphotransferase reactions catalyzed by creatine kinases (CK) of the CrP shuttle (Fig. 1a) [4].

In the heart, ATP is required for cell viability and myocardial pump function. Cleavage of the terminal phosphate by ATPases releases chemical energy that is converted into work, such as contraction, ion pumping, synthesis and degradation of large and small molecules, and molecular trafficking; indeed, all cell functions [5-7].

Because the amount of ATP in the heart is small compared with demand, the myocardial cell must continually resynthesize ATP to maintain normal cardiac pump function and cellular viability.

In striated muscle, not only myofibril contraction is benefited by the CrP, which comes from the mitochondria, but also glucose uptake [8] and the protein synthesis system [9]. Creatine phosphokinase, which uses CrP to fuel protein synthesis, is bound to the microsomes

(which synthesize muscle protein) (Fig. 1b) [4]. Creatine phosphokinase enzymes, which use CrP for fueling glucose transport, are bound to the cell membrane [4].

According to Bessman and Mohan [4], CrP can stimulate microsomal protein synthesis more effectively than ATP. Cr liberation, occurring during exercise, causes an increased influx of CrP to the microsome, which in turn can stimulate microsomal protein synthesis. Exercise is, therefore, equivalent to insulin stimulation of protein synthesis, both causing increased delivery of phosphate energy to the microsome.

ENERGETICS OF THE ISCHEMIC AND FAILING HEART: HIGH-ENERGY PHOSPHATE INVOLVEMENT

An altered high-energy phosphate metabolism has been shown in many in vitro and in vivo experimental models of cardiac pathology [10-12]. Hearse [10], using isolated, anoxia-perfused, working rat hearts, observed a fast reduction of myocardial CrP and contractile activity, and a slower fall of ATP. Similar results were obtained by Whitman et al. [11] in isolated rabbit hearts. Pool et al. [12], in cats with right ventricular hypertrophy and congestive heart failure,

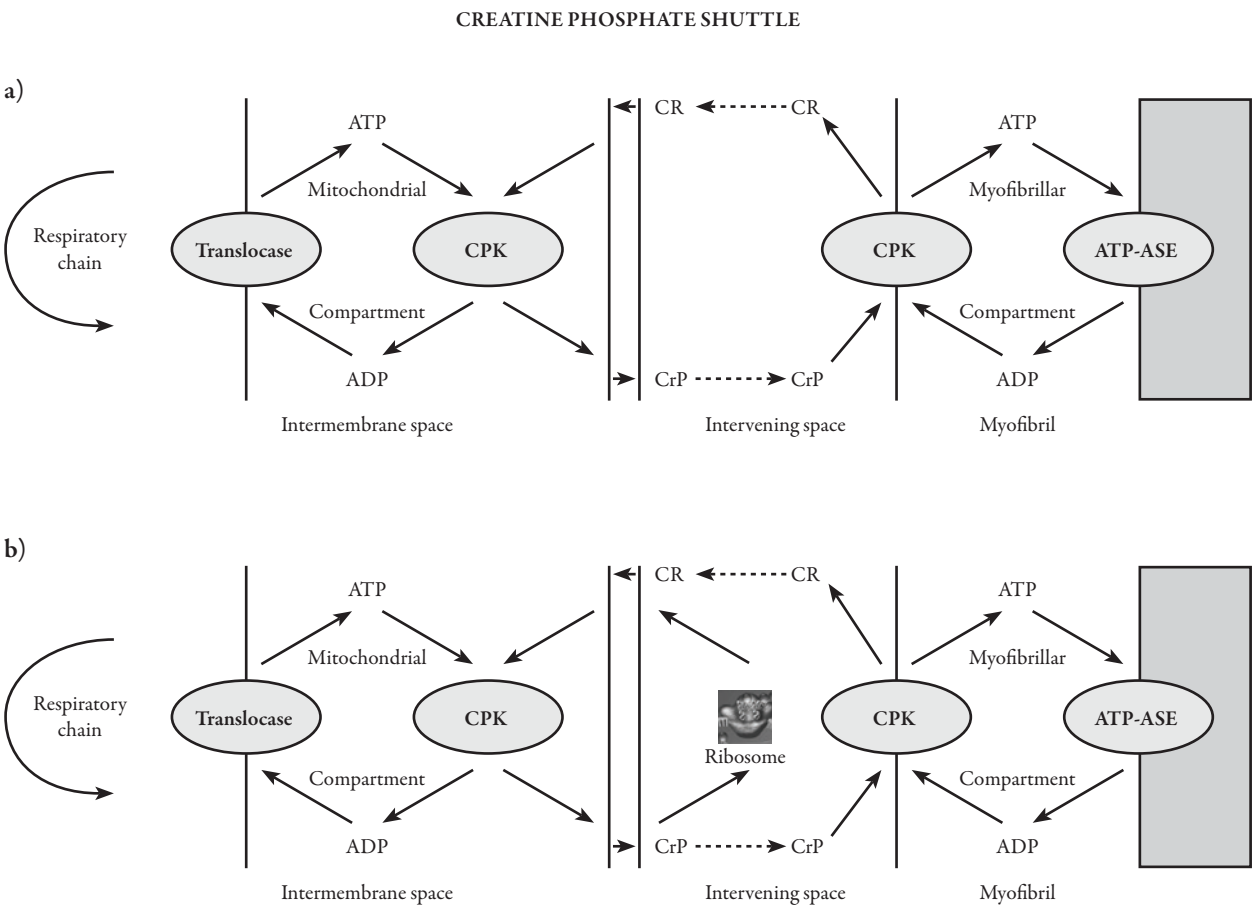

Fig. 1. (a) CrP shuttle and myofibrillar contraction. (b) CrP shuttle and protein synthesis. ADP=adenosine monophosphate; ATP=adenosine triphosphate; CPK=creatine phosphate kinase; CrP=creatine phosphate; CR=creatine. *Reproduced with permission from Bessman SP, Mohan C. Phosphocreatine, exercise, protein synthesis, and insulin. In: PP De Deyn, B, Marescau, V. Stalon an IA Qureshi, eds. Guanidino Compounds in Biology and Medicine; 1992;181-186.*

induced by pulmonary artery ligature, observed a reduction of right ventricular CrP levels; the reduction was greater in cats with congestive failure. In addition, Ye et al. [13] in a porcine model with cardiac hypertrophy and failure due to aortic stenosis, observed a reduction of myocardial CrP, which was related to the severity of the cardiac hypertrophy.

The data in experimental animal models have also been confirmed in human pathology. Decreased CrP/ATP ratios and decreased absolute levels of CrP and ATP have been shown in hypertrophied and failing myocardium, due to a wide variety of etiologies. Weiss et al. [14] showed that the ratio of CrP/ATP in the left ventricular wall did not change during hand-grip exercise in normal subjects and in patients with nonischemic heart disease, but significantly decreased by 40% in patients with coronary heart disease and ischemia, due to severe stenosis of the left anterior descending or left main coronary arteries.

Similarly, Yabe et al. [15,16] observed a significant decrease in the ratio of subendocardial CrP/ATP during hand-grip exercises in a group of patients with severe coronary artery disease. A low CrP/ATP ratio has been shown by Conway [17] in the failing hypertrophied myocardium of patients with aortic valve disease.

In patients with dilated cardiomyopathy, Hardy et al. [18] observed a reduction in the myocardial CrP/ATP ratio. Neubauer et al. [19] reported that the reduction in the myocardial CrP/ATP ratio, measured noninvasively with phosphorus-31 (31P)-nuclear magnetic resonance (NMR) spectroscopy, correlates with the clinical severity of heart failure and improves during clinical recompensation. These data were also confirmed by Naveri et al [20].

Moreover, according to Neubauer [21], the myocardial CrP/ATP ratio is a significant, independent multivariate predictor of cardiovascular mortality in patients with heart failure. In 2½ years, in patients with a normal

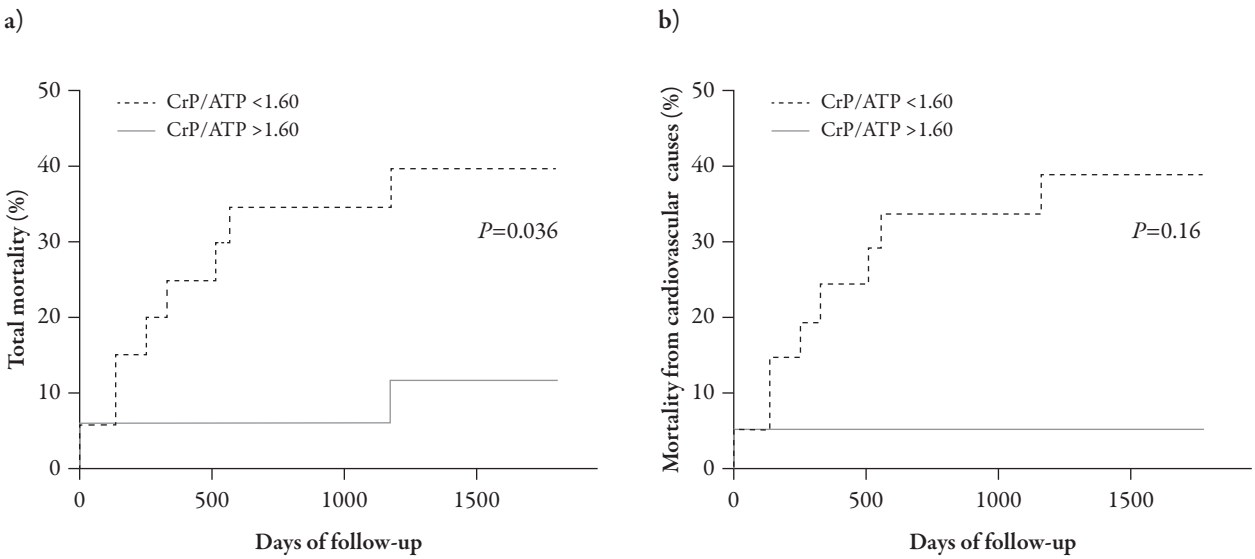

Fig. 2. Kaplan-Meier life-table analysis for total mortality (in percent; a) and mortality from cardiovascular causes (in percent; b) of dilated cardiomyopathy patients divided into two groups split by CrP/ATP ratio (<1.60 vs. >1.60). ATP=adenosine triphosphate; CrP=creatine phosphate. *Reproduced with permission from Neubauer S, Horn M, Cramer M, et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation. 1997;96:2190-2196.*

CrP/ATP ratio (superior to 1.60), cardiovascular mortality was 5% and total mortality was 10%, while in patients with reduced CrP/ATP ratio (inferior to 1.60), cardiovascular and total mortality were 40% (Fig. 2) [21]. In fact, over the course of several years, a low cardiac CrP/ATP ratio is a better predictor of overall and cardiovascular mortality than the New York Heart Association (NYHA) class and left ventricle ejection fraction.

Recently, Chida et al. [22] have demonstrated that myocardial energy metabolism is also correlated with the B-type natriuretic peptide level in heart failure. These investigators have evaluated the correlation between the plasma B-type natriuretic peptide levels and the myocardial CrP/ATP ratio, determined using rapid 31P-NMR spectroscopy in patients with dilated cardiomyopathy. Chida et al. have shown that the CrP/ATP ratio negatively correlates with the severity of heart failure, estimated using the plasma B-type natriuretic peptide (r=–0.54, P=0.06) (Fig. 3) [22].

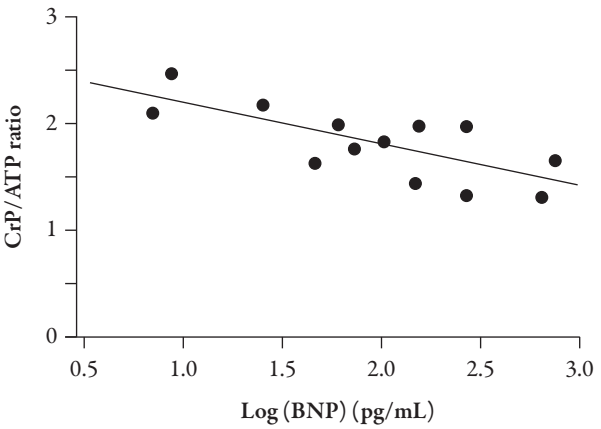

Fig. 3. Correlation between myocardial CrP/ATP ratio and the log of plasma BNP in patients with DCM. ATP=adenosine triphosphate; BNP=B-type nutriuretic peptide; CrP=creatine phosphate; DCM= Dilated cardiomyopathy. *Reproduced with permission from Chida K, Otani H, Kohzuki M, et al. The relationship between plasma BNP level and the myocardial phosphocreatine/adenosine triphosphate ratio determined by phosphorus-31 magnetic resonance spectroscopy in patients with dilated cardiomyopathy. Cardiology. 2006;106:132-136.*

Based on the analysis of biopsy specimens, ATP is 25%-30% lower in the failing human heart [23,24].

According to animal models, the total Cr pool is as much as 60% lower in left ventricular hypertrophy and heart failure [25,26]. This result was confirmed in human myocardial biopsy specimens in the mid-1980s in patients with severe aortic stenosis [27], and in the 1990s in patients with severe heart failure [28].

A study using proton (1H)-NMR spectroscopy demonstrated Cr depletion in human heart failure and, furthermore, that the magnitude of the decrease was related to heart failure severity [29].

All these observations have renewed interest in the energy starvation hypothesis and in the role of energy depletion in heart failure at basic and clinical levels [5,30,31].

Skeletal Muscle Energy Production in Congestive Heart Failure

Patients with congestive heart failure present a reduction in skeletal muscle functional capacity, which appears to have only a weak relation with central hemodynamic parameters and is mainly associated with changes in the periphery [32-35].

NMR spectroscopy with 31P shows that (separate from blood flow changes in skeletal muscle) there is a primary metabolic abnormality. Under both aerobic and ischemic conditions, repetitive finger-flexion exercises at increasing workloads will increase CrP depletion and lower pH of the flexor digitorum superficialis muscle in patients with mild-to-moderate congestive heart failure, relative to control subjects [36]. Mancini et al. [37] reported the prevalence of skeletal muscle atrophy and its relation to exercise capacity and abnormal muscle metabolism in a large cohort of patients

with chronic heart failure. Mancini's findings indicate that patients with chronic heart failure frequently develop significant skeletal muscle atrophy and metabolic abnormalities; atrophy contributes to both the reduced exercise capacity and altered muscle metabolism [37].

Abnormal skeletal muscle metabolism in patients with heart failure usually occurs in the absence of myoglobin deoxygenation, suggesting that abnormalities are not a result of cellular hypoxia during exercise with minimal cardiovascular stress [38].

Furthermore, results of noninvasive studies using 31P-NMR have shown that skeletal muscle energy production may be defective in congestive heart failure patients because of the intrinsic abnormalities of skeletal muscle. According to Lunde et al. [39], there is a more rapid breakdown of CrP and lower intramuscular pH during exercise in patients with heart failure in comparison with healthy subjects. In addition, the rate of CrP resynthesis is significantly delayed [39]. This indicates that the major factor limiting exercise performance in patients with heart failure during vigorous exercise is likely to be skeletal muscle fatigue, which is similar to healthy subjects, but that changes are seen at a much lower exercise level.

CrP AND CARDIAC PROTECTION

Myocardial preservation in acute myocardial infarction and ischemia during cardioplegia represent a problem of great concern from a clinical standpoint, and influence both the short- and long-term prognosis of patients. The extent of infarcted myocardium is the main cause of severe cardiac arrhythmias and development of heart failure.

Experimental and clinical studies of the biochemical mechanism during myocardial ischemia have shown a reduction of high-energy phosphates, such as CrP and ATP, in the myocardial cell. This reduction is correlated with the severity of structural and functional alterations of the myocardium.

Based on these considerations, multiple experimental studies have been performed to evaluate possible cardioprotective effects of CrP administration. Evidence has been now accumulated that CrP is able to protect myocardium in many experimental models of cardiac pathology.

The first demonstration of a cardioprotective effect of CrP was shown in the 1970s in studies by Parratt and Marshall [40,41]. Parratt and Marshall observed that the addition of CrP enabled isolated cardiac muscle to withstand the effects of anoxia; in isolated guinea-pig cardiac muscle preparations, acute ischemia resulted in a gradual decrease both in developed tension and in the rate of tension development. In the presence of CrP (4.64 mM), the time taken to reach 25%, 50%, and 75% of the control resting values was significantly increased; approximately 60% more. This ability to maintain contraction during anoxia was not seen in the presence of Cr or inorganic phosphate only [40]. In another in vivo study, the same authors also observed an antiarrhythmic effect of CrP during cardiac ischemia; pretreatment with CrP significantly reduced ventricular arrhythmias following acute coronary ligation in the dog [41].

The antiarrhythmic protective effects of CrP have also been confirmed in other studies [42-44]. In particular, Hearse et al. [44], observed a striking protection against reperfusion-induced arrhythmias; they used an isolated, perfused, working rat heart preparation to assess the effect of including CrP (10 mmol/L) in the perfusion fluid of hearts subjected to aerobic perfusion (20 minutes), regional ischemia (15 minutes), and reperfusion (2 minutes). In this study, the incidence of ventricular fibrillation was reduced

from over 80% in the control group to 10% in the CrP-treated group (P<0.001) (Fig. 4) [44].

The cardioprotective role of CrP was evaluated by Sharov et al. [45] using an in vivo model of experimental infarction in rabbit (ligation of circumflex coronary artery), and total ischemia in vitro in hearts of small pigs (50-70 kg of body weight). In the in vivo model, CrP reduced the size of the necrotic zone by 40%, with a significant protection of the cardiomyocyte sarcolemma in the perinecrotic zone. The in vitro study of the model of total ischemia showed significant protection of cardiac sarcolemma from irreversible ischemic injury and reduction in the rate of high-energy phosphate depletion.

Recently, in a rodent model of a transient coronary occlusion of myocardial ischemia, Woo et al. [46] showed that a strategy of intravenous CrP administration successfully prevents ventricular dysfunction. In CrP-treated animals, there was a significantly greater preservation of myocardial ATP levels (luciferin/luciferase bioluminescence assay) in comparison with control patients. Multiple hemodynamic parameters, such as maximum pressure, maximum left ventricular dP/dt ejection fraction, and stroke work, found an improvement at multiple time points which was statistically significant; interestingly, minimum left ventricular dP/dt, a measure of diastolic relaxation, was also significantly improved (Fig. 5) [46].

CrP and Experimental Cardioplegia

The introduction of cold-chemical cardioplegia has enhanced myocardial protection and improved survival following cardiac procedures. Studies have

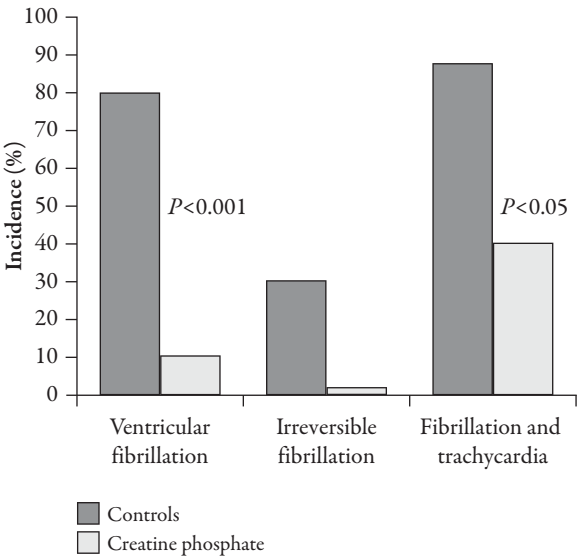

Fig. 4. The effect of CrP (10 mmol/L) on the incidence of reperfusion-induced rhythm disturbances on the isolated working rat heart. CrP=creatine phosphate. *Reproduced with permission from Hearse DJ, Tanaka K, Crome R, Manning AS. Creatine phosphate and protection against reperfusion-induced arrhythmias in the rat heart. Eur J Pharmacol. 1986;131:21-30.*

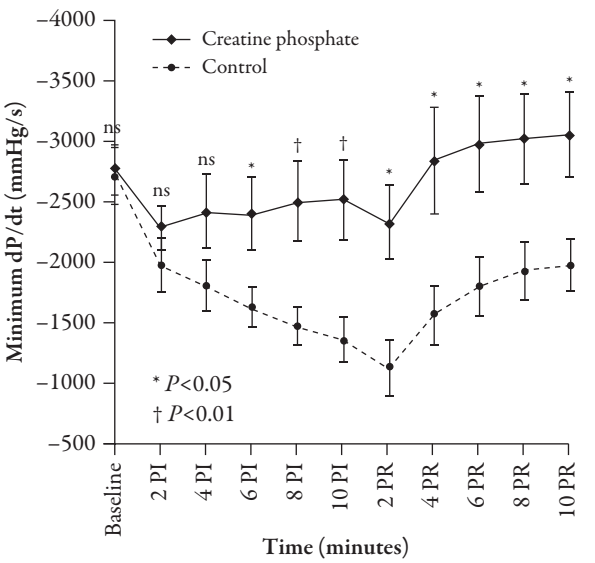

Fig. 5. Mean minimum left ventricular dP/dt of control animals (n=8) versus creatine phosphate animals. Control animals experienced marked declines six minutes following the initiation of ischemia. *Reproduced with permission from Woo YJ, Grand TJ, Zentko S, et al. Creatine phosphate administration preserves myocardial function in a model of off-pump coronary revascularization. J Cardiovasc Surg. 2005;46:297-305.*

demonstrated a positive correlation between post-ischemic recovery of myocardial performance and the cellular content of high-energy phosphates, such as ATP and CrP. This suggests that maintaining ATP and CrP during ischemia may help to provide optimal myocardial protection.

On this basis, Robinson et al. [47] investigated the potential for enhancing myocardial protection by adding CrP to the St. Thomas' Hospital cardioplegic solution, in a rat heart model of cardiopulmonary bypass and ischemic arrest. Dose-response studies (CrP 0-50 mmol/L) revealed 10.0 mmol/L as the optimal concentration. This concentration improved recovery of aortic flow and cardiac output after a 40-minute period of normothermic (37°C) ischemic arrest from 21.2% and 32.8% in the CrP-free control group to 82.5% and 82.6% (P<0.001), respectively, in the CrP-treated group. CK leakage was reduced by 68.7% in the CrP group (P<0.001). With hypothermic (20°C) ischemia (240 minutes) and multidose (every 30 minutes) cardioplegia, recoveries of aortic flow and cardiac output were improved from 33.1% and 42.3% in the CrP-free control group to 77.9% and 79.6% (P<0.001), respectively, in the drug group. In addition to improving function and decreasing CK release, CrP reduced reperfusion arrhythmias, significantly decreasing the time between cross-clamp removal and return of regular rhythm, and completely obviating the need for electrical defibrillation. Robinson et al. conclude: '… despite its alleged inability to enter the myocardial cell, exogenous CrP exerts potent protective and antiarrhythmic effects when added to the St. Thomas' Hospital cardioplegic solution [47].'

31P-NMR spectrometry was used by Sharov et al. [48] to study the effects of CrP on the isolated, perfused rat heart. The hearts were chemically arrested using St. Thomas' Hospital solution and made totally ischemic for 35 minutes at 37°C. In the presence of CrP 10 mmol/L, almost complete recovery of heart function and CrP myocardial content and 61% recovery of ATP content were observed after 30 minutes of post-ischemic reperfusion (Fig. 6) [48]. Sarcolemma protection and integrity were

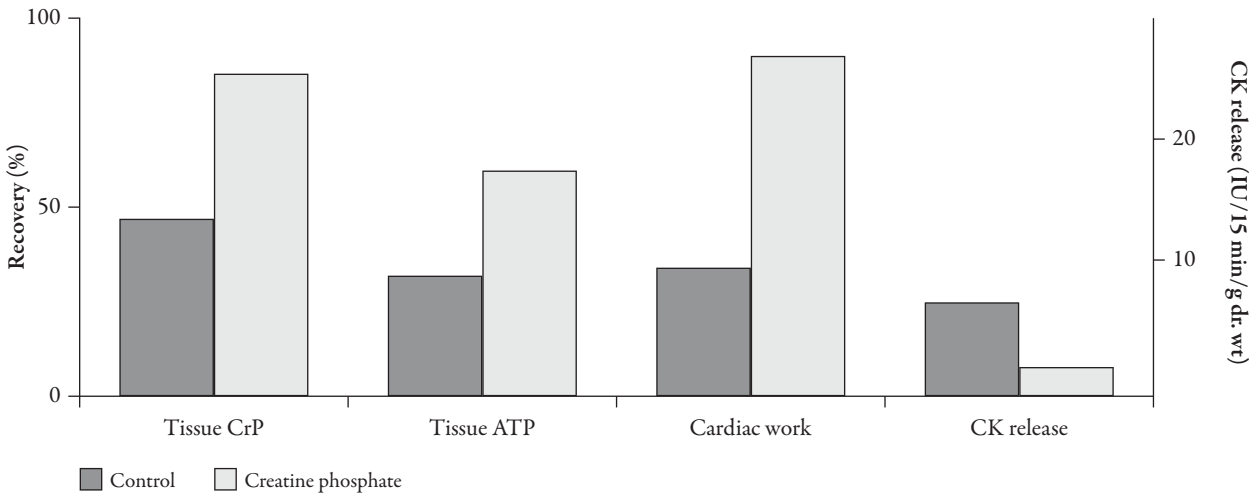

Fig. 6. Effect of CrP on post-ischemic level of high-energy phosphate, cardiac work, and CK release in perfused rat hearts: 35 minutes of total ischemia at 37°C and 30 minutes of repurfusion. ATP=adenosine triphosphate; CK=creatine kinase; CrP=creatine phosphate. *Reproduced with permission from Sharov VG, Saks VA, Kupriyanov VV, et al. Protection of ischemic myocardium by exogenous creatine phosphate: morphologic and phosphorus 31-nuclear magnetic resonance studies. J Thorac Cardiovasc Surg. 1987;94:749-761.*

also proved using morphological analyses with a lanthanum tracer method. Ischemic and reperfusion damage to membranes allows tracer lanthanum to penetrate cells during cardiac biopsies fixation. In CrP nontreated rat myocardium, after ischemia and reperfusion, lanthanum penetrated the cells and the tracer was located on the outer mitochondrial membrane. In contrast, in CrP-perfused rat myocardium, lanthanum did not enter the cells and was visible, only inside the intercalated discs, giving further proof of sarcolemma protection and integrity.

Thelin et al. [49] demonstrated improved myocardial protection using CrP-enriched cardioplegic solution in an in vivo study in porcine, during normothermic ischemia. In the CrP-treated group, a higher number of animals were successfully weaned from bypass, with a greater heart performance and statistically significantly higher adenilate charge potential.

CrP: Mechanism of Action

Different mechanisms have been proposed for CrP cardioprotective effects. Several authors have studied the problem of penetration of CrP into cardiomyocytes through the sarcolemma.

Down et al. [50] and Breccia et al. [51] have found that administration of 32P-CrP or double-labeled CrP in in vitro experiments significantly increased the tissue levels of ATP, and that labeled phosphate was incorporated into ATP; however, the rate of tissue uptake was low.

These data were confirmed by Preobrazhensky et al. [52], who found that uptake was no more than 200 nmol/min/g dry weight, and increased only by a factor of 2 after ischemia. This rate is approximately three to four orders of magnitude lower than that of the metabolic turnover of ATP in the working heart, which is approximately 100-1000 µmol/min/g dry weight [53].

However, at this low rate, intracellular penetration of exogenous CrP may be important in maintaining some subsarcolemmal pools of CrP or ATP. Moreover, in this local pool, CrP may affect the metabolism of adenine nucleotides in an important way, through inhibition of nucleotide catabolism or activation of the de novo synthesis and salvage pathways [54]. In fact, CrP inhibits the enzymes of adenosine monophosphate catabolism, adenosine monophosphate deaminase [55], and 5'-nucleotidase [56,57].

CrP can provide a protection against oxidative damage, as shown in many studies [58-60]. Furthermore, CrP reduces the degradation of membrane phospholipids and slows the formation of lysophosphoglycerides (LPG) [56]. Accumulation of LPGs in the ischemic zone, due to degradation of phospholipids, is the major factor in electrical instability of the ischemic myocardium [61,62].

According to Saks [63], the membrane stabilizing effect of CrP may be explained by it being a zwitterionic molecule (Fig. 7) [63]. In fact, as CrP carries positive and negative charges, it can interact with opposite charges of phospholipid polar heads in the membrane surface interphase. This results in the transition of the mobile domain (fluid phase) of a membrane into a structured domain (gel phase) leading to a decrease in the rate of phospholipid degradation into lysophospholipids and lipid peroxidation.

In a recent review, the same author and co-workers [64] have reaffirmed the hypothesis that CrP "may play two important roles in the regulation of muscle energetics: first by maintaining local ATP pools via compartmentalized CK reactions, and secondly by stabilizing cellular membranes due to electrostatic interactions with phospholipids. The second mechanism decreases the production of LPG in hypoxic heart, protects the cardiac

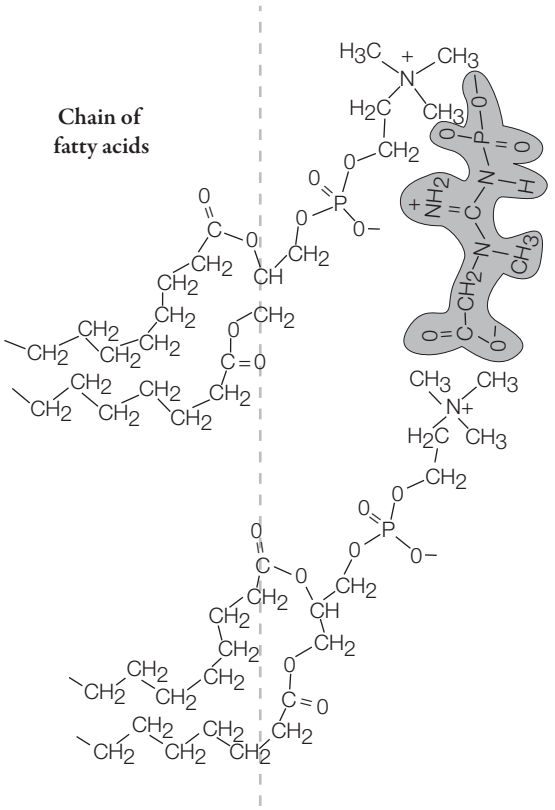

Fig. 7. Hypothetical zwitterionic interaction of CrP with polar heads of phospholipid molecules in the interphases of sarcolemma. CrP=creatine phosphate. *Reproduced with permission from Saks VA, Kapelko VI, Ruda MY, Semenovski ML, Strumia E. Phosphocreatine as effective drug in clinical cardiology. In: De Deyn PP, Marescau B, Stalon V, Qureshi IA, eds. Guanidino Compounds in Biology and Medicine. London: John Libbey & Company;1992:239-248.*

cells sarcolemma against ischemic damage, decreases the frequency of arrhythmias and increases the post-ischemic recovery of contractile function" [64].

Biochemical evidence gives reasons to believe that the mechanism of CrP protective action is complex and includes many components, such as: (a) some extent of CrP penetration into the cells and participation in the energy transport system with subsequent maintenance of high local ATP; (b) stabilization of sarcolemma, due to: (i) zwitterionic interaction of CrP with polar heads of phospholipids in the interphases of sarcolemma, (ii) prevention of phospholipids degradation and inhibition of LPG accumulation, and (iii) 'inhibition of adenine nucleotide degradation during the 5'-nucleotidase reaction in the sarcolemma of cardiac cells.

The total result of the action of these components is the preservation of the adenine nucleotide pool, delay of irreversible sarcolemmal damage, more efficient recovery of contractile function, and decrease of arrhythmia frequency.

CrP: CLINICAL STUDIES AND APPLICATIONS

CrP for Cardioprotection in Heart Surgery

The protective properties of cardioplegic solutions depend on three main components: chemical arrest, hypothermia, and additional protection with metabolic agents.

The St. Thomas' Hospital cardioplegic solutions contain elevated concentrations of potassium to induce rapid diastolic arrest of the myocardium [65]. In addition to chemical arrest, hypothermia confers significant myocardial protection, which has been shown to be additive to that given by chemical arrest.

For the third component of cardioplegia, metabolic protection, many compounds have been proposed to preserve the high-energy phosphate content of the myocardium.

The potential for further myocardial protection through addition of CrP was demonstrated by Robinson et al. [47] in rat hearts during cardiopulmonary by-pass and ischemic arrest, under conditions of normothermia or hypothermia.

On this basis, the cardioprotective effects of CrP added preoperatively both to crystalloid and blood cardioplegic solution have been extensively studied in adults and children during surgery (such as valve replacement,

coronary artery by-pass grafting, and the repair of congenital heart defects). In all these investigations, CrP was added to the cardioplegic fluids at a concentration of 10 mM in accordance with the dose derived from animal studies [47].

The first human trials investigating CrP cardioprotection were conducted by Semenovsky et al. [66] and D'Alessandro et al. [67]. In the former, the addition of CrP to blood cardioplegic fluids resulted in more efficient restoration of sinus rhythm and a decreased need for defibrillation. In contrast to controls, in whom the myocardial ATP content decreased by 25% at the end of surgery, the CrP-treated group maintained the concentration of high-energy compounds at preoperative levels. Ultrastructural analysis demonstrated good preservation of the sarcolemmal structure, which was clearly seen using the lanthanum method, despite the high degree of heterogeneity of the human heart material studied.

Very similar results were obtained by D'Alessandro et al. [67], who demonstrated that in the CrP-treated group there were significant differences in the number of watts required for defibrillation, time to recovery of cardiac activity after aortic declamping, inotropic drug support in the postoperative period, and presence of electrocardiographic anomalies.

Two clinical studies have been performed at the St. Thomas' Hospital, London, by Chambers et al [68,69]. In the first study [68], patients were mainly undergoing coronary artery by-pass and CrP 10 mM was added to St. Thomas' Hospital cardioplegic solution no. 2. Birefringence assessment of changes in myocardium demonstrated a significant improvement of myocardial protection in the cardioplegic solution plus CrP group in both the end-ischemic and the post-ischemic period.

In the second study [69], the majority of patients underwent valve replacement. St. Thomas' Hospital cardioplegic solution no. 1 plus CrP enhanced myocardial protection and conferred a direct benefit to patients by reducing postoperative arrhythmias and need for prolonged inotropic support. There was a significant (P<0.02) reduction in the number of direct-current shocks required to convert to sinus rhythm in the St. Thomas' Hospital cardioplegic solution no. 1 plus CrP group compared with the control (St. Thomas' Hospital cardioplegic solution no. 1 alone) (Fig. 8) [69]. Therefore, the total joules required for conversion were also significantly (P<0.02) reduced. The incidence of spontaneous sinus rhythm recovery was significantly (P<0.05) increased in the CrP-supplemented patients compared with the control patients. Furthermore, the incidence of severe postoperative arrhythmias (atrial fibrillation, supraventricular tachycardia, ventricular tachycardia) was significantly (P<0.05) reduced in the St. Thomas' Hospital cardioplegic solution no. 1 plus CrP group.

Furthermore, the study also investigated the effect of CrP on inotropic support. Patients from the CrP-supplemented group who required inotropic support postoperatively appeared better able to respond to this stimulation in comparison with the control group. In particular, the CrP-treated group required a significantly shorter duration of inotropic support in comparison with the duration required by the control group (6.5 ± 1.1 hours vs. 24.8 ± 7.3 hours, P<0.05).

Results from several other studies support these findings [70-76]. In particular, Cossolini et al. [76] carried out a study in newborn infants and pediatric patients from 9 days to 13 years (mean age 33.9 months) undergoing open heart surgery for congenital heart disease.

In summary, all the trials with CrP added to both crystalloid and blood cardioplegia confirm the cardioprotective actions of CrP by showing the following effects: (a) preservation

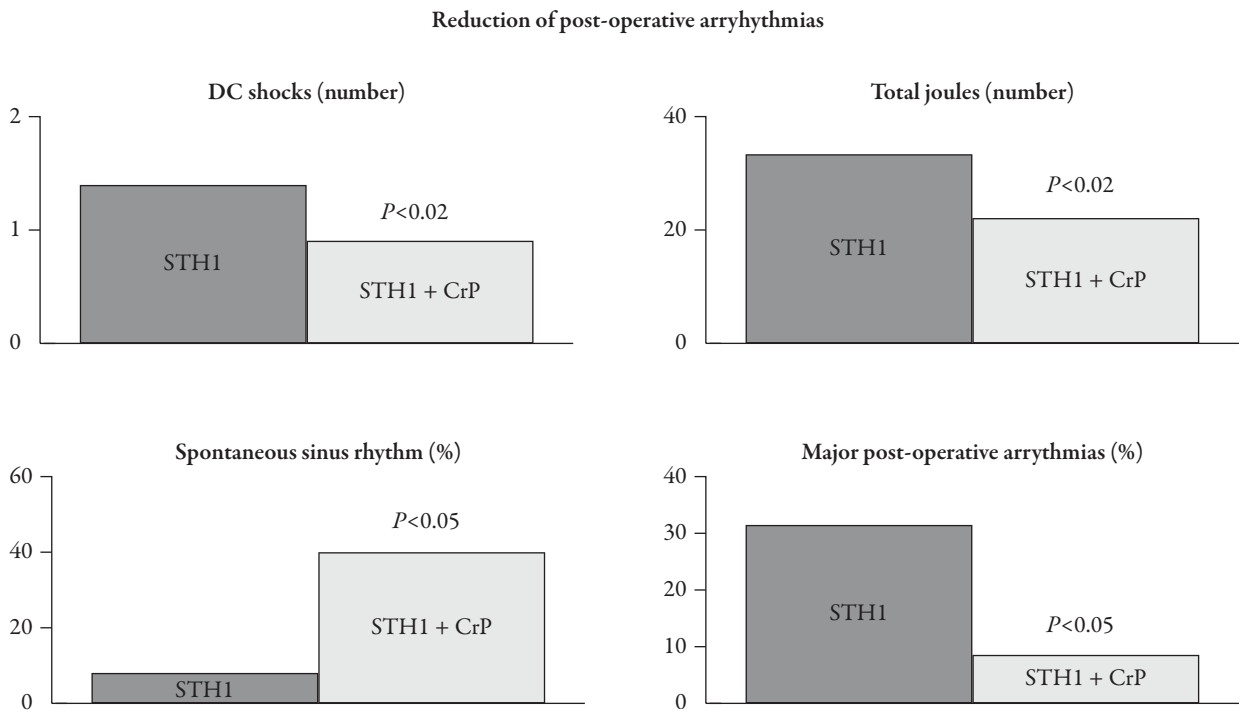

Fig. 8. St. Thomas' Hospital cardioplegic solution enhanced protective effects with CrP: antiarrhythmic effect. CrP=creatine phosphate; DC=direct-current; STH1=St. Thomas' hospital cardioplegic solution 1. *Reproduced with permission from Chambers DJ, Haire K, Morley N, et al. St. Thomas' Hospital cardioplegia: enhanced protection with exogenous creatine phosphate. Ann Thor Surg. 1996;61:67-75.*

of myocardial CrP and ATP levels; (b) higher spontaneous recovery of sinus rhythm; (c) minor incidence of postoperative arrhythmias, particularly lower incidence of major arrhythmias (III and IV Lown class; III grade atrioventricular blocks); (d) decreased frequency of defibrillation and/or direct-current shocks number required to convert to sinus rhythm; (e) decreased need of inotropic support (reduced number of patients needing inotropic administration; posology reduction), shorter period of administration; (f) greater response to inotropic support (in the subset requiring this treatment); and (g) reduction of enzyme leakage (CK, muscle-brain [MB]-CK).

Myocardial Infarction

The cellular mechanism underlying the cardioprotective effects of CrP [54] (stabilization of sarcolemma, prevention of LPGs formation, and inhibition of adenine-nucleotide degradation) and experimental animal researches in vivo demonstrating a reduction in the extent of damage in experimental acute myocardial infarction lesions [45], suggest that CrP may be useful during the early hours post-myocardial infarction.

Indeed, the experimental evidence of an antiarrhythmic action of CrP in models of acute ischemia has been confirmed in the clinical setting by Ruda et al. [77], who carried out a clinical trial of CrP in 60 randomized patients with acute myocardial infarction (30 patients in the treatment group and 30 in the control group). CrP was administered intravenously (i.v.) within 6 hours of symptom onset (2 g i.v. bolus followed by a 2-hour infusion at a rate of 4 g/hour). Twenty-four hour Holter monitoring

showed a significant decrease in the frequency of ventricular premature beats and of the number of ventricular tachycardia paroxysms in the CrP-treated group (Fig. 9) [77].

In other acute myocardial infarction trials, CrP infusion took place over a more prolonged period, according to or similar to the following scheme; day 1, 4 g bolus i.v. injection, followed by an i.v. infusion at a rate of 4 g/hour over 2 hours; days 2-6, 4 g i.v. injection twice daily. Using this posology, Reimers et al. [78] observed markedly reduced CK and MB-CK release, statistically significant for patients with a MB-CK peak greater than 100 U/L.

Reduced enzyme release and/or reduction of arrhythmias have been observed in the CrP-treated patients in many other trials. In 1987, Scattolin et al. [79] reported that the percentage reduction in total R-waves in the leads with ST humps >0.15 mV was 44% in the treated group versus 63% in the control group. In the study by Coraggio et al. [80], lower peaks of markers of myocyte damage, ie, CK and MB-CK levels, were observed in those patients who received CrP. Cini et al. [81] observed a more rapid improvement in ST changes (P=0.027 at 48 hours) and a reduction in Lown's grade of ventricular arrhythmias, in comparison with control patients. In 1989, Raisaro et al. [82] reported that treated patients showed lower scores of electrocardiographic and echocardiographic indexes of myocardial damage in comparison with control patients.

The use of CrP during acute myocardial infarction has never been associated with any significant side effects in any trial, and there is evidence by Camilova et al. [83] that it can be safely administered with vasodilators, such as nifedipine, improving its therapeutic efficiency.

Finally, it is worth pointing out that the cardioprotective effects that CrP has initially shown in the so-called "thrombolytic era" have been confirmed recently in a case of primary angioplasty [84,85]. Indeed, it has become evident in the last decade that mechanical reperfusion of the infarct-related artery is the most effective means to limit the consequences

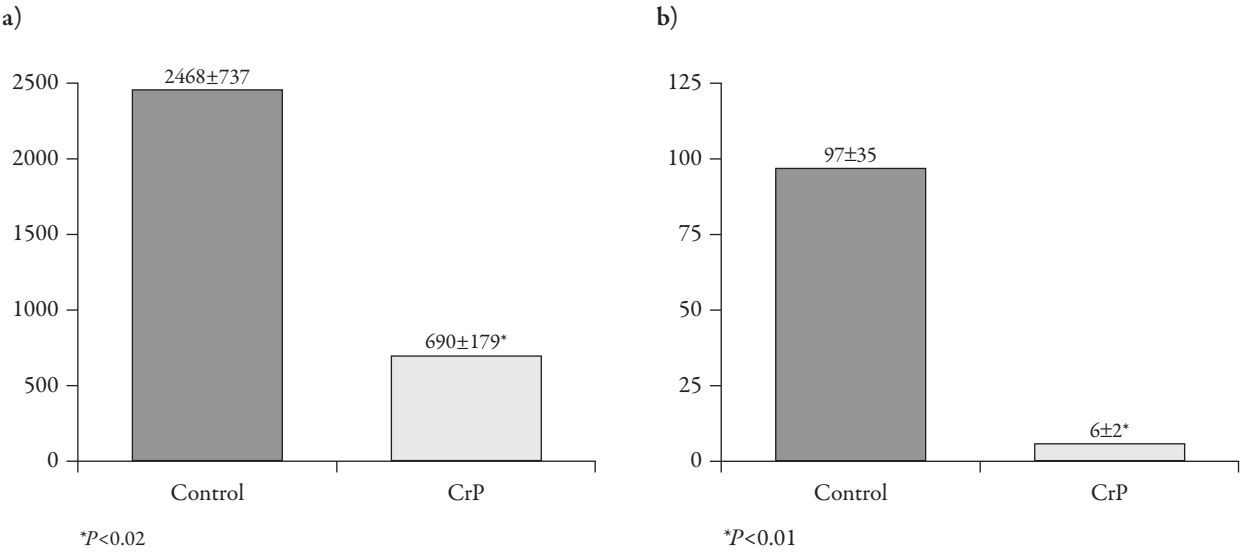

Fig. 9. Mean number of (a) ventricular premature beats and (b) paroxysmal ventricular tachycardia episodes in the CrP group compared with the control group. CrP=creatine phosphate. *Reproduced with permission from Ruda MY, Samarenko MB, Afonskaya NI, Saks VA. Reduction of ventricular arrhythmias by creatine phosphate in patients with acute myocardial infarction. Am Heart J. 1988;116:393-397.*

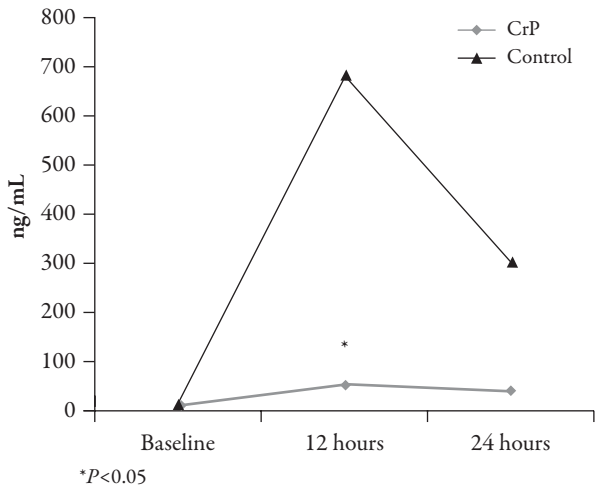

Fig. 10. Changes of serum troponin I level in the CrP group compared with control group. CrP=creatine phosphate. *Reproduced with permission from Iosseliani DG, Koledinsky AG, Kuchkina NV. Does intracoronary injection of phosphocreatine prevent myocardial reperfusion injury following angioplasty of infarct-related artery in acute-stage of myocardial infarction? J Intervent Cardiol. 2004;6:10-14.*

of acute myocardial infarction. Thanks to the elegant works of Iosseliani et al. [84,85], it has been shown that further benefits from emergency intervention can be obtained when CrP administration is used. These investigators have shown that patients randomized to receive intracoronary injections of CrP during percutaneous coronary intervention for acute myocardial infarction have a significantly lower extent of myocardial necrosis. This is indicated by the significantly lower peaks in troponin I levels (Fig. 10) [84], which in turn correlate with the subsequent evidence of a significantly greater left ventricular ejection fraction in comparison with patients who did not receive any metabolic agent in the acute phase.

Chronic Myocardial Ischemia: Cardiac Failure

The use of CrP can be of value in the chronic phase of ischemic heart disease, and its effects have been carefully investigated in this condition. The hemodynamic effects of CrP have been characterized by Smilari et al. [86], Ferraro et al. [87], Cafiero et al. [88], Strozzi et al. [89] and Scattolin et al [90].

Smilari et al. [86] performed an open echocardiographic study to evaluate the effect of CrP acute infusion (1 g) on left ventricular function in 20 patients with congestive cardiomyopathy, ischemic cardiomyopathy, and chronic pulmonary heart disease. Three hours after the end of CrP infusion, a significant reduction in end-diastolic and end-systolic diameters along with significant increases in fractional shortening (+30%), ejection fraction (+18%), and cardiac output (+17%) were observed. Ferraro et al. [87] studied the effects of CrP in patients with chronic heart failure, where all the patients were on stabilized pharmacological therapy. Echocardiographic measurements were performed at baseline, after acute infusion, and after the end of short-term therapy (3 days). No significant changes occurred after placebo treatment, but the CrP-treated group demonstrated a significant increase in ejection fraction and fractional shortening, and reduced end-systolic diameter and systemic vascular resistance. Similarly, Cafiero et al. [88] noticed that a bolus administration of 5 g i.v. induced a significant amelioration of all indexes of cardiac contractility (wall stress, ejection fraction, and fractional shortening). Further significant increases in cardiac function were observed when treatment was continued for up to 6 days. Strozzi et al. [89], in patients with chronic ischemic heart failure treated with a CrP infusion (1 g/day for 7 days), found a statistically significant decrease of end-diastolic and end-systolic volumes as well as an improvement in left ventricular wall motion.

In primary ischemia, diastolic dysfunction may be the first change to appear. The effect of CrP on diastolic parameters has been studied by Scattolin et al. [90]. Acute infusion of CrP (5 g i.v.) to 20 patients suffering from chronic ischemic cardiomyopathy produced a general improvement in diastolic function parameters as measured by echo Doppler. There was a statistically significant modification in the isovolumetric relaxation time and protodiastolic deceleration slope rate. According to the authors, these data suggest that CrP can be usefully employed in case of transient, but reversible, impairment in diastolic function, to prevent progression to systolic dysfunction.

Furthermore, Gelfgat [91] observed an improvement in exercise tolerance with i.v. infusion of CrP in patients with ischemic heart disease. Administration of CrP (6 g) just before an exercise stress test was associated with an increase in the exercise capacity by 385 kg/m, along with a lower increase in arterial pressure during exercise.

The activity and safety of the drug in heart failure has been studied extensively and, in particular, more than 2000 patients have been considered in two controlled Italian multicenter studies by Grazioli et al. [92,93].

In 49 cardiology or medicine departments, Grazioli and Strumia [92] reported the effects of CrP (2 g/daily i.v. for 3 weeks), in addition to conventional therapy (digitalis, diuretics, nitrates). A total of 1174 patients with heart failure were randomly assigned to CrP treatment (739 patients) or no treatment (435 patients). The CrP treatment group showed significant improvement in clinical symptoms of heart failure (dyspnea, pulmonary stasis, and peripheral edema), and signs of ischemia (angina pectoris, use of sublingual nitroglycerin, negative T-waves).

Grazioli et al. [93] conducted their multicenter trial in 58 cardiology or medicine departments. Clinical symptoms and NYHA classes, electrocardiographic signs of ischemia, and the use of sublingual nitroglycerin were monitored. A total of 1007 patients were studied, of whom 508 were randomized to receive CrP i.v. (1 g twice daily) for 2 weeks, followed by a 1-month course of therapy with CrP intramuscularly (i.m.) (500 mg daily). During the study period, the number of patients in NYHA classes III and IV decreased significantly in the CrP group compared with the control. Specifically, the number of patients in NYHA classes III and IV was significantly lower ($P<0.001$) in the CrP group than in the control group after 15 days (17% vs. 26%) and after 45 days (9% vs. 24%) (Fig. 11) [93]. The difference in NYHA classes in the CrP group was also statistically significant ($P<0.001$) between day 15 and 45 of treatment, while the results in the control group were almost unchanged. The main symptoms and signs of ischemia (angina pectoris, need for sublingual nitroglycerin, and T-wave inversion on electrocardiogram), and the incidence of ventricular premature beats improved significantly in the CrP-treated patients.

Further studies on CrP in patients with heart failure were carried out by Andreev et al. [94], Galyautdinov et al. [95], and Wang et al. [96]. Andreev et al. [94] examined the effects of CrP and digoxin in 67 patients aged over 60 years with NYHA class II-III chronic heart failure (ischemic etiology). Combined treatment resulted in an increase in left ventricular ejection fraction, decrease in systemic vascular resistance, as well as in the frequency of ventricular extrasystoles and paroxysmal ventricular tachycardia. Improvements in the patients' clinical status with a decrease in the severity of dyspnea, frequency of angina attacks, and reduction in the need for nitroglycerin were also obtained together with a substantial improvement in exercise tolerance.

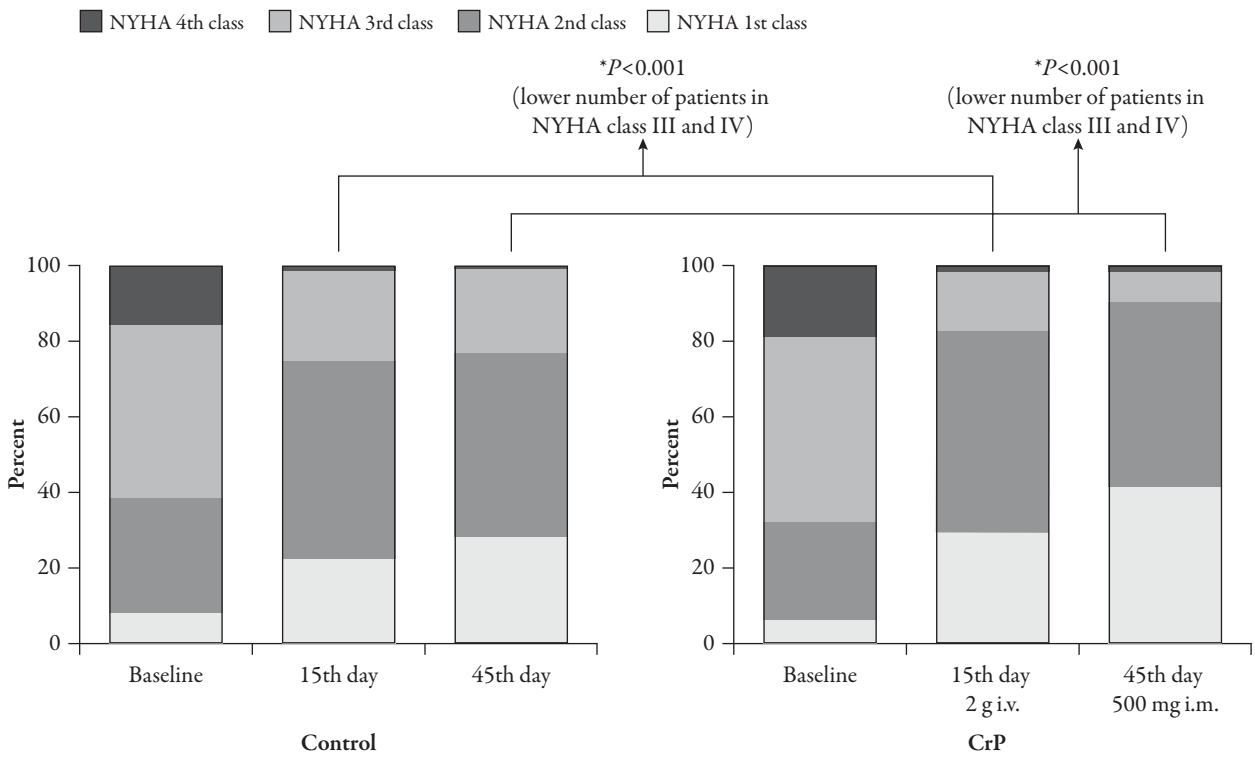

Fig. 11. Significant reduction of NYHA classes III and IV patients in CrP groups compared with controls. CrP=creatine phosphate; NYHA=New York Heart Association. *Reproduced with permission from Grazioli I, Melzi G, Strumia E. Multicentre controlled study of creatine phosphate in the treatment of heart failure. Curr Ther Res. 1992;52:271-280.*

Similar findings have been reported by Galyautdinov et al. [95] who evaluated the effects of CrP given in a total i.v. dose of 40 g over 5 days to ischemic patients with heart failure.

It has been recently confirmed that CrP can be used in adjuvant treatment of chronic heart failure and can improve heart functions, in particular left ventricular systolic function. Wang et al. [96] studied the influences of CrP on left ventricular function and plasma-brain natriuretic peptide levels of patients with heart failure. The investigators selected 64 cases of patients with chronic heart failure who were divided randomly into control and treatment groups. The treatment group was given CrP, 2 g once daily for 14 days, and showed significant improvements in left ventricular ejection fraction, stroke volume, and cardiac output in comparison with the control group, along with a significant lowering in B-type natriuretic peptide levels.

Another possible indication of CrP may be constituted by pediatric myocarditis. Yang et al. [97] observed that the use of CrP in children (mean age 7 years and 4 months) with this pathology was more effective to control symptoms of heart failure, to reduce the number of ventricular arrhythmias, decrease left ventricular dimension, and improve cardiac systolic pump function.

CrP in Skeletal Muscle Performance, Muscle Hypotonotrophy, and Muscle Rehabilitation

Based on the possible correlation between deficiency in CrP and compromised skeletal

muscle function, several trials were carried out to evaluate possible effects of CrP administration on skeletal muscle performance, both in patients with muscle hypotonotrophy and healthy individuals during high-intensity exercise.

In healthy subjects, CrP was shown to increase the muscle force-generating capacity and to delay the onset of muscle fatigue. In patients with muscle hypotonotrophy, CrP was shown to allow a more efficient recovery of muscle trophism and function.

In a double-blind study against a placebo, Dal Monte et al. [98] evaluated the effect of CrP on the maximum anaerobic power in healthy volunteers. CrP was administered at a dose of 200 mg i.m. twice a day for 10 consecutive days. When compared with the placebo, CrP administration significantly potentiated the ability of muscle to produce a burst output.

During maximal exercise, such as "cronoscalata" (timed uphill cycling), enhanced performance has been observed in amateur cyclists treated with CrP [99]. These data have been confirmed by Vorobiev et al. [100] in healthy subjects during maximal and prolonged submaximal physical exercise. When compared with placebo, CrP administration improved total work and anaerobic threshold; moreover, CrP improved effort tolerance during submaximal prolonged exercise (70% of maximum O_2 capture).

CrP administration can also be useful in the recovery of muscle trophism and function in several pathologic conditions, and during muscular rehabilitation.

Three studies involving a total of 159 patients with muscle hypotonotrophy of the thigh, due to prolonged post-trauma immobilization, have been published. These studies compared the efficacy of exogenous CrP treatment (between 0.5 g i.m. and 1 g i.v. per day for a mean period of 20-30 days) in addition to physiokinesitherapy versus physiokinesitherapy training alone.

CrP use in the recovery of skeletal muscle following surgery and cast immobilization has been evaluated by Satolli and Marchesi [101]. Satolli and Marchesi studied 69 patients with muscle hypotonotrophy of the thigh due to knee osteoarticular lesions who underwent a rehabilitative training with or without the addition of CrP. During physiokinesitherapy, 38 patients treated with CrP showed a significantly faster and greater muscle recovery of strength and power than 31 control patients. After 30 days of treatment, the difference between the two groups was 12% in flexion and 16% in extension for strength, and 14% in flexion and 19% in extension as far as the power is concerned (Fig. 12) [101].

Similarly, Agnese et al. [102] evaluated the recovery from muscular hypotrophy of quadriceps and triceps surae after immobilization with a plaster cast due to surgical reconstruction of capsuloligamentous knee joint. Following surgery and plaster cast, 60 patients undergoing physiotherapy were given (30 patients) or not given (30 patients) CrP 500 mg/day i.m. for 25 days. Measurements of the circumference of the thigh at 18 and 10 cm from the kneecap superior pole, and of the calf at 15 cm from the kneecap inferior margin, showed a significant increase in muscle mass in the CrP-treated group in comparison with the control group, and an improved recovery in comparison with the sound limb.

Finally, it has been shown that CrP use yields a significant improvement of muscle mass recovery even in older patients with hypotrophy of the lower extremity, due to femur fracture. Pirola et al. [103] studied 30 patients aged >60 years with muscle hypotrophy of the lower limb due to femur fracture, who underwent physiokinesitherapy with or without the addition of CrP (500 mg/day i.m.) for 20 days. At the end of the

treatment period, echotomography showed a net muscle mass recovery in 15 patients treated with CrP in comparison with 15 patients who received only physiokinesitherapy (4.4 mm vs. 1.5 mm, $P<0.01$).

CrP and Neurological Conditions

Recently interest has been observed in the possible neuroprotective effects of CrP. Weber et al. [104] reported on the effects of CrP for the treatment of cardiocerebral syndrome in acute myocardial infarction in the elderly. The aim of Weber's open investigation was to verify if the use of CrP might influence the general neuropsycologic status, described as a cardiocerebral syndrome, during the first 3 days of an acute myocardial infarction in old age. Fifty patients admitted to the coronary unit due to myocardial infarction were randomized to receive or not receive treatment with CrP (total dose 18 g i.v. in 3 days). Evaluation of mental deterioration by means of a test of cognitive function (Mini-Mental State Examination) during the first 3 days after myocardial infarction revealed a favorable effect of CrP on mental function in comparison with the control group. The CrP-treated group also showed a lower incidence of angina and ventricular arrhythmias than the control group. Administration of CrP did not cause any side effect.

According to Skrivanek et al. [105], CrP can play a role in the treatment of acute ischemic stroke. This multicenter study evaluated 119 patients, in whom treatment of cerebral ischemia was initiated within 8 hours after onset of symptoms. CrP was given to 64 patients (total dose 34 g i.v. in 3 days), while 55 patients were treated with standard cerebrovascular medication. All patients underwent quantitative scoring of stroke damage by the Stroke Assessment System (SAS) and the Toronto Stroke Scale (TSS) at days 1, 5, 12, and 20, and had a computerized tomography scan and

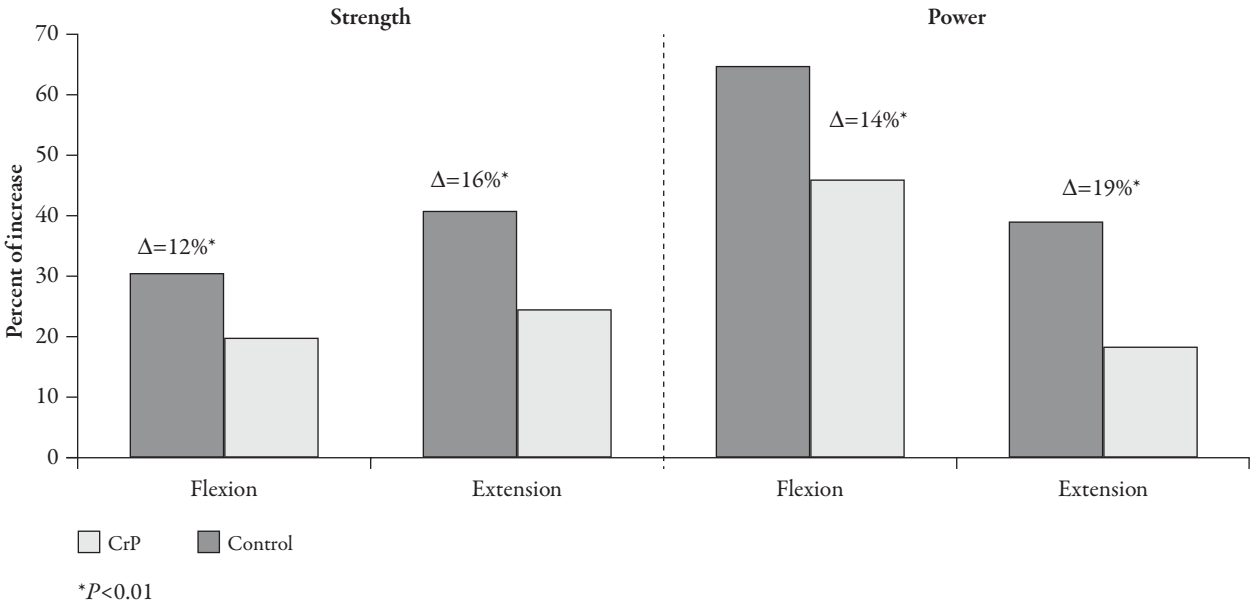

Fig. 12. Increase of strength and power peak torque mean values in flexion and extension of impaired extremity after 30 days of physiokinesitherapy training with and without CrP. CrP=creatine phosphate. *Reproduced with permission from Satolli F, Marchesi G. Creatine phosphate in the rehabilitation of patients with muscle hypotonotrophy of the lower extremity. Curr Ther Res. 1989;46:67-73.*

an electroencephalograph on days 1, 2, and 20. Death occurred in 4 of the 64 patients in the CrP group (6.3%) and in 9 of the 55 control patients (16.4%). A comparison of clinical improvement by means of the SAS and TSS scales revealed a superior score trend in the CrP group; even if not statistically significant, the difference being even more marked in the group of patients in whom treatment was started within 3 hours. As far as functional damage is concerned, the asset of CrP was more marked in categories "consciousness," "paresis," and "dysarthria/dysphagia."

The theory that CrP is well tolerated and can improve the treatment of ischemic stroke has also been suggested in a noncontrolled study by Bakala and Kalita [106]. Using the single-photon-emission computed tomography method (technetium-99m Technegas hexamethylpropyleneamine oxime) these investigators observed amelioration of cerebral blood flow and a superior clinical evolution in patients treated with CrP (32 g in 5 days).

Neonatal Pediatric Conditions

Recently, investigators have disclosed possible alternative protective effects and possible uses of CrP in pediatric patients, other than its addition to cardioplegic solutions.

Zhang et al. [107] have shown that CrP can improve blood-gas changes in neonates with moderate or severe hypoxic ischemic encephalopathy. This condition is a major cause of neonatal mortality, as hypoxia in the uterus or at time of delivery can cause metabolic damage of neuronal cells, leading to cerebral edema and necrosis. Thus, it is particularly important to initiate early and appropriate therapy. Zhang et al. enrolled 60 neonates who had moderate or severe ischemic encephalopathy, who were randomized into either a control group (n=30) or treatment group (n=30). The control group received conventional therapy for the maintenance of good ventilation, stabilization of blood flow to organs, improvement of microcirculation, control of blood glucose at the upper limit of the normal value, maintenance of homeostasis, control of convulsion, depressurization of intracranial pressure, and elimination of brain stem disorders. Along with conventional therapies, the treatment group received CrP 1 g for 5-7 days. The results showed that CrP use was associated with a significant improvement in neurological symptoms, ie, conscious state recovery, convulsion disappearance, normal muscular tension, and primitive reflex recovery, as well as in blood-gas analysis.

CONCLUSION

The physiological role of Cr and CrP in the regulation of cardiac function has been recently revaluated and revised. A decrease in the intracellular contents of Cr and CrP results in a hypodynamic state of muscle and muscle pathology. In this situation, exogenous CrP administration has a clear protective effect.

Many pharmacological studies support this view, showing the clear protective effects of CrP administration on ischemic and/or failing myocardium in different experimental models. The mechanism of CrP protective action is complex and includes many components, resulting in a delay of irreversible sarcolemma damage, preservation of adenine nucleotide pool, and superior functional recovery and antiarrhythmic action.

Clinical studies also are in agreement. In controlled studies, the clear protective effects of CrP have been shown by addition of CrP to cardioplegic solutions for protection of the heart against intraoperational injury, such as

minor incidence of postoperative arrhythmias, decreased frequency of defibrillation, and a decreased need for inotropic support.

In medical cardiology, i.v. or i.m. CrP administration in acute and chronic myocardial ischemic conditions can improve the hemodynamic response and clinical conditions of the patients.

Furthermore, the clear membrane-stabilizing effect of CrP, and the possible role of CrP administration in supporting regeneration of local ATP pools for isoforms of CK reactions in myofibrils and cellular membranes may be important, and should be further considered and investigated.

This review has summarized the clear-cut scientific evidence supporting the meaningful and therapeutic value of CrP supplementation, not only for protection during heart surgery, but also for targeting cellular energy impairment in a wide spectrum of other human diseases, ranging from congestive heart failure, ischemic heart disease, skeletal muscle hypotonotrophy, and cerebral ischemia.

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Giuseppe D'Ambrosio reports being an officer and employee of Alfa Wassermann.

Ettore Strumia is the guarantor for this article, and takes responsibility for the integrity of the work as a whole.

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