High-Energy Phosphates and Ischemic Heart Disease: From Bench to Bedside

Hao Yi-Dan, Zhao Ying-Xin, Yang Shi-Wei* and Zhou Yu-Jie*

The Key Laboratory of Remodeling-Related Cardiovascular Disease, Ministry of Education, Beijing Institute of Heart, Lung and Blood Vessel Disease, Beijing Anzhen Hospital, Capital Medical University, Beijing, China

The purpose of this review is to bridge the gap between clinical and basic research through providing a comprehensive and concise description of the cellular and molecular aspects of cardioprotective mechanisms and a critical evaluation of the clinical evidence of high-energy phosphates (HEPs) in ischemic heart disease (IHD). According to the well-documented physiological, pathophysiological and pharmacological properties of HEPs, exogenous creatine phosphate (CrP) may be considered as an ideal metabolic regulator. It plays cardioprotection roles from upstream to downstream of myocardial ischemia through multiple complex mechanisms, including but not limited to replenishment of cellular energy. Although exogenous CrP administration has not been shown to improve long-term survival, the beneficial effects on multiple secondary but important outcomes and short-term survival are concordant with its pathophysiological and pharmacological effects. There is urgent need for high-quality multicentre RCTs to confirm long-term survival improvement in the future.

Keywords: high-energy phosphates, creatine phosphate, energy metabolism, ischemic heart disease, cardioprotection

INTRODUCTION

The heart is more than a hemodynamic pump. It is also an organ that needs energy from metabolism (1). In fact, altered cardiac metabolism is the primary and upstream pathophysiological manifestation of myocardial ischemia in humans (2). After coronary blood flow blockage, energy metabolism disorder occurs within a few seconds, followed by mechanical, electrophysiological and structural abnormalities of the myocardium. To date, standard treatments for ischemic heart disease (IHD), including revascularization (thrombolysis, percutaneous coronary intervention, and coronary artery bypass grafting), antithrombotic therapy (antiplatelet and anticoagulant agents), stabilization/reversal of atherosclerosis progression (control of atherosclerotic risk factors), and inhibition of myocardial remodeling (sympathetic and renin-angiotensin-aldosterone system inhibitors), focus on coronary anatomy and on the results of changes in myocardial metabolism rather than on the metabolic changes themselves (2–8). In addition, almost all of the above treatments exert cardioprotection by directly or indirectly affecting heart rate, blood pressure or myocardial perfusion. In contrast, myocardial energy metabolic therapy (MEMT) plays a protective role by regulating the energy synthesis and utilization of myocardial cells without significant impacts on heart rate, blood pressure and perfusion (9, 10). Because of residual cardiovascular risk, MEMT is promisingly emerging as an upstream treatment for IHD (11).
Since the discovery of creatine phosphate (CrP) in 1927 (12) and adenosine triphosphate (ATP) in 1929 (13), the biochemical, physiological, and pharmacological properties of high-energy phosphates (HEPs) have been gradually uncovered. Unlike the single metabolic process of glucose, free fatty acids or amino acids, the pathways and regulations of HEPs biosynthesis and degradation are involved in all metabolic substrates. Moreover, due to the production and consumption of HEPs in different cells and subcellular organelles, the transmembrane transport of HEPs is also a complex process requiring the assistance of many special transporters and catalytic enzymes (14). Therefore, although HEPs have been known for nearly a 100 years, clinicians still have a lot to learn. In recent years, a series of basic and clinical studies have shown potent protection for IHD by exogenous HEPs (15–19). These results have been confirmed in our laboratories (16, 20, 21).

Previous reviews focused either on the cellular and molecular mechanisms of HEPs which is too complex for clinical application (14, 22), or on presenting the clinical evidence which in turn is too simple for clinicians to understand their pathophysiological and pharmacological effects (15, 16). The purpose of this article is to bridge the gap between clinical and basic research.

OVERVIEW OF HIGH-ENERGY PHOSPHATES AND THEIR TRANSFORMATION

It is believed that energy would be concentrated in the chemical bond containing phosphate groups, which yields energy upon hydrolysis (23). Low-energy phosphates are usually linked to phosphoester bonds, which will release 2 and 3 kcal/mol energy. HEPs include a variety of phosphate compounds with energies of hydrolysis higher than 7 kcal/mol (24). ATP and CrP are considered to be the primary HEPs in human body. ATP is the intracellular energy currency, majority of which is not synthesized de novo but generated from adenosine diphosphate (ADP) by oxidative phosphorylation (OP) of mitochondria and cytoplasmic substrate phosphorylation (SP) (Figure 1) (25, 26). Thus, at any given time, the total amount of ATP and ADP remains fairly constant and recycled continuously (27). While, CrP is the storage and transport carrier of energy, which serves to transfer the HEP-bond from the site of ATP production to the site of ATP utilization through “CrP shuttle” (Figure 1) (28–35). Normally the total quantity of ATP in human body is about 0.1 mole (~50 g). However, the energy used by human cells requires the hydrolysis of 100–150 moles (around 50–75 kg) of ATP daily (36). This means that each ATP molecule is recycled 1,000–1,500 times during a single day. The ATP and CrP activity combined, also referred to as the phosphagen system, is the most rapidly available source of energy (37). Unfortunately, the energy available from the store of phosphagen system is limited and can provide energy for a few seconds of maximal activity.

CrP, also known as phosphocreatine or phosphorylated creatine, is a small molecular compound with the formula of C4H10N3O5P, having a molecular weight of 211 daltons. There is one high-energy phosphate bond (N~P) in the chemical structure. As compared, ATP has a relatively more complex molecular structure (C10H16N5O13PS), larger molecular weight (507 daltons), and two high-energy phosphate bonds (O~P). However, the N~P bond of CrP has more energy than either one O~P bond of ATP, 10.3 kcal/mol in comparison with 7.3 kcal/mol (Figure 2) (38). Therefore, CrP can easily provide enough energy and serve as a HEP-bond donor for ATP reconstitution through “CrP shuttle” (28).

The contents of HEPs vary significantly in different tissues. The highest levels of HEPs are found in muscle, heart, brain, spermatozoa, and retina (14). The concentration and distribution of HEPs in vivo can be determined non-invasively by 31P-magnetic resonance spectroscopy (MRS) (39, 40). The myocardial CrP/ATP ratio measured by 31P-MRS reflects the viability and energy metabolic status of cardiomyocytes (41). Over a wide range of cardiac workloads, the CrP/ATP ratio is essentially invariant and consistent with a constant free ADP concentration (42, 43). The cutoff point for CrP/ATP ratio (>1.60 and <1.60), which was established retrospectively and need to be evaluated prospectively, is a stronger predictor of cardiovascular death (44). The ratio is decreased upon myocardial ischemia (45, 46).

THE BIOSYNTHESIS, DEGRADATION AND TURNOVER OF ENDOGENOUS CREATINE PHOSPHATE

The biosynthesis of CrP begins by formation of creatine from three essential amino acids: arginine, glycine, and methionine (Figure 3) (14). The entire glycine molecule is incorporated whereas arginine furnishes its amidino group to yield guanidinoacetic acid (GAA), which then methylated at the amidino group to give creatine. It is postulated, but largely accepted, that the main route of creatine synthesis involves formation of guanidinoacetate in kidney, and methylation in liver (47–49). These reactions are respectively catalyzed by two rate-limiting enzymes, i.e., L-arginine-glycine amidinotransferase (AGAT) and S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase (GAMT) (47–50). To complete the phosphorylation process, creatine is then transported to tissues such as muscle, heart, and brain by a specific Na+/Cl−-dependent plasma membrane transporter (51). CrP production is catalyzed by creatine kinase (CK), which is a dimer of M and B (M = muscle, B = brain) subunits produced by different structural genes. Three isozymes are possible: BB, MB, and MM. Cardiac muscle contains significant amounts of CK-MB (25–46% of total CK activity, as opposed to

**Abbreviations:** ADP, adenosine diphosphate; AGAT, L-arginine-glycine amidinotransferase; AMP, adenosine monophosphate; APD, action potential duration; ATP, adenosine triphosphate; CK, creatine kinase; CrP, creatine phosphate; ERP, effective refractory period; GAA, guanidinoacetic acid; GAMT, S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase; HEPs, high-energy phosphates; IHD, ischemic heart disease; KATP, ATP-sensitive K+ channels; LPLs, lysophospholipids; MDA, malondialdehyde; MEMP, myocardial energy metabolic therapy; MRS, magnetic resonance spectroscopy; OP, oxidative phosphorylation; RCTs, randomized controlled trials; SP, substrate phosphorylation.
FIGURE 1 | An overview of synthesis of ATP and “CrP shuttle” in cardiomyocyte. ATP is the intracellular energy currency, majority of which is synthesized from ADP by oxidative phosphorylation of mitochondria (predominant) and cytoplasmic substrate phosphorylation (subordinate). CrP is the storage and transport carrier of energy, which serves to transfer the HEP-bond from the site of ATP production to the site of ATP utilization through “CrP shuttle.” ADP, adenosine diphosphate; ATP, adenosine triphosphate; CK, creatine kinase; CrP, creatine phosphate; HEP, high-energy phosphate; OP, oxidative phosphorylation; SP, substrate phosphorylation.

FIGURE 2 | Transfer of HEP-bond through “CrP shuttle.” There is one HEP-bond (N\(\sim\)P) in the chemical structure of CrP. As compared, ATP has a relatively more complex molecular structure and two HEP-bonds (O\(\sim\)P). However, the N\(\sim\)P bond of CrP has more energy than either one O\(\sim\)P bond of ATP, 10.3 kcal/mol in comparison with 7.3 kcal/mol. ATP, adenosine triphosphate; CrP, creatine phosphate; HEP, high-energy phosphate; \(\Delta G\), Gibbs free energy change.
giving an overall conversion rate for total creatine pool (creatine and CrP) of 1.1%/day and 2.6%/day is converted into creatinine, +CrP) of glycolysis (Table 1) (57–61). However, the HEPs synthesized by glycolysis are far from meeting the energy requirements of heart. Under such condition, the ischemic myocardium preferentially utilizes the energy contained in endogenous CrP, followed by ATP, ADP, and adenosine monophosphate (AMP) (Figure 4) (62–67). AMP can also be decomposed into adenosine, hypoxanthine, etc. under the action of 5’-nucleotidase (Figure 4) (62, 68). The above reaction ultimately leads to a decrease in intracellular adenine nucleotide pool (ATP + ADP + AMP), resulting in a significant reduction in high-energy phosphate precursors. If the myocardium recover aerobic oxidation in a short period of time, AMP can be reoxidized to ADP and ATP to replenish energy. If not, it is no longer possible to reoxidize AMP to ADP or ATP. Furthermore, the lactic acid and other intermediate products produced by glycolysis accumulate in cardiomyocytes (Figure 4) (57, 58). After 10 min of ischemia, the intracellular pH will drop to 5.8–6.0 (69, 70). The rate of ADP reporphosphorylation to ATP by anaerobic glycolysis is slowed down by acidosis (71).

Secondary to the metabolic changes, myocardial ischemia/reperfusion injuries occur as follows: intracellular Ca²⁺ overload, accumulation of arrhythmogenic intermediates and oxygen free radicals, myocardial membrane instability, electrophysiological changes in cardiomyocytes, mitochondrial damage, and platelet aggregation, etc (Figure 4).

PATHOPHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF EXOGENOUS CREATINE PHOSPHATE ON MYOCARDIAL ISCHEMIA

The clinical effects of ATP in patients with cardiovascular disorders have been evaluated in early studies (72–74). Intravenous administration of ATP can interrupt the reentry pathways through the atrial ventricular node and restore normal sinus rhythm accompanied by relatively high incidences of advanced atrioventricular block and other adverse reactions, which makes paroxysmal supraventricular tachycardia the primary cardiovascular indication (75). And it seems quite paradoxical that oral administration of ATP may lead to a progressive diminution of plasma ATP level (76). Furthermore, exogenous ATP is a charged molecule containing three negative charges that is not freely permeable through cell membranes (77–79). In addition, there are enzymes that decompose ATP on the surface of cell membrane, including ATPase, adenylate kinase and AMP deaminase, which can split ATP into ADP, AMP, adenosine, and inorganic phosphate (80, 81). Since the first publication by Parrat and Marshall (82), CrP has been substantially demonstrated to be effective in protection of ischemic myocardium. The following we will focus on the pathophysiological and pharmacological effects of exogenous CrP, including but not limited to supplementing cellular energy.

Replenishment of Intracellular ATP

It has been observed that the exogenous CrP could be incorporated into intracellular ATP molecules and increase the

MYOCARDIAL METABOLIC CHANGES DURING ISCHEMIA/REPERFUSION: SUBSTRATES, PATHWAYS, METABOLITES, AND PURINE NUCLEOTIDE CYCLE

Within a few seconds after coronary blood flow blockage, the oxygenated hemoglobin in ischemic zone rapidly depletes. The main pathway used to generate energy in myocardium changes from aerobic oxidation of mitochondria to cytoplasmic anaerobic glycolysis (Table 1) (57, 58). And the primary substrate of myocardial energy metabolism also changes from free fatty acids to glucose (Table 1) (57–61). However, the HEPs synthesized by glycolysis are far from meeting the energy requirements of heart. Under such condition, the ischemic myocardium preferentially utilizes the energy contained in endogenous CrP, followed by ATP, ADP, and adenosine monophosphate (AMP) (Figure 4) (62–67). AMP can also be decomposed into adenosine, hypoxanthine, etc. under the action of 5’-nucleotidase (Figure 4) (62, 68). The above reaction ultimately leads to a decrease in intracellular adenine nucleotide pool (ATP + ADP + AMP), resulting in a significant reduction in high-energy phosphate precursors. If the myocardium recover aerobic oxidation in a short period of time, AMP can be reoxidized to ADP and ATP to replenish energy. If not, it is no longer possible to reoxidize AMP to ADP or ATP. Furthermore, the lactic acid and other intermediate products produced by glycolysis accumulate in cardiomyocytes (Figure 4) (57, 58). After 10 min of ischemia, the intracellular pH will drop to 5.8–6.0 (69, 70). The rate of ADP reporphosphorylation to ATP by anaerobic glycolysis is slowed down by acidosis (71).

Secondary to the metabolic changes, myocardial ischemia/reperfusion injuries occur as follows: intracellular Ca²⁺ overload, accumulation of arrhythmogenic intermediates and oxygen free radicals, myocardial membrane instability, electrophysiological changes in cardiomyocytes, mitochondrial damage, and platelet aggregation, etc (Figure 4).

PATHOPHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF EXOGENOUS CREATINE PHOSPHATE ON MYOCARDIAL ISCHEMIA

The clinical effects of ATP in patients with cardiovascular disorders have been evaluated in early studies (72–74). Intravenous administration of ATP can interrupt the reentry pathways through the atrial ventricular node and restore normal sinus rhythm accompanied by relatively high incidences of advanced atrioventricular block and other adverse reactions, which makes paroxysmal supraventricular tachycardia the primary cardiovascular indication (75). And it seems quite paradoxical that oral administration of ATP may lead to a progressive diminution of plasma ATP level (76). Furthermore, exogenous ATP is a charged molecule containing three negative charges that is not freely permeable through cell membranes (77–79). In addition, there are enzymes that decompose ATP on the surface of cell membrane, including ATPase, adenylate kinase and AMP deaminase, which can split ATP into ADP, AMP, adenosine, and inorganic phosphate (80, 81). Since the first publication by Parrat and Marshall (82), CrP has been substantially demonstrated to be effective in protection of ischemic myocardium. The following we will focus on the pathophysiological and pharmacological effects of exogenous CrP, including but not limited to supplementing cellular energy.

Replenishment of Intracellular ATP

It has been observed that the exogenous CrP could be incorporated into intracellular ATP molecules and increase the
TABLE 1  | Myocardial energy metabolism: source, process and site of ATP production.

| Source of ATP production | Pathway of ATP production | Oxygen consumption (per unit ATP) | Accumulation of acid metabolites | Rate of ATP production | Net ATP yield (per unit substrate) | Site of ATP production |
|--------------------------|---------------------------|----------------------------------|---------------------------------|------------------------|----------------------------------|------------------------|
| CrP                      | CrP ⇌ ATP shuttle         | None                             | –                               | Very fast              | 1                                | Cytoplasm              |
| Glucose                  | Anaerobic glycolysis      | None                             | + + +                           | Fast                   | 2                                | Cytoplasm              |
| Glucose                  | Aerobic oxidation         | Less                             | –                               | Moderate               | 38                               | Mitochondria (predominant) and cytoplasm |
| Free fatty acids         | Aerobic oxidation         | More                             | –                               | Slow                   | Usually > 100 (depending on the number of carbon atoms in the molecule of free fatty acid) | Mitochondria (predominant) and cytoplasm |

ATP, adenosine triphosphate; CrP, creatine phosphate.

FIGURE 4 | The primary metabolic changes and the secondary cellular injuries during myocardial ischemia/reperfusion. Ischemic myocardium preferentially utilizes the energy contained in CrP, followed by ATP, ADP, and AMP. And AMP can be further decomposed into adenosine and hypoxanthine, which leads to a decrease in intracellular adenine nucleotide pool. Furthermore, the lactic acid produced by glycolysis accumulate in cardiomyocytes, resulting intracellular acidosis. The loss of HEPs eliminates three of the four mechanisms of cellular calcium homeostasis, leading intracellular Ca\(^{2+}\) overload. Mitochondrial sequestration, the remaining mechanism, causes overloading of the mitochondria with Ca\(^{2+}\) and diminished capacity for oxidative phosphorylation. And overloaded intracellular Ca\(^{2+}\) induces the conversion of xanthine dehydrogenase to xanthine oxidase. The latter can produce oxygen free radicals, which in turn oxidize the membrane phospholipids and produce MDA, causing the membrane instability. In addition, intracellular accumulation of metabolic intermediates, including AMP, lactic acid, Ca\(^{2+}\), and H\(^+\), etc, may activate membrane phospholipase to make cell membrane degrade to LPLs, which also contribute to myocardial membrane instability. Increased ADP can induce platelet adhesion and aggregation. ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CrP, creatine phosphate; HEPs, high-energy phosphates; LPLs, lysophospholipids; MDA, malondialdehyde.

tissue level of ATP (83). Although exogenous CrP uptake was 3–4 orders of magnitude lower than ATP conversion in the case of normal cardiac work, it may be important in maintaining subsarcolemmal pools of CrP or ATP (Figure 4) (35, 83). The exogenous CrP uptake rate can be markedly increased in hypokinetic segments of ischemic myocardium (35, 83–86).
Low-dose CrP may promote intracellular ATP synthesis mainly through substrate. After reaching a certain concentration of 10 mmol/L, it can significantly inhibit S'-nucleotidase and AMP deaminase, thereby maintaining the nucleotide pool level, indicating that CrP does not only act as an energy substrate but also a regulator able to bind to the active sites of the enzymes and change their activity (62, 68, 87–90).

**Attenuation of Intracellular Ca\(^{2+}\) Overload in Cardiomyocytes**

Normally, extracellular fluid has a concentration of Ca\(^{2+}\) 10,000 times higher than intracellular fluid (91). Furthermore, there is an electrical force driving Ca\(^{2+}\) into the cell because of the negative resting membrane potential (91, 92). However, there is little leakage of Ca\(^{2+}\) into the cardiomyocyte except during the action potential. Even the Ca\(^{2+}\) that enters the cell during action potentials must be removed from the cell otherwise an accumulation of Ca\(^{2+}\) would lead to cellular dysfunction (92). Main mechanisms maintaining the intracellular to extracellular concentration and charge gradients include: (1) pumping Ca\(^{2+}\) out of the cytoplasm by the plasma membrane Ca\(^{2+}\) ATPase (93), (2) exchange of Ca\(^{2+}\) for Na\(^+\) driven by the intracellular to extracellular concentration gradient of Na\(^+\) as a result of the plasma membrane Na\(^+\)-K\(^+\) ATPase (94), (3) sequestration of cytoplasm Ca\(^{2+}\) in sarcoplasmic reticulum (SR) by the SR Ca\(^{2+}\) ATPase (95), and (4) accumulation of intracellular Ca\(^{2+}\) by oxidation-dependent calcium sequestration inside the mitochondria (96). The loss of HEPs during ischemia eliminates three of the four mechanisms of cellular calcium homeostasis (Figure 4). Mitochondrial sequestration, the remaining mechanism, causes overloading of the mitochondria with Ca\(^{2+}\) and diminished capacity for oxidative phosphorylation (Figure 4) (97). Furthermore, activation of phospholipases and protein kinases (98), production of arachidonic acid (99, 100), and oxygen free radicals (101) are all involved in the destruction of membrane integrity. This, in turn, causes a massive and rapid influx of Ca\(^{2+}\) into the cell.

Several studies have shown that intracellular Ca\(^{2+}\) overload is a major cause of myocardial cell damage and cardiac dysfunction in IHD. CrP can reduce Ca\(^{2+}\) influx by providing energy to ATP-dependent Ca\(^{2+}\) ATPase and Na\(^+\)-K\(^+\) ATPase on the plasma membrane (102, 103). At the same time, the Ca\(^{2+}\) ATPase activity on the sarcoplasmic reticulum is restored, and Ca\(^{2+}\) enter the sarcoplasmic reticulum to avoid the myocardial stiffness contracture (104). Furthermore, CrP binds to membrane phospholipids through zwitterionic interaction, which can enhance membrane stability (105, 106). In addition, CrP can also provide energy for the sliding of actin-myosin filaments, promoting the rapid recovery of myocardial contractility (107).

**Protection of Heart From Oxidative Stress-Induced Myocardial Injury**

And overloaded intracellular Ca\(^{2+}\) induces the conversion of xanthine dehydrogenase to xanthine oxidase (108–111). The latter can produce superoxide and xanthine from hypoxanthine upon reperfusion (Figure 4) (112). Furthermore, more damaging free radicals could be produced by the metal catalyzed Haber-Weiss reaction (113–115). The large amount of oxygen free radicals generated by the above reactions can in turn oxidize the membrane phospholipids and produce malondialdehyde (MDA), causing the membrane instability (Figure 4) (116). Zucchi et al. (117) found that supplementation of exogenous CrP could reduce the product of phospholipid peroxidation, MDA, by inhibiting ADP/AMP degradation and Ca\(^{2+}\) accumulation in cardiomyocytes. Myocardial peroxidation damage is alleviated through all of the above mechanisms.

**Stabilization of Membrane Structure**

Maintaining the integrity of the phospholipid bilayer membrane is a basic requirement for preserving overall cell viability. Myocardial membrane instability due to the decrease of ATP production and accumulation of acid metabolites plays a key role in the pathogenesis of ischemia-reperfusion injury, especially the electrophysiological manifestation of ischemia (118). The possibility that lysophospholipids (LPLs) contribute to myocardial membrane instability was first reported by Hajdu (119). Normally their concentration is maintained very low, but LPLs in sufficient quantities are potent detergents, which can alter general properties of the membrane such as fluidity and permeability (120). Furthermore, LPLs have been shown to affect the activities of plasma membrane Na\(^+\)-K\(^+\) ATPase (121). Upon myocardial ischemia, intracellular accumulation of metabolic intermediates, including AMP, lactic acid, Ca\(^{2+}\), and H\(^+\), etc, may activate membrane phospholipase to make cell membrane degrade to LPLs (Figure 4). At 8 min after ischemia, a 60% increase in LPLs levels occurred, which could either be reacylated or transacylated to form precursor phospholipids or further degraded, depending on the energy state of the cell (121–123). Supplementation of exogenous CrP can provide energy to ATP-dependent Ca\(^{2+}\) ATPase and Na\(^+\)-K\(^+\) ATPase on the plasma membrane and reduce the activation of anaerobic glycolysis, which blocks the process of phospholipids degradation and stabilizes the cell membrane. In addition, the integrity of the mitochondrial structure during ischemia is the basis for oxidative phosphorylation to synthesize ATP after reperfusion. CrP also has protective effects on the mitochondrial membrane and its oxidative phosphorylation function (124–126).

**Broad Spectrum Antiarrhythmic Effects**

Normally, the electrophysiological properties of cardiomyocytes require cell membrane integrity and maintaining of intracellular to extracellular concentration and charge gradients. Metabolic changes after myocardial ischemia, including the decrease of ATP production and accumulation of acid metabolites, lead to decreased activity of ATP-dependent transport systems. ATP-sensitive K\(^+\) channels (KATP), inactivated by normal cellular ATP levels, will open and permit K\(^+\) to leave the cell upon ischemia (127, 128). Furthermore, decreased activity of Na\(^+/\)K\(^+\)-ATPase leads to extracellular accumulation of K\(^+\) and inactivation of fast Na\(^+\) channels that are responsible for the rapid depolarization (129). These mechanisms lead to a series of electrophysiological changes in cardiomyocytes, including: (1) the resting membrane potential and the action potential
amplitude are significantly decreased; (2) the depolarization speed is slowed down; (3) the action potential duration (APD) is shortened; (4) the distance from the resting membrane potential to the K⁺ equilibrium potential is increased; (5) the conduction velocity rate is slowed down (130). All of the above changes ultimately can contribute to arrhythmias.

Studies have shown that in myocardial ischemia and reperfusion, CrP can play a broad spectrum antiarrhythmic effects through several electrophysiological mechanisms, including but not limited to ATP replenishment (131). Firstly, by providing energy to ATP-dependent KATP channels and Na⁺/K⁺-ATPase, exogenous CrP can reduce extracellular accumulation of K⁺ and reactivate the fast Na⁺ channels, suggesting a Class I antiarrhythmic role (132). Secondly, by prolonging ventricular myocardium APD and effective refractory period (ERP) under normoxic but not ischemic conditions, exogenous CrP can prevent reentrant circuits forming between the ischemic and non-ischemic zone and play a class III antiarrhythmic role (132, 133). Thirdly, by attenuating intracellular Ca²⁺ overload, exogenous CrP can inhibit Ca²⁺-mediated activation of inward current channels and triggered activity, exerting a class IV antiarrhythmic role (134, 135). Furthermore, exogenous CrP can also play an antiarrhythmic role by reducing the accumulation of arrhythmogenic lysophosphoglycerides and increasing the threshold of ventricular fibrillation (136–138).

Inhibiting Platelet Aggregation and Improving Microvascular Function

It is known that ADP can not only induce platelet adhesion and aggregation, but also amplify the aggregation effects of collagen, thrombin and other inducers (Figure 4) (139, 140). ADP may still affect the platelets when the arachidonate pathway is blocked (141). Exogenous CrP can inhibit platelet aggregation and then improve the microvascular function by rapid removal of ADP and formation of ATP, which is an inhibitor of ADP-induced platelet aggregation (19, 142).

CLINICAL APPLICATION OF EXOGENOUS CREATINE PHOSPHATE IN ISCHEMIC HEART DISEASE: EVIDENCE AND EVALUATION

As mentioned above, energy metabolic abnormalities are the upstream and primary pathophysiological manifestation of myocardial ischemia. Whereas, hemodynamic, electrophysiological, morphological, clinical, biochemical and imaging changes are the downstream, and secondary consequence of myocardial energy metabolic abnormalities. The depletion of HEPs is involved in both upstream and downstream changes in myocardial ischemia. As demonstrated in vitro and animal experiments, CrP was suggested to be potentially beneficial in patients with acute and chronic myocardial ischemic injury through multiple mechanisms, including but not limited to ATP replenishment. In fact, results from a large number of clinical studies substantially support that supplementation of exogenous CrP is associated with improved short-term survival (143, 144), enhancement of cardiac systolic and diastolic function (145–147), lower peak CK-MB/troponin release (20, 148–152), reduction in the incidence of major arrhythmias (144, 151, 153–156), etc. There is still uncertainty, however, whether the administration of exogenous CrP can improve long-term outcomes, rather than just the secondary endpoints or pathological process of IHD.

LIMITATIONS AND PERSPECTIVES

According to a meta-analysis performed by Landoni et al. (16), although more than 4,000 articles were screened, only 12 studies comparing CrP with placebo or standard treatment in patients with IHD met the design requirements for controlled or case-matched clinical trials. Unfortunately, there is insufficient statistical power to obtain results on long-term survival due to the common limitations, including:

| Indications                                      | Contraindications and relative contraindication | Side effects          | Instructions of administration and dosage |
|-------------------------------------------------|-------------------------------------------------|-----------------------|--------------------------------------------|
| Cardiac metabolic abnormalities during myocardial ischemia. | Chronic renal failure (in high doses, for example, daily dose of 5–10 g). | Allergic reactions, Lowering of arterial pressure. | Cardiac metabolic abnormalities during myocardial ischemia: 0–24 h—intravenous bystry infusion of 2–4 g of CrP divided in water for injections of 50 ml with the subsequent intravenous infusion for 2 h 8–16 g in 250 ml of 5% of solution of glucose; during second day 2 times a day intravenously kepeln (infusion duration of 30 min) enter 2–4 g of the drug divided in 50 ml of water for injections; during third day the drug is administered according to the same scheme in a dose 2 g (if necessary treatment is continued for 6 days). |
| Cardioprotection during heart surgery.           | Hypersensitivity to drug components. | Cardiovascular protection during heart surgery: intravenously kepeln (infusion duration of 30 min) 2 g of the drug divided in 50 ml of water for injections with frequency rate of introduction 2 times a day. The course is begun in 3–5 days prior to surgical intervention and continued 1–2 more days after its carrying out. During operation it is necessary to add to composition of usual cardioplegic solution in concentration 10 mmol/l just before introduction. |

CrP, creatine phosphate; IHD, ischemic heart disease.

A table showing the indications, contraindications, side effects, and application instructions of CrP supplement for IHD.
(1) single center trial; (2) small sample size; (3) short-term follow-up; (4) secondary endpoints; (5) choice of standard treatment rather than placebo as the comparator; (6) administration routes and doses of CrP varying significantly among the studies; (7) inadequate baseline information or baseline bias (20, 143, 144, 146, 150, 151, 153, 156). In addition, majority of the studies were published before the “era of revascularization” and patients were recruited from those undergoing non-revascularization therapy or mixed, significantly different from the current practice (143, 144, 146, 150, 153, 156).

At first glance, it is surprising that exogenous CrP has not been shown to improve long-term survival in clinical studies. In fact, there are two sides to the same issue. On one side, CrP may play extensive roles in every physiological and pathophysiological process from upstream to downstream of myocardial ischemia. On the other side, the myocardial intracellular actions of CrP lack target and pathway specificity. Furthermore, the uptake and distribution of exogenous CrP in vivo lack of tissue and cell specificity. Such non-specificities lead to uncertainties in the dominant pharmacological mechanism, optimal administration route and dose, as well as treatment window of exogenous CrP in individualized patients with IHD. Moreover, the cardioprotection of exogenous CrP may be limited by endogenous CrP levels. However, owing to the physiological, pathophysiological, and pharmacological plausibility of its effects and to the concordance of the beneficial effects of exogenous CrP on multiple secondary but important outcomes and short-term survival, there is urgent need for high-quality multicentre randomized controlled trials (RCTs) to confirm long-term survival improvement. In addition, further studies are needed to investigate the causality between changes in endogenous/exogenous CrP levels and IHD progression and prognosis (157).

To better understand the pathophysiological and pharmacological effects, we specified the context for all cited researches as cell study (19, 23–27, 29–35, 48–54, 69, 70, 91–103, 118, 128), animal study (12, 41, 42, 45, 46, 65, 66, 68, 71–74, 76–78, 81–90, 99, 104, 111, 116–119, 129, 133–135) and human study (15–18, 20, 21, 37–40, 44, 58, 108–110, 143–156).

Furthermore, we detailed the indications, contraindications, side effects, and application instructions of CrP supplement in Table 2.

CONCLUSIONS

The purpose of this article is to provide a comprehensive and concise description of the cellular and molecular aspects of cardioprotective mechanisms and a critical evaluation of the clinical evidence of HEPs in IHD. According to the well-documented physiological, pathophysiological and pharmacological properties of HEPs, exogenous CrP may be considered as an ideal metabolic regulator. It plays cardioprotection roles from upstream to downstream of myocardial ischemia through multiple complex mechanisms, including but not limited to replenishment of cellular energy. Although exogenous CrP administration has not been shown to improve long-term survival, the beneficial effects on multiple secondary but important outcomes and short-term survival are concordant with its pathophysiological and pharmacological effects. There is urgent need for high-quality multicentre RCTs to confirm long-term survival improvement in the future.

AUTHOR CONTRIBUTIONS

HY-D, ZY-X, and YS-W contributed toward drafting and critically reviewing the document and agree to be accountable for all aspects of the work. YS-W and ZY-J provided his views and comments on the manuscript, made the final decision about the journal selection as well as approved the submission of the manuscript to the journal. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Opie LH. Proof that glucose-insulin-potassium provides metabolic protection of ischaemic myocardium. Lancet. (1999) 353:768–9. doi: 10.1016/S0140-6736(98)00385-7

2. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G. Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. N Engl J Med. (1999) 323:1593–600. doi: 10.1056/NEJM199912063232304

3. Fihn SD, Blankenship JC, Alexander KP, Bittl JA, Byrne JG, Fletcher BJ, et al. 2014 ACC/AHA/ACCP/AATS/PCNA/SCAI/STS Focused Update of the guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, and the American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol. (2014) 64:1929–49. doi: 10.1016/j.jacc.2014.07.017

4. Amsterdam EA, Wenger NK, Brindis RG, Casey DE, Ganiats TG, Holmes DR, et al. 2014 AHA/ACC Guideline for the Management of Patients with Non-ST-Elevation Acute Coronary Syndromes: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. (2014) 64:e139–228. doi: 10.1016/j.jacc.2014.09.016

5. Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, et al. 2015 ACC/AHA/SCAI Focused Update on Primary Percutaneous Coronary Intervention for Patients With ST-Elevation Myocardial Infarction: an Update of the 2011 ACCF/AHA/SCAI Guideline for Percutaneous Coronary Intervention and the 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction. J Am Coll Cardiol. (2016) 67:1235–50. doi: 10.1016/j.jacc.2015.10.005

6. Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Fumagalli R, Ciferno P, et al. 2018 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. (2020) 41:407–77. doi: 10.1093/eurheartj/ehz425

7. Ibáñez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. (2018) 39:119–77. doi: 10.1093/eurheartj/ehx393
16. Landoni G, Zangrillo A, Lomivorotov VV, Likhvantsev V, Ma J, De Simone M, D’Alessandro M, Melandri BA. ATP hydrolysis in ATP synthases can
25. Rosca MG, Hoppel CL. Mitochondria in heart failure. Cardiovasc Res. (2010) 88:46–50. doi: 10.1093/cvr/cvq240
27. Klingenberg M. The ADP and ATP transport in mitochondria and its carrier. Biochim Biophys Acta. (2008) 1778:1978–2021. doi: 10.1016/j.bbamem.2008.04.011
37. Kerksick CM, Roberts MD, Dalbo VJ, Sunderland KL. Intramuscular phosphagen status and the relationship to muscle performance across the age spectrum. Eur J Appl Physiol. (2016) 116:115–27. doi: 10.1007/s00421-015-3246-1
45. McDonald KM, Yoshiyama M, Francis GS, Ugurbil K, Cohn JN, Assmann G, et al. Residual macrovascular risk in 2013: what have we learned. Cardiovasc Diabetol. (2012) 11:e004800. doi: 10.1161/CIRCHEARTFAILURE.117.004800
46. Liao R, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Circ Heart Fail. (2018) 11:e004800. doi: 10.1161/CIRCHEARTFAILURE.117.004800
50. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation. (1997) 96:2190–6. doi: 10.1161/01.CIR.96.6.2190
52. McDonald KM, Yoshiaima M, Francis GS, Uguribl K, Cohn JN, Zhang J. Myocardial bioenergetic abnormalities in a canine model of left ventricular dysfunction. Am J Cardiol. (1994) 73:876–93. doi: 10.1016/0002-9149(94)90769-2
54. Rao L, Nascimben L, Friedrich J, Gwathmey JK, Ingwall JS. Decreased energy reserve in an animal model of dilated cardiomyopathy. Circulation. (1999) 100:2869–76. doi: 10.1161/01.CIR.96.6.2190
48. Popolo A, Adesso S, Pinto A, Autore G, Marzocco S. L-Arginine and its metabolites in kidney and cardiovascular disease. Amino Acids. (2014) 46:2271–86. doi: 10.1007/s00726-014-1825-9

49. Barcelos RP, Stefanello ST, Maurizi JL, Gonzalez-Gallego J, Soares FA. Creatine and the liver: metabolism and possible interactions. Mini Rev Med Chem. (2016) 16:162–8. doi: 10.2174/138955751666105720102613

50. Iqbal F, Hoeger H, Lubec G, Bodamer O. Biochemical and behavioral phenotype of AGAT and GATM deficient mice following long-term creatine monohydrate supplementation. Metab Brain Dis. (2017) 32:1951–61. doi: 10.1007/s11011-017-0099-3

51. Sora I, Richman J, Santoro G, Wei H, Lubec G, Bodamer O. Biochemical and behavioral phenotype of AGAT and GATM deficient mice following long-term creatine monohydrate supplementation. Metab Brain Dis. (2017) 32:1951–61. doi: 10.1007/s11011-017-0099-3

52. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger HM. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the ‘phosphocreatine circuit’ for cellular energy homeostasis. Biochem J. (1992) 281 (Pt 1):21–40. doi: 10.1042/bj2810021

53. Saks VA, Ventura-Clapier R, Aliev MK. Metabolic control and metabolic capacity: Two aspects of creatine kinase functioning in the cells. Biochim Biophys Acta. (1996) 1274:61–8. doi: 10.1016/0005-2728(96)00011-4

54. Brosnan ME, Brosnan JT. Renal arginine metabolism. J Nutr. (2004) 134:2791S–5; discussion 2796S–2797S. doi: 10.1093/jn/134.13.2791S

55. Kashani K, Richman J, Santoro G, Wei H, Wang Y, Vanderah T, et al. The intracellular pH on K+ stimulated, hypoxic and underperfused rat heart. J Mol Cell Cardiol. (2011) 50:598–605. doi: 10.1016/j.yjmcc.2011.01.007

56. Kopf GS, Chaudry I, Condos S, Baue AE. Reperfusion with ATP-MgCl2 following prolonged ischemia improves myocardial performance. J Surg Res. (1987) 43:114–7. doi: 10.1016/0022-4807(87)90152-1

57. Thelin S, Hultman J, Ronquist G, Hansson HE. Myocardial high-energy phosphates, lactate and pyruvate during moderate or severe normothermic ischemia in rat hearts perfused with phosphoeno-lpyruvate and ATP in cardioplegic solution. Scand J Thorac Cardiovasc Surg. (1987) 21:245–9. doi: 10.3109/14017438701906033

58. Martinis L, Belli C. [Experimental study on the effects of polarizing solutions with and without ATP on the electrocardiographic pattern of myocardial ischemia caused by pitressin]. G Clin Med. (1967) 48:720–26.

59. Camm AJ, Garratt CJ. Adenosine and supraventricular tachycardia. N Engl J Med. (1991) 325:1621–9. doi: 10.1056/NEJM199110053252306

60. Popolo A, Adesso S, Pinto A, Autore G, Marzocco S. L-Arginine and its metabolites in kidney and cardiovascular disease. Amino Acids. (2014) 46:2271–86. doi: 10.1007/s00726-014-1825-9

61. Kichenin K, Seman M. Chronic oral administration of ATP modulates nucleoside transport and purine metabolism in rats. J Pharmacol Exp Ther. (2000) 294:1263–33.

62. Glynn IM. Membrane adenosine triphosphatase and cation transport. Br Med Bull. (1968) 24:165–9. doi: 10.1093/oxfordjournals.bmb.a017062

63. Kichenin K, Seman M. Chronic oral administration of ATP modulates nucleoside transport and purine metabolism in rats. J Pharmacol Exp Ther. (2000) 294:1263–33.

64. Fedelesová M, Zieglerhoffer A, Krause EG, Wollenberger A. Effect of exogenous adenosine triphosphate on the metabolic state of the excised hypothermic dog heart. Circ Res. (1969) 24:617–27. doi: 10.1161/01.RES.24.6.617

65. Parratt JR, Marshall RJ. The response of isolated cardiac muscle to acute anoxia: protective effect of adenosine triphosphate and creatine phosphate. J Pharmacol. (1976) 264:27–33. doi: 10.1111/j.1092-7157.1974.tb09308.x

66. Down WH, Chasseaud LF, Ballard SA. The effect of intravenously administered phosphocreatine on ATP and phosphocreatine concentrations in the cardiac muscle of the rat. Arzneimittelforschung. (1983) 33:552–4.

67. Thelin S, Hultman J, Ronquist G, Juhlin C, Hansson HE, Lindgren PG. Improved myocardial protection by creatine phosphate in cardioplegic solution. An in vivo study in the pig during normothermic ischemia. Thorac Cardiovasc Surg. (1987) 35:377–42. doi: 10.1055/s-2007-1020217

68. Thelin S, Hultman J, Ronquist G, Hansson HE. Metabolic and functional effects of creatine phosphate in cardioplegic solution. Studies on rat hearts during and after normoxic ischemia. Scand J Thorac Cardiovasc Surg. (1987) 21:39–45. doi: 10.3109/14017438709116917

69. Rosenkranz R, Vask A, Yurievich IA, Nesterenko VV, Smirnov VN, et al. Effect of creatine phosphate on the slow inward calcium current, action potential, and contractile force of frog atrium and biventricle. Biochem Med. (1979) 21:1–5. doi: 10.1016/0006-2944(79)90049-8

70. Schoepf G, Rumpold H, Müller MM. Alterations of purine salvage pathways during differentiation of rat heart myoblasts towards myocytes. Biochim Biophys Acta. (1986) 884:319–25. doi: 10.1016/0006-4165(86)90180-7

71. Lewandowski ED, White LT. Pyruvate dehydrogenase influences posts ischemic heart function. Circulation. (1995) 91:2071–9. doi: 10.1161/01.CIR.91.7.2071

72. Smith CD, Wright G, Lofkin M. The effects of aspartate and 2-oxoglutarate upon glycolytic energy metabolites and mechanical recovery following global ischaemia in isolated rat hearts. J Mol Cell Cardiol. (1992) 24:305–15. doi: 10.1016/0022-2828(92)93167-1
106. Dawson AP. Regulation of intracellular Ca2+. *Cell Calcium* (1976) 3:63–76.

107. Günther J, Oddoy A, Schubert E. [Mechanical characteristics of isotonic cardiac muscle in situ]. *Biochem. Biophys. Acta* (1978) 559:259–87. doi: 10.1016/B978-0-306-41779-0.0002-3

108. Srivastava SK, Ansari NH, Liu S, Izban A, Das B, Szabo G, et al. The effect of hypoxanthine on mitochondrial respiration and the production of reactive oxygen species. *Am J Physiol. Lung Cell Mol Physiol.* (2005) 289:L101–9. doi: 10.1152/ajplung.00020.2005

109. Neumeier D, Widmer HR, et al. Phosphocreatine interacts with cardiac muscle myofibrils. *J Mol Cell Cardiol.* (1994) 26:71–213. doi: 10.1006/jmcc.1994.0145

110. Ronca-Testoni S, Raggi A, Ronca G. Muscle AMP aminohydrolase. 3. A comparative study on the regulatory properties of skeletal muscle enzyme from various species. *Biochem Biophys Acta.* (1970) 198:101–12. doi: 10.1016/0005-2744(70)90038-0

111. Gnesi L, Manovukov VV, Bogoslovskii VA. [Hypoxanthine content of peripheral venous blood in infant and ischemia of the myocardium]. *Ter Arkh.* (1978) 50:24–4.

112. Golfman LS, Haughey NJ, Wong JT, Jiang JY, Lee D, Geiger JD, et al. Calcium overload and cardiac myocyte cell damage induced by arachidonate lipoxygenation. *J Mol Cell Cardiol.* (1994) 26:71–213. doi: 10.1006/jmcc.1994.0145

113. Fridovich I. Superoxide radical: an endogenous toxicant. *Annu Rev Pharmacol Toxicol.* (1985) 23:239–57. doi: 10.1146/annurev.pa.23.040185.001323

114. McCord JM. The superoxide free radical: its biochemistry and pathophysiology. *Surgery.* (1983) 94:412–4.

115. Tien M, Svingen BA, Aust SD. An investigation into the role of hydroxyl radical in xanthine oxidase-dependent lipid peroxidation. *Arch Biochem Biophys.* (1982) 216:142–51. doi: 10.1016/0003-9863(82)90198-9

116. Ballagi-Pordany G, Richtler J, Koltai M, Aranyi Z, Pogátsa G, Schaper W. Is malondialdehyde a marker of the effect of oxygen free radicals in rat heart tissue. *Basic Res Cardiol.* (1981) 86:266–72. doi: 10.1007/BF02190606

117. Zucchi R, Poddishe R, Limbruno U, Mariani M, Ronca-Testoni S, Ronca G. Protection of isolated rat heart from oxidative stress by exogenous creatine phosphate. *J Mol Cell Cardiol.* (1989) 21:67–73. doi: 10.1002/0228-891494-6

118. Houang EM, Bartos J, Hackel BJ, Lodge TP, Yannopoulos D, Bates FS, et al. Cardiac muscle membrane stabilization in myocardial reperfusion injury. *JACC Basic Transl Sci.* (2019) 4:275–87. doi: 10.1016/j.jatbs.2019.01.009

119. Hajdu S, Weiss H, Titus E. The isolation of a cardiac active principle from mammalian tissue. *J Pharmacol Exp Ther.* (1957) 120:99–113.

120. Weltzien HU. Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. *Biochim Biophys Acta.* (1979) 559:259–87. doi: 10.1016/0306-4457(79)90042-0

121. Shaikh NA, Downar E. Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. *Circ Res.* (1981) 49:316–23. doi: 10.1161/01.RES.49.2.316

122. van den Bosch H. Phosphoglyceride metabolism. *Annu Rev Biochem.* (1974) 43:243–77. doi: 10.1146/annurev.bi.43.070174.001331

123. Sobel BE, Corr PB. Biochemical mechanisms potentially responsible for lethal arrhythmias induced by ischemia: the lysolipid hypothesis. *Adv Cardiol.* (1979) 26:76–85. doi: 10.1159/000402593

124. Scott ID, Nicholls DG. Energy transduction in intact synaptosomes. Influence of plasma-membrane depolarization on the respiration and membrane potential of internal mitochondria determined in situ. *Biochem J.* (1980) 186:21–31. doi: 10.1042/bj186021

125. Nakahara T, Takeo S. Irreversible changes in oxidative phosphorylation activity of the mitochondrial membrane from hearts subjected to hypoxia and reoxygenation. *Can J Cardiol.* (1986) 2:24–33.

126. Berkich DA, Salama G, LaNoue KF. Mitochondrial membrane potential of internal mitochondria determined in situ. *J Bioenerg Biomembr.* (1989) 21:67–73. doi: 10.1016/0022-2828(89)91494-6

127. Chowdhry IU, Witt RC, Nance PN, Rozanski GJ, et al. Heart failure mediated by inhibition of cardiac muscle Na,K-pump rate in isolated sheep cardiac Purkinje fibers. *J Mol Cell Cardiol.* (1997) 29:297–212. doi: 10.1016/j.jmcc.1997.0455

128. Dhall A, Singh JN, McNamara DB, Bernatsky A, Singh A, Harrow JA. Energy production and utilization in contractile failure due to intracellular calcium overload. *Adv Exp Med Biol.* (1983) 161:305–16. doi: 10.1007/BF004-2

129. Kléber AG. Resting membrane potential, extracellular potassium concentrations of hypoxanthine, xanthine, uric acid and allantoin in the peripheral blood of normals and patients with acute myocardial infarction and other ischaemic diseases. *Eur J Clin Chem Clin Biochem.* (1994) 32:837–42. doi: 10.1515/ccm.1994.32.11.837

130. Klabunde RE. Cardiac electrophysiology: normal and ischemic pathophysiology. *Circ Arrhythm Electrophysiol.* (2019) 12:1011–11. doi: 10.1016/j.jacbts.2019.01.009

131. Rosenshtraukh LV, Witt RC, Nance PN, Rozanski GJ, et al. Heart failure mediated by inhibition of cardiac muscle Na,K-pump rate in isolated sheep cardiac Purkinje fibers. *J Mol Cell Cardiol.* (1997) 29:297–212. doi: 10.1016/j.jmcc.1997.0455

132. Malis CD, Bonventre JV. Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. A model for post-ischemic and toxic mitochondrial damage. *J Biol Chem.* (1986) 261:14201–8. doi: 10.1016/S0021-9258(86)70404-8

133. Kock R, Delvoux B, Sigmund M, Greiling H. A comparative study of the concentrations of hypoxanthine, xanthine, uric acid and allantoin in the peripheral blood of normals and patients with acute myocardial infarction and other ischaemic diseases. *Eur J Clin Chem Clin Biochem.* (1994) 32:837–42. doi: 10.1515/ccm.1994.32.11.837
1. Du XH, Liang FY, Zhao XW. Effects of phosphocreatine on plasma brain activity in the dog. *Circ Res.* (1983) 52:566–79. doi: 10.1161/01.RES.52.5.566
2. El-Shref N, Gough WB, Zeiler RH, Mehran R. Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. *Circ Res.* (1985) 56:184–9. doi: 10.1161/01.RES.56.2.184
3. Ferrier GR, Moffat MP, Lukas A. Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion. Studies on isolated canine ventricular tissues. *Circ Res.* (1985) 56:14–20. doi: 10.1161/01.RES.56.2.14
4. Kryzhnovskii SA, Kacharava VG, Marko R, Kelemen K, Kaverina NV, Sakhs VA. [Electrophysiological study of the anti-arrhythmic mechanism of action of phosphocreatine in acute myocardial ischemia and reperfusion]. *Kardiolgia.* (1991) 31:66–9.
5. Anyukhovsky EP, Javadov SA, Preobrazhensky AN, Beloshapko GG, Rosenshtraukh LV, Sakhs VA. Effect of phosphocreatine and related compounds on the phospholipid metabolism of ischemic heart. *Biochem Med.* (1986) 35:327–34. doi: 10.1016/0885-4505(86)90090-3
6. Robinson LA, Braimbridge MV, Hearse DJ. Creatine phosphate: an additive myocardial protective and antiarrhythmic agent in cardioplegia. *J Thorac Cardiovasc Surg.* (1984) 87:190–200. doi: 10.1016/S0022-5223(19)7413-6
7. Niewiarowski S, Thomas DP. Platelet aggregation by ADP and thrombin. *Nature.* (1966) 212:1544–7. doi: 10.1038/2121544a0
8. Packham MA, Guccione MA, Chang PL, Mustard JF. Platelet aggregation and release: effects of low concentrations of thrombin or collagen. *Am J Physiol.* (1973) 225:338–47. doi: 10.1152/ajplegacy.1973.225.1.338
9. Zucker MB, Peterson J. Effect of acetylsalicylic acid, other nonsteroidal anti-inflammatory agents, and dipyridamole on human blood platelets. *J Lab Clin Med.* (1970) 76:66–75.
10. Macfarlane DE, Mills DC. The effects of ATP on platelets: evidence against the central role of released ADP in primary aggregation. *Blood.* (1975) 46:309–20. doi: 10.1182/blood.V46.3.309.309
11. Golikov AP, Riabinin VA. [Neoton in the treatment of myocardial infarction]. *Nan Fang Yi Ke Da Xue Xue Bao.* (2009) 29:154–55, 159.
12. Gough WB, Zeiler RH, Mehran R. Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. *Circ Res.* (1983) 52:566–79. doi: 10.1161/01.RES.52.5.566
13. El-Shref N, Gough WB, Zeiler RH, Mehran R. Triggered ventricular rhythms in 1-day-old myocardial infarction in the dog. *Circ Res.* (1983) 52:566–79. doi: 10.1161/01.RES.52.5.566
14. Chambers DJ, Braimbridge MV, Kosker S, Yamada M, Jupp RA, Crowther A. Creatine phosphate [Neoton] as an additive to St. Thomas’ Hospital cardioplegic solution (Plegisol). Results of a clinical study. *Eur J Cardiothorac Surg.* (1991) 5:74–81. doi: 10.1016/1010-7940(91)90004-4
15. Cheng SX, Hu QH. [Cardioprotective effect of exogenous phosphocreatine in patients undergoing open heart surgery]. *Huanan Yi Ke Da Xue Xue Bao.* (2001) 26:353–5.
16. Lisowsky M, Bochenek A, Kuczewicz E, Wnuk-Wojnar AM, Morawski W, Skalski J, et al. The use of exogenous creatine phosphate for myocardial protection in patients undergoing coronary artery bypass surgery. *J Cardiovasc Surg.* (1996) 37:75–80.
17. Guo-han C, Jian-hua G, Xuan H, Jinyi W, Rong L, Zhong-min L. Role of creatine phosphate as a myoprotective agent during coronary artery bypass graft in elderly patients. *Coron Artery Dis.* (2013) 24:48–53. doi: 10.1097/MCA.0b013e32835a6b95
18. Pagani L, Musiani A. [The use of systemic phosphocreatine in heart surgery]. *Minerva Anestesiol.* (1992) 58:199–205.
19. Cerny J, Nemec P, Bucek J, Cerny E, Papousek F, Lojek A. The effect of creatine phosphate in patients after surgery in ischemic heart disease. *Vnitri Lek.* (1993) 39:153–9.
20. Chambers DJ, Haire K, Morley N, Fairbanks L, Strumia E, Young CP, et al. St. Thomas’ Hospital cardioplegia: enhanced protection with exogenous creatine phosphate. *Ann Thorac Surg.* (1996) 61:67–75. doi: 10.1016/0003-4975(95)00819-5
21. Zhiukov IL, Ivanov VA, Kozhevnikov VA, Charnaia MA, Mukhamedzhanova AR, Trekova NA. [Intraoperative myocardial protection with extracellular cardioplegic solutions in patients with cardiac valve diseases]. *Anesteziol Reanimatol.* (2007) 2:38–42.
22. Mya R, Samarenko MB, Afonskaya NI, Sakhs VA. Reduction of ventricular arrhythmias by phosphocreatine (Neoton) in patients with acute myocardial infarction. *Am Heart J.* (1988) 116:393–7. doi: 10.1016/0002-8703(88)90611-4
23. Kitzenberg D CSP, Glover LE. Creatine kinase in ischemic and inflammatory disorders. *Clin Transl Med.* (2016) 5:31. doi: 10.1186/s40169-016-0114-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Yi-Dan, Ying-Xin, Shi-Wei and Yu-Jie. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.