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REVIEW ARTICLE

Pharmacological Basis for Abrogating Myocardial Reperfusion Injury Through a Multi-Target Combined Antioxidant Therapy

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Abstract

The main goal of the treatment for acute myocardial infarction is to achieve reperfusion of the affected myocardial tissue, with percutaneous coronary angioplasty being the gold standard procedure. However, this strategy has been associated with additional heart damage termed "lethal reperfusion injury," which is responsible for up to half of the final infarct size. Among the possible underlying mechanisms that are likely to explain this damage, studies suggest that oxidative stress plays a key role. Although this has not been translated into clinical benefits in most studies, recent preclinical studies reported promising results and a possible synergy with the combined use of vitamin C (VC), *N*-acetylcysteine (NAC), and deferoxamine (DFO). However, to implement a combined therapy with these drugs for patients requires further studies to understand their pharmacokinetic properties. Available data of the clinical trials have not been validated by looking into the pharmacokinetics in their design. Therefore, this article presents an update and comparison of the evidence for the efficacy of these administration schemes for each drug in cardioprotection, their pharmacokinetic properties and mechanisms of action for their use against "lethal reperfusion injury." To achieve a cardioprotective effect using a new pharmacological strategy before the onset of reperfusion, it is helpful to consider the pharmacokinetics of each drug. In this regard, to design a fast and short pharmacologic therapeutic strategy, theoretically VC and DFO concentrations could be modeled by a one-compartment model whereas NAC could be modeled by a three-compartment model with an initial short half-life.

Key Points

Evidence suggests that oxidative stress plays an important role in reperfusion injury due to acute myocardial infarct management, but many clinical trials that have assessed the benefits of antioxidants have been unable to translate this evidence into clinical benefits.

Clinical trials have utilized different administration designs for each antioxidant, making it difficult to compare their outcomes and establish a consensus for an optimal therapeutic strategy.

Pharmacokinetics, mechanisms of action, and efficacy of vitamin C, N-acetylcysteine, and deferoxamine reviewed here can help to support and develop a pharmacologic strategy based on these antioxidants.

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1 Introduction

Cardiovascular diseases (CVDs) are the major cause of death worldwide, with ischemic heart disease being the leading cause of mortality among them [1, 2]. Percutaneous transluminal coronary angioplasty (PTCA) has long remained the most widely used procedure and the one that has given the best results in the clinical outcome of patients with acute myocardial infarction (AMI) [3]. However, it has been demonstrated that reperfusion can also potentiate myocardial damage. This phenomenon, called reperfusion injury (RI), can manifest as reperfusion arrhythmias, myocardial stunning, microvascular non-reflow, and lethal reperfusion injury [4]. Lethal reperfusion injury refers to death of myocardial tissue due to RI [4, 5]. The importance of this undesirable additional injury following reperfusion lies in the association of final infarct size to the prognosis after PTCA [4, 6]. Consequently, lethal reperfusion injury should explain why surviving patients undergoing PTCA still have high heart failure and mortality rates [4, 7, 8]. It has been claimed that RI accounts for up to 50% of the final infarct size [5].

Several pathophysiological mechanisms have been suggested to explain the cell damage involved in RI [5], among which oxidative stress plays an important role due to reported increased reactive oxygen and nitrogen species occurring early after reperfusion onset [5, 9, 10]. Accordingly, pharmacological antioxidants appear to be a promising strategy against RI by interfering at different levels in the mechanism of redox imbalance [11]. Although animal models have shown promising results, the outcomes of most clinical trials have not yet been translated into significant clinical benefits and myocardial infarct size reduction. In this regard, the strategy to design clinical studies based on preclinical models has been questioned. It is advisable that the dosing and design of pharmacological therapies are validated previously, as are dosing and designs of pharmacological therapies [5, 12, 13]. In this sense, to design an optimal strategy it is useful to have a good knowledge of the pharmacokinetic (PK) properties of the drugs to be assessed, as the outcomes of any clinical trial will depend on the drug concentrations achieved.

Recently, it has been hypothesized that, to obtain greater efficacy when implementing these interventions in clinical practice, the approach needs to be multi-targeted, focusing on the different pathophysiological mechanisms [13]. In agreement with this view, a recent in vitro pre-clinical study in rat cardiac cells described the efficacy and synergy of coadministration of vitamin C (VC), *N*-acetylcysteine (NAC), and deferoxamine (DFO) [14], since they act through different mechanisms of action. Roughly summarized, VC acts as a ROS scavenger [15, 16]; NAC contributes to reinforce levels of reduced glutathione (GSH), a physiological antioxidant [17, 18]; and DFO acts as an iron chelator, preventing these metal ions from participating in ROS formation through the Fenton reaction [19]. Further insights about the evidence and underlying mechanisms of these antioxidants have been recently reviewed in other articles [20, 21].

The aim of this article was to gather information to help propose a feasible multitarget pharmacological strategy based on VC, NAC, and DFO, which could enhance the effectiveness of these drugs in decreasing reperfusion injury due to PTCA. To achieve this aim, we give a description of the main ROS sources and, for each drug, we review clinical and preclinical studies that have assessed their efficacy in abrogating reperfusion injury and their PK properties.

2 Oxidative Stress and Myocardial Reperfusion Injury

Mitochondrion is the major source of intracellular ROS, occurring especially through the mitochondrial respiratory chain and mitochondria-localized proteins that produce superoxide anion and H_2O_2. In a physiological state, cardiac ROS signaling regulates heart development, cellular maturation, cardiac calcium handling, contraction, and vascular tone [22]. However, ROS also plays a key role in pathological conditions. In several animal models it has been shown that mitochondrion up-regulation of ROS pathways or down-regulation of mitochondrial scavenging systems lead to increased infarct size [23] and cardiac disease [22], both acute and chronic.

During myocardial ischemia, an imbalance between ROS production and cellular antioxidant systems occurs in favor of the first, leading to oxidative stress [24]. Also, the ischemic process prior to reperfusion sensitizes myocardial cells to ROS production, which is accompanied by impaired antioxidant defenses. Consequently, a restoration of blood flow, with the ensuing massive oxygen supply, generates bursts of ROS and reactive nitrogen species (RNS) [15], causing mitochondrial dysfunction. In this regard, it has been described that thioredoxin-interacting protein (Txnip) inhibits thioredoxin 2 (Trx2), which is the mitochondrial isoform of the thioredoxin antioxidant system [25, 26]. From in vitro studies, it has been shown that knockdown of Txnip in cardiomyocytes reduces apoptosis under conditions of oxidative stress [25]. Also, the inhibition of Trx2 in cardiac tissue results in increased H_2O_2 emission in rat hearts [27].

AMI patients undergoing PTCA have an acute and transient increase in ROS production, which tends to decrease spontaneously after a few hours [28–30]. This ROS increase peaks within the first 5 min after reperfusion [31]. In addition, ischemic-damaged cardiomyocytes, ferritin, and heme group degradation lead to the release of iron, which participates in the formation of even more ROS through the Fenton

reaction and various complex mechanisms, as extensively reviewed previously [21, 32].

ROS production is strongly related to RNS production and ensuing nitrosative stress. As superoxide anion is produced, it can directly react with nitric oxide (NO), an important physiological free radical produced by NO synthases [33]. This reaction leads to peroxynitrite anion ($ONOO^-$), a highly oxidizing agent that can further react to produce nitrogen dioxide radical. These molecules can nitrate several biomolecules, among which protein tyrosine-nitration seems to play an important role. Finally, nitrosative stress leads to cell injury through many mechanisms, also playing a relevant role in the pathophysiology of myocardial RI [34]. Overall, ROS and RNS sources include cardiomyocytes, macrophages, neutrophils, endothelial cells, and fibroblasts of the heart [21, 35].

Antioxidant defenses include direct or nonenzymatic antioxidants, such as ascorbic acid (VC), α-tocopherol (vitamin E), and reduced glutathione (GSH) [15]. A study that evaluated oxidative stress and antioxidant defenses in patients with AMI showed that serum VC and vitamin E levels are lower in these patients than in a healthy group [36]. Additionally, decreased levels of GSH have been reported to participate in RI, so its reinforcement could play a fundamental role in its prevention [37], which can be achieved with the administration of NAC [17, 18]. On the other side, counteracting free radicals by up-regulation of antioxidant enzymes through the Nrf2-ARE (nuclear factor erythroid 2-related factor–antioxidant-response element) pathway takes more time than available before PTCA [26, 38]. Thus, administration of direct antioxidants appears to be a reasonable approach.

3 Pharmacokinetic Background

As discussed below, one of the main difficulties when establishing comparisons between clinical trials that have studied the effectiveness of VC, NAC, or DFO in preventing RI is the use of varied administration schemes across different studies. Furthermore, among the studies found in the bibliographic search, only two of them reported plasma drug concentrations [29, 39]. Thus, among the studies reviewed here, there is no available information of the concentrations reached for each drug utilizing the different administration schemes. This prevents establishing a relationship between a drug concentration and the observed outcomes, therefore allowing the design of an optimal concentration-related therapy based on these studies. To overcome the difficulties when designing a new pharmacologic therapy, the use of model-informed drug development has been promoted, with PK being an essential step in the "learning phase" of clinical drug development [40–43].

This way, reviewing the PK properties of each drug could be useful to establish comparisons among trials within a single framework to design an optimized therapeutic strategy based on existing evidence. In this sense, considering the short time window available for drug administration prior to reperfusion and the precise time of the occurrence of ROS and RNS burst during AMI management, visualization of concentration-time curves for each drug could be helpful. Estimated simulations of concentration-time curves can be constructed from compartmental PK (CPK) models. CPK models assume that a drug distributes uniformly within different compartments, and distribution from a central compartment of the body (e.g., the blood in intravenous (IV) administration) to peripheral compartments takes a certain amount of time. It should be noted that different compartments do not necessarily correspond to specific tissues. Peripheral compartments of multiple-compartment models are used essentially to model successive phases of varied exponential decreases or increases in drug concentrations. This can also be seen as more than one half-life in the PK curves of a drug [44].

These CPK models can be constructed from volume of distribution (V_d) and clearance (CL). The V_d for each compartment is a constant of proportionality, which establishes a relationship between the quantity of the drug in a certain compartment and the actual concentration within that compartment [45]. On the other hand, intercompartmental clearance and elimination clearance refer to the volume of blood that is cleared for a drug per unit time from a certain compartment to a peripheral one or outside the body, respectively [42]. For multiple IV infusions, the functions used to model concentration-time curves for the models of one, two, and three compartments are the sum of the increasing phase of the current nth infusion and the decreasing phase of the previous ith infusions (i.e., $i \in [1;n-1]$), as if they were stopped from the initiation of the nth infusion. Functions used in this article to model concentration over time were extracted from Bertrand et al. [46]

4 Antioxidants in Reperfusion Injury Cardioprotection

This section summarizes and compares clinical evidence about the efficacy of VC, NAC, and DFO in reducing RI due to PTCA. PK and safety trials are also reviewed given their importance in designing drug-based therapeutic strategies. Table 1 summarizes the results of the clinical trials that have studied the use of each drug in the setting of AMI. Figure 1 illustrates the approximate mean tendency of achieved concentrations for each study based on PK trials reviewed here.

Table 1 Clinical trials of VC, NAC and DFO administration in myocardial reperfusion injury prevention administration scheme and outstanding outcomes

Articles	Administration scheme	Outstanding outcomes
Vitamin C		
Shafaei-Bajestani et al. 2019	Before reperfusion: IV infusion: 30 g/h VC (151.43 mmol/h) for 6 min IC administration of VC through PCA: 0.10 g (0.50 mmol) Total administration until reperfusion: 3 g IV, 0.1 g IC	6 h after reperfusion, significantly lower CK-MB in the VC supplemented group 12 h after reperfusion, significantly lower troponin T in the VC supplemented group The increase by fivefold and threefold the upper limit of normal of troponin T and CK-MB was less frequent in the VC group than in the control group No significant differences between the groups in contrast induced acute kidney injury or P-selectin levels
Ramos et al. 2017	Before reperfusion: Fast infusion of IV VC: 38.04 g/h (192 mmol/h) during 1 h Slow infusion of IV VC: 11.41 g/h (57.60 mmol/h) during 2 h After reperfusion Oral VC 0.5 g/12h (2.52 mmol/12h) Oral Vitamin E 400 UI/day Total administration until reperfusion: 60.6 g IV	No significant differences in infarct size, LVEF, indexed end-systolic volume and CK-MB. LVEF difference between 1 week and 2–3 months after reperfusion significantly higher in the VC group compared with placebo. Measured VC plasmatic concentration in VC group: 9.63 mM (6.25–11.64) immediately after 0.72 mM (0.23–2.43) at 6–8 h 0. 2 mM (0.01–0.05) at hospital discharge Oxidative stress biomarkers: FRAP significantly higher in VC group immediately and at 6–8 h after reperfusion compared with placebo, Erythrocyte GSH significantly lower in VC group immediately after and 6–8 h after reperfusion
Valls et al. 2016	Before reperfusion: Fast infusion of IV VC: 38.04 g/h (192 mmol/h) during 1 h Slow infusion of IV VC: 11.41 g/h (57.60 mmol/h) during 2 h 800 IU Oral Vitamin E After reperfusion: 0.5 g/12h (2.52 mmol/12h) oral VC 400 UI daily oral Vitamin E Total administration until reperfusion: approximately 60.86 g IV	Significantly lower microvascular dysfunction (measured by TIMI myocardial perfusion grade) in patients with high plasma VC concentration after reperfusion (HA) 84 days after reperfusion, significantly higher LVEF in HA group LVEF difference between days 6 and 84 in HA group Measured VC plasmatic concentration in HA group: 9.79 ± 3.87 immediately after reperfusion 1.79 ± 1.51 at 6 to 8h after reperfusion 0.06 ± 0.06 upon discharge Oxidative stress biomarkers: FRAP significantly higher in HA group immediately after reperfusion, and at 6–8 h Erythrocyte GSH significantly lower in HA group at 6 to 8 h after reperfusion 8-isoprostanes (lipid peroxidation) significantly higher in HA group No significant differences between groups in CK-MB levels
Gasparetto et al. 2005	Before reperfusion: 1 g (5.05 mmol) VC IV bolus After reperfusion [for 1 month]: 1 g (5.05 mmol) daily VC 300 mg/day Vitamin E 50.000 UI/day Vitamin A Total administration until reperfusion: 1 g IV	48 h after reperfusion, significantly higher levels of: sVCAM-1 and ROS in placebo group (P) vs antioxidant therapy group (AT) TAS in AT vs P 1 week and 1 month after reperfusion significantly higher TLVV in P vs AT No significant differences in sVCAM-1, ROS and TAS between the two groups after 1 month
Guan et al. 1999	Before reperfusion: 2 g (10.10 mmol) VC IV bolus 0.02 g/min (0.10 mmol/min) constant VC IV infusion maintained throughout the study period Total administration until reperfusion: 2 g IV	3–4 weeks after reperfusion: no significant differences in LVEF and cardiac index between control and VC groups In control and VC groups in the setting of AMI, 8-epi $PGF_{2\alpha}$ excretion reached a similar peak value at 60–90 min after the onset of reperfusion and then decreased towards baseline levels at 120–150 min. This course in 8-epi $PGF_{2\alpha}$ excretion levels was not seen in elective coronary angioplasty

Table 1 (continued)

Articles	Administration scheme	Outstanding outcomes
N-Acetylcysteine		
Pasupathy et al. 2017	Before reperfusion: 1.2 g/h (7.35 mmol/h) IV infusion during 1 h After reperfusion: 0.01 g/min (0.06 mmol/min) IV infusion during the following 47 h Total administration until reperfusion: 1.2 g	5.5% absolute decrease vs placebo in infarct size, measured through CMR Other outcomes: Lower CK-MB AUC in NAC group vs placebo Quicker relief of angina in NAC group Higher percentage of saved myocardium in NAC group No significant differences in coronary artery permeability before PCA
Thiele et al. 2010	Before reperfusion: 1.2 g (7.35 mmol) IV bolus After reperfusion: 1.2 g (7.35 mmol) two times daily, in the first 2 days Total administration until reperfusion: 1.2 g	No significant differences in saved myocardium index Lower levels of oxidative stress biomarkers: up to 20% of oxidized LDL and activated oxygen protein products
Nozari et al. 2018	Before reperfusion: 0.2 g/kg/h (1.23 mmol/kg/h) IV bolus for 30 min IC administration of NAC through PCA: 0.48 g (2.94 mmol) 12 h after reperfusion: 0.01 g/kg/h (0.06 mmol/kg/h) Total administration until reperfusion: 0.1 g/kg IV, 0.48 g IC	No significant differences in peak levels of: CK-MB, MBG or cTFC Significantly lower levels of hsTnT and higher number of TIMI 3 patients in NAC group
Ozaydin et al. 2008	Before surgical procedure (coronary artery bypass and/or valve surgery): 0.05 g/kg/h (0.306 mmol/kg/h) IV infusion for 1 h After surgical procedure: 0.05 g/kg/day (0.31 mmol/kg/day) IV infusion for 48 h Total administration until reperfusion: 0.05 g/kg (0.306 mmol/kg)	Significant lower postoperative AF incidence in the NAC group compared with placebo No significant differences in duration of hospitalization or postoperative complications
Deferoxamine		
Chan et al. 2012	Before reperfusion: 0.5 g (0.76 mmol) IV bolus After initial bolus: 4.17×10^{-3} g/kg/h (0.0063 mmol/kg/h) IV infusion for 12 h	Significant reduction in oxidative stress (plasma F2-isoprostane levels) immediately after PCA There was no difference in infarct size (CMR), myocardial salvage index at 3 days or at 3 months, or the area-under-the-curve for creatine kinase or troponin I versus placebo

Reviewed trials included participants in the context of AMI with the exception of Ozaydin et al. [99]

8-epi $PGF_{2\alpha}$ 8-epi prostaglandin F2alpha, *AF* atrial fibrillation, *AMI* acute myocardial infarction, *AT* antioxidant therapy group, *AUC* area under the curve, *CK-MB* creatine kinase-MB, *CMR* cardiac magnetic resonance, *cTFC* corrected thrombolysis in myocardial infarction frame count, *DFO* deferoxamine, *FRAP* ferric reducing ability of plasma, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *HA* high ascorbate group, *hsTnT* high-sensitivity troponin T, *IC* intracoronary, *IV* intravenous, *LVEF* left ventricle ejection fraction, *MBG* myocardial blush grade, *NAC* *N*-Acetylcysteine, *P* Placebo group, *PCA* percutaneous coronary angioplasty, *sVCAM-1* soluble vascular adhesion molecules, *TAS* total antioxidant status, *TIMI* thrombolysis in myocardial infarction, *TLVV* telediastolic left ventricular volume, *VC* vitamin C

4.1 Vitamin *C*

4.1.1 Previous Studies

A recent systematic review evaluated eight clinical trials that studied the effectiveness of VC in reducing myocardial reperfusion injury in the context of myocardial infarction [30]. Table 1 incorporates the clinical trials included in this systematic review that have studied the use of VC in the context of AMI [28, 29, 39, 47, 48].

It is difficult to establish comparisons among these studies for various reasons. Firstly, the administration scheme of VC differs from one trial to the next, ranging from a 1 g IV bolus to 60 g IV infusion before reperfusion. Additionally, as previously mentioned, not every study measured the same outcomes [30].

Among these trials, only two of them reported VC concentrations, reaching around 9.63 mM after total administration of 60 g [29, 39]. Figure 1 illustrates an approximate concentration-time curve of VC, based on PK parameters proposed by Nielsen et al. [49] and the reported administration scheme for each trial.

All the trials that measured oxidative stress biomarkers found a decrease in oxidative stress levels and an increase

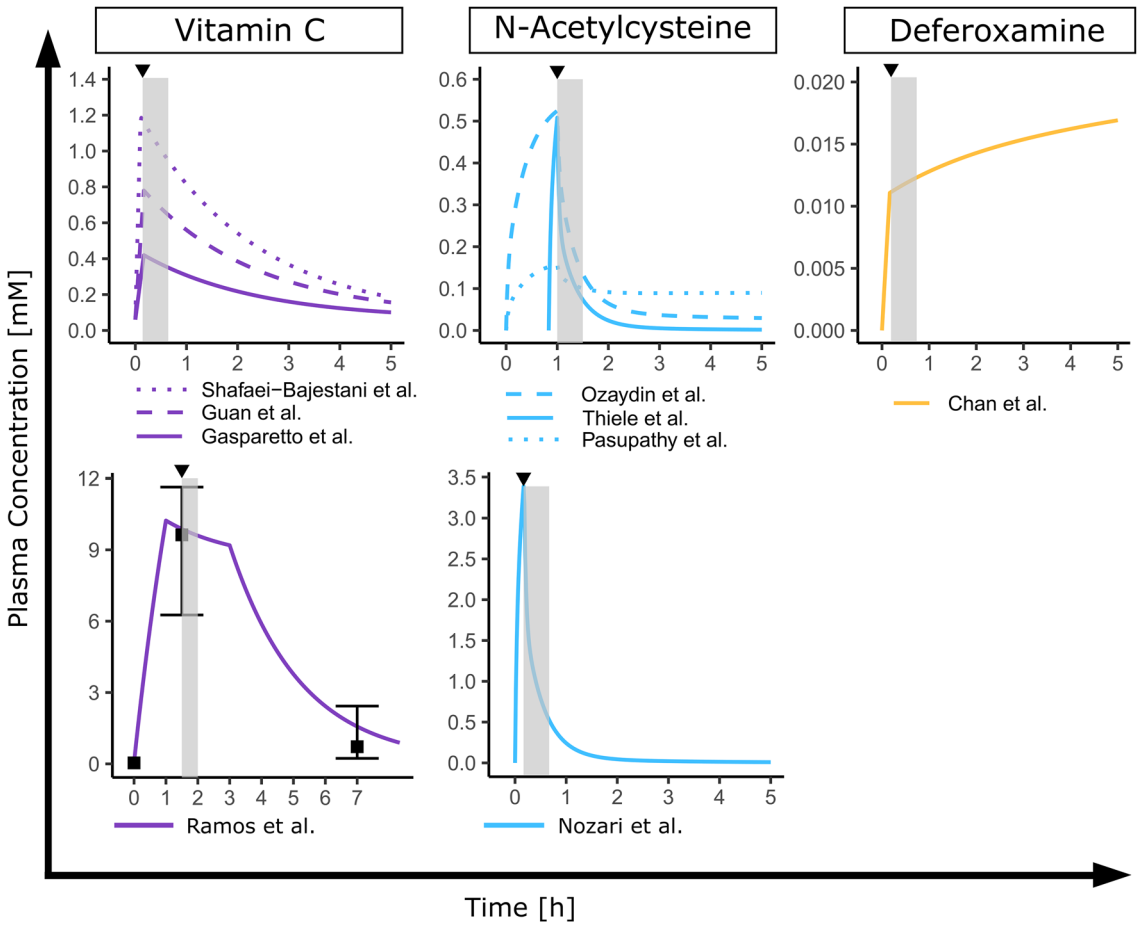

Fig. 1 VC, NAC and DFO plasma concentration-time curves from previous clinical trials addressing myocardial reperfusion injury prevention. Estimated plasma concentrationtime curves comparison among clinical trials reviewed in Table 1. VC (left), NAC (middle) and DFO (right). Each curve represents hypothetical mean concentrations of the drugs, based on pharmacokinetic parameters shown in Table 2. Whenever possible, pharmacokinetic parameters used here were calculated for a 70 kg person. Concentrations were estimated by equations given by Bertrand and Mentré [46] and software R (v. 4.1.1). The black triangles represent the onset of reperfusion for each curve, and the light grey rectangles represents the critical period of ROS burst after the onset of reperfusion (30 minutes). Ramos et al. and Nozari et al. simulations were graphed apart due to their higher estimated concentrations. Details of the administration scheme are shown in Table 1. The black square and error bars of the lower left graph represent the median and interquartile range of VC concentration informed by Ramos et al. [29]. Since pharmacodynamics of a drug is usually a function of that drug's plasma concentration, it should be expected that different administration schemes resulting in different plasma concentration (as shown in the graphs) influence the outcomes obtained in the studies. As it can be seen, in the studies here presented there was a high variability in the concentrations achieved, which could influence the measured outcomes. It is also of interest to notice the concentration of the drugs during the period of ROS burst. As an example, NAC appears to have an initial short half-life that could hinder to maintain the concentration of this drug above the lowest effective concentration during the entire period of ROS burst. Having this visual estimate of the concentration-time curves could help to interpretate the results of these clinical trials and compare them with equivalent pre-clinical trials

in antioxidant status, except for Valls et al. [39] and Ramos et al. [29], who found a decrease in GSH levels between 6 and 8 h after reperfusion and an increase in lipid peroxidation immediately after reperfusion measured by 8-isoprostanes [39]. According to their proposal, this could be due to GSH-dependent VC recycling, in agreement with what Martins et al. suggested [50], and a pro-oxidative effect of high-dose VC, respectively [29, 39]. In terms of myocardial injury biomarkers (CK-MB and troponin T), only Shafaei-Bajestani et al. reported a lower level in the VC group [48], and Valls et al. [39] and Ramos et al. [29] found no difference between groups in CK-MB levels. A better ventricular function in VC groups was reported by Gasparetto et al. [47], Valls et al. [39], and Ramos et al. [29], whereas Guan et al. reported no difference 3–4 weeks after reperfusion [28]. Considering these clinical trials, the systematic review concluded that VC administration should be considered as an intervention to prevent reperfusion injury in the context of AMI treated with PTCA, but more studies are needed to determine the optimal therapeutic dosage [30].

4.1.2 Pharmacokinetics

It has been described that the cellular uptake across cell membranes of VC, due to its water solubility, occurs mainly through sodium vitamin C transporters (SVCT), SVCT1 and SVCT2, SVCT1 being of low affinity and high capacity and SVCT2 of high affinity and low capacity. Additionally, dehydroascorbic acid (DHA), one of its oxidized metabolites that can be recycled to VC, is transported by facilitated diffusion through GLUT1 and GLUT3 transporters [51]. The mechanism of VC efflux from intracellular to extracellular space is still unclear. Differential expression of these transporters in different tissues determines the complex PK of VC [52]. In addition, stress conditions for the organism, such as AMI followed by reperfusion, could increase VC usage [53].

Plasma VC physiological concentrations vary between 50 and 70 µM. Intestinal absorption occurs mainly through a saturable SVCT1 transporter, which makes it impossible to reach supraphysiologic concentrations with oral administration of VC [51, 54]. This is in agreement with a study that showed a major bioavailability of liposomal-encapsulated VC compared with un-encapsulated VC [55]. Daily consumption of five servings of fruits and vegetables should provide around 200 mg of VC, which would translate into the physiological plasma concentration mentioned above [56, 57].

One implication of the tissue differential expression of SVCT1 and SVCT2 is that their distribution is largely compartmentalized and regulated to maintain homeostasis in different clinical contexts according to VC requirements (e.g., under stress conditions) [51, 57]. A clinical trial in critically ill patients (defined by severe sepsis, after major surgery, trauma with SOFA (sequential organ failure assessment) score > 6 and expected ICU stay in > 96 h) found that the plasma dose-concentration relationship could be represented by a two-compartment model (mean V_d for central and peripheral compartment of 31.6 and 39.6 L, respectively); however, this study group was unable to assess a three- or more compartment model [53]. Hudson et al., in a clinical trial in septic shock patients, performed a non-compartmental analysis of the data, obtaining a V_d 23.3 L for a first dose and 39.9 L for consecutive doses, which does not differ broadly from the trial by Grooth et al. [58]. In contrast, clinical trials performed in healthy and cancer patients demonstrated a lower V_d [49, 59] of around 0.18 L/kg [49], which is consistent with the hypothesis of a lower tissue uptake in lower stress conditions. In addition, in line with the hypothesis of red blood cells being an important VC pool, a recent study demonstrated that erythrocyte VC concentration remains high after infusion, possibly affecting the plasma VC status [60].

Finally, VC elimination is mainly renal: ascorbate is freely filtered in the glomerulus, and reabsorbed in the proximal renal tubule by saturable SVCT1 transporters [51]. De Grooth et al. found that ascorbate clearance depends on creatinine clearance [53]. Moreover, concordant with reabsorption saturation [51], given the dose-elimination relationship, studies have described this to be an order 1 clearance [49, 53, 56, 58]. The mean clearance found in these studies was 6.02 L/h [49], 4.2 L/h [53], and 5.2 L/h for the first dose, respectively, and 3.6 L/h for subsequent doses [58]. The half-life described in the Hudson et al. study was 4.3 h for the first dose and 6.9 h for subsequent doses [58].

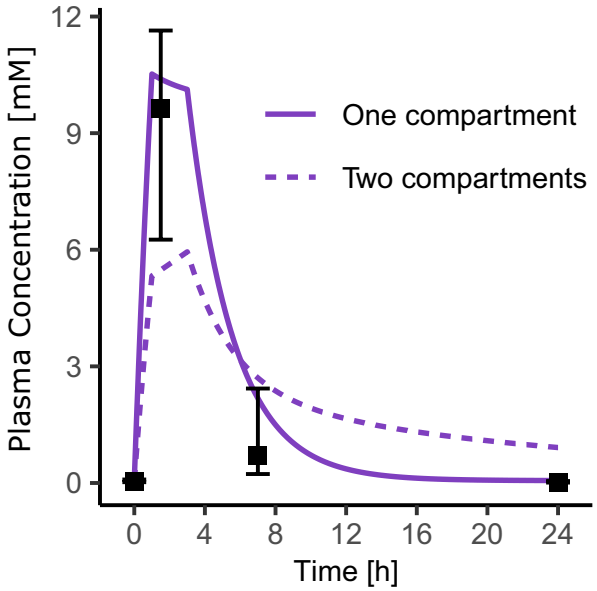

Fig. 2 One-compartment vs two-compartment model comparison for VC in AMI setting. Curves represent simulations of concentration-time curves for reported administration scheme by Ramos et al. [29]. Black boxes and error bars respectively represent median and interquartile range values for VC concentration measured by Ramos et al. 2017 [29]

For the administration scheme given by Valls et al. [39] and Ramos et al. [29], a one-compartment model simulation [49] predicts a similar concentration-time curve with a mean peak concentration around 10 mM and a decay to basal concentrations at almost 12 h after reperfusion. By contrast, a two-compartment model [53] for this scheme predicts a lower mean peak concentration at around 6 mM and a lower rate of decay, returning to basal levels after 12 h (Fig. 2). Therefore, simulations presented in Fig. 1 were constructed based on Nielsen et al.'s PK parameters (summarized in Table 2) [49].

4.1.3 Safety

No significant adverse events associated with VC infusion have been reported, even at high IV infusions given to

Table 2 VC, NAC and DFO pharmacokinetic parameters used for simulation graphs

	Vitamin C (sodium ascorbate)	*N*-Acetylcysteine	Deferoxamine mesylate
Molecular weight [g/mol]	198.11	163.20	656.79
Pharmacokinetic compartment model	One-compartment	Three-compartments	Two-compartments
Distribution volume [L]			
Central compartment (V_1)	15.2	4.48	77.4
Second compartment (V_2)		8.75	238
Third compartment (V_3)		9.8	
Clearance [L/h]			
Of elimination (CL)	6.84	40.6	19.3
From first to second compartment (Q or Q_2)		70.7	17.6
From first to third compartment (Q_3)		4.41	
Plasma physiologic concentration [mM]	0.05–0.07		

Pharmacokinetics data was extracted from Nielsen et al. [49], Brown et al. [73], Bellanti et al. 2016 [86] and normalized to a 70 kg person for VC and NAC. Only mean values are shown

patients in the context of sepsis and cancer [60, 61]. It has been suspected that high VC doses can cause renal stones, given that oxalate is one of its metabolites [62]. Even so, a long-term prospective study showed that there was neither renal stone formation nor renal impairment due solely to high-dose VC administration [63]. This was supported by a recent scoping review, which found no consistent evidence suggesting a major risk of renal stones [64]. A large study by Padayatty et al. [65], who reviewed available literature and collected data from surveys given to practitioners, suggested that high doses of VC are safe in almost every patient. However, due to possible adverse events attributed to VC use, it should not be administered to patients with some specific conditions (pre-existing renal disease, glucose 6-phosphate dehydrogenase deficiency, and paroxysmal nocturnal hemoglobinuria, among others). Finally, it should be mentioned that the articles by Valls et al. and Ramos et al. reported no adverse events attributable to VC supplementation in the setting of PTCA [29, 39]. Overall, VC infusion in the context of interest appears to be safe, given that it would be administered for a relatively short period of time and in lower doses than those given in cancer treatment.

4.2 *N*-Acetylcysteine

4.2.1 Previous *Studies*

Clinical trials suggest improvements in certain outcomes by using NAC as part of the treatment of patients with AMI undergoing PTCA. One of them, the NAC in acute myocardial infarction trial (NACIAM), has shown that a continuous infusion schedule of NAC (20 mg/min during the first hour, followed by 10 mg/min over the next 47 h) leads to a 5.5% decrease in the absolute infarct size compared to the control group, as well as improvements in the percentage of salvaged myocardium, also associated with a decrease in the area under the curve of CK and decrease in the time for resolution of angina [17]. Furthermore, the LIPSIA study, which used a schedule of 6 g of NAC in five doses (detailed in Table 1), showed that, even with no significant differences in the rate of myocardial salvage between the two groups, there was a decrease of up to 20% in markers of oxidative stress [66]. A study by Nozari et al. demonstrated a significant increase in the percentage of patients with TIMI 3 in the NAC-treated group [67].

A meta-analysis involving a total of nine clinical trials that used NAC as part of the treatment of AMI patients undergoing PTCA described the group of patients undergoing NAC therapy as having a decrease in all-cause mortality, a lower frequency of major adverse cardiovascular events, and high-sensitivity troponin T (hsTnT) levels [68].

Moreover, a study performed in an ischemia-reperfusion model in rats has shown that the use of NAC in conjunction with NO increases GSH levels and decreases total infarct size [69].

Figure 1 illustrates the estimated NAC concentration-time curves based on the administration-scheme reported by these studies.

4.2.2 Pharmacokinetics

NAC is an acetylated cysteine amino acid that deters its oxidation in solutions and also allows the molecule to increase its lipid solubility, improving its entry into the cell [70]. Despite this, some studies suggest that the polarity determined by the –COOH and –SH groups would make its passage through biological membranes unlikely [71].

The total concentration of NAC in plasma will be in its reduced or oxidized state. The oxidized state can be presented in different ways: as disulfides (*N*-*N*′ diacetylcysteine)

or bound to other molecules with thiol groups (such as glutathione, cysteine, or plasma proteins). That is why NAC concentrations can be measured by various methodologies to assess the different forms of NAC (total NACT, reduced NAC, non-protein-bound NAC). The methodology used to measure plasma concentrations influences the estimation of PK parameters [72]. Since reduced NAC has antioxidant properties, here we focus on reduced NAC properties rather than NACT properties whenever possible.

It has been determined that NAC PK are linear, meaning that its concentration in plasma would be proportionally dependent on the dose administered [73]. With regard to its transport state through the blood, 50% of NACT is covalently bound to thiol groups of plasma proteins [72].

Clinical trials that aimed to determine the PK parameters of NAC for its subsequent use in future protocols have proposed one-compartment and three-compartment models to predict the plasma concentrations of NAC [73, 74]. It should be noted that as healthy patients were assessed in these studies, PK parameters may be different in pathological situations like patients undergoing AMI.

With respect to V_d, Brown et al. determined a mean value for reduced NAC of 0.064 L/kg for the central compartment, and 0.125 L/kg and 0.14 L/kg for two peripheral compartments [73]. A study by Olsson et al. used a non-compartmental model analysis to evaluate reduced NAC, NAC as disulfides, and NACT. This group determined a mean distribution volume for reduced NAC of 0.59 L/kg [72]. Borgström et al. [75] evaluated non-compartmental PK of non-protein-bound NAC, obtaining a mean total distribution volume of 0.327 L/kg. Prescott et al. [76] assessed NACT PK in patients undergoing overdosage of paracetamol, calculating a V_d of 0.536 L/kg. Even if these results are slightly different, Hong et al. reported a distribution volume of 3.07 L/kg [74], which is much higher than the previously mentioned value [72, 73, 75, 76], attributed by the authors to the different doses administered [74]. Among all the studies that measured reduced NAC and NACT, it seems to be that reduced NAC has a higher V_d [73, 74].

The drug elimination is determined in 30% by renal clearance [75], while the total body clearance could be defined by a three-compartment model with three different half-lives and clearances [72, 73]. Brown et al. [73] reported mean half-lives for reduced NAC of 1.3 min, 15.9 min, and 101.6 min. Additionally, the clearances for reduced NAC calculated in this study were 0.58 L/kg/h, 1.01 L/kg/h, and 0.063 L/kg/h. However, they emphasized that they only measured the drug concentration over a short period of time after infusion (2 h in the exercise study group), which could imply they were not able to find a longer terminal half-life [73]. The study by Olsson et al. [72] reported a mean a biphasic decline in reduced NAC concentration, with half-lives of 8.74 min and 1.95 h ; the estimated total body clearance was 0.84 L/kg/h. Borgström et al. [75] defined a value of 2.27 h for the overall half-life of non-protein-bound NAC, as well as a total body clearance of 0.207 L/kg/h [75]. Finally, Prescott et al. [76] determined a half-life of 5.7 h and a clearance of 66.6 mL/kg/h for NACT. In agreement with this study, Coles et al. [77] measured the half-life of NACT as 5.9 h and clearance of 66.6 L/h. Therefore, the global half-life appears to be between 2 and 6 h [72, 75, 76]. A relevant fact to consider is that the studies in which parameters of reduced NAC were measured separately reported a higher total body clearance of reduced NAC in comparison to NACT [72, 73]. Overall, the differences in methodologies among studies to measure NAC concentration makes it difficult to make accurate and precise conclusions pooling all the studies.

An important aspect to consider is the intervals at which blood samples were drawn in the different studies. The studies that reported a lower initial half-life were those that obtained blood samples at shorter intervals of time, making it possible to observe an initial faster decrease in plasma concentration [72, 73]. The rest of the studies obtained blood samples at higher intervals (10–30 min as a minimum interval) [74–77]. Since this article focuses on the acute phase of reperfusion (15 min), PK parameters of reduced NAC reported by Brown et al. were used to construct the concentration-time curves from Fig. 1. However, it should be noted that this trial reported a lower V_d compared to the other studies, which means that Fig. 1 could be overestimating NAC concentrations.

4.2.3 Safety

In regard to the occurrence of adverse effects during NAC administration, a meta-analysis by Jiang et al. showed no significant differences between the standard of care and NAC study group [68]. Data from published literature indicate that the frequency of adverse reactions with the use of different schedules of IV NAC varies from 0.2 to 20.8%. The clinical trial conducted by Sanaei-Zadeh used a three-dose schedule of NAC, the first of which was 150 mg/kg, the second 50 mg/kg, and the last 100 mg/kg, all over a period of 20–21 h. From this, the most common adverse reactions (facial flushing, pruritus, dyspnea, rash, cough, and tachycardia) were determined, and no fatal or severe reactions were reported [78]. Another study in patients with underlying systemic inflammatory response syndrome, which evaluated safety of 100 mg/kg IV bolus followed by 50 mg/kg/day maintenance infusion (similar to Nozari et al. [67]), concluded that this administration scheme was safe in an acute phase [79]. NACIAM study found no differences in adverse events between the two study groups [17].

Severe adverse reactions have only been described in cases of NAC overdose. In addition, both severe and mild

reactions are related to the initiation of treatment with NAC, which suggests that the adverse effects are proportional to the initial dose of the drug, constituting a direct pharmacological consequence [76]. Accordingly, the frequency of severe adverse reactions should be very low with appropriate use of IV NAC dosage.

4.3 Deferoxamine

4.3.1 Previous Studies

Several animal models studies have demonstrated the role of DFO in the prevention of RI, thus resulting in less morphological alterations, smaller infarct size, and less morphological repercussion when DFO is administered in murine, rabbit, and canine hearts [80–82]. Magni et al. [83] demonstrated in 1993 in an animal model that DFO can prevent a burst of lipid peroxidation seen in myocardial tissue undergoing redox imbalance due to rewarming after hypothermia, a model that has some similarities to the mechanisms of ischemia reperfusion [83]. However, these outcomes have not been observed in clinical studies in the setting of patients with AMI. To our knowledge, there is only one clinical trial that evaluated the safety and effectiveness of DFO therapy in these patients. Chan et al. showed that treatment with DFO (administration scheme in Table 1) as monotherapy in patients with AMI reduces oxidative stress biomarkers but does not lead to a reduction of either infarct size or biomarkers of myocardial damage in patients with STEMI undergoing PTCA [84]. This study did not report serum concentrations of the drug, but Fig. 1 illustrates the estimated concentration-time curve based on its administration-scheme.

4.3.2 Pharmacokinetics

The absorption of DFO in the digestive tract is minimal, so it must be administered through intramuscular, subcutaneous, or IV route [85]. Studies have concluded that it follows a zero-order absorption model [86], reaching a steady plasma concentration 12 h after a 50 mg/kg/24 h in 48 h infusion [87] and 100 mg/kg of body weight administered in 24 mL, given at 1 mL/h [88].

Due to its low lipid solubility and high molecular weight [89], DFO is not able to cross the cell membrane, but enters the intracellular compartment slowly through endocytosis, and stimulates ferritin degradation through lysosomal enzymes to bind to the released iron [86]. DFO distribution follows a bicompartmental model [86], and its metabolism is mainly due to plasma enzymes [90]. With regard to its V_d, results of the studies differ. Bellanti et al. [86] reported an apparent V_d of 77.4 L for the central compartment and of 238 L for the second compartment after a 40 mg/kg of DFO as an 8 h subcutaneous infusion, based on the model of Porter et al. [89]. Lee et al. reported a V_d of 1.88 L/kg (131.6 L for a 70 kg person) after an IV infusion at a dose 50 mg/kg/24 h for 48 h in 11 iron-overloaded thalassemic patients [87].

In terms of its elimination, ferrioxamine, which is the complex formed once DFO binds to iron, is excreted mainly through glomerular filtration [88]. Pharmacokinetic studies suggest that it follows a first-order elimination model following a biphasic elimination [86]. The half-life has been described to be 5–10 min after an IV bolus [88, 91]. After an IV infusion (50 mg/kg/day for 48 h), a biphasic elimination is described: after 48 h of infusion, the initial half-life was 0.28 h and the terminal half-life was 3.05 h [87]. Since the article by Bellanti et al. was the most recent one, these PK parameters were used to construct the simulation of Fig. 1. These PK parameters are shown in Table 2.

It is relevant to consider that the existing PK studies are based on protocols in other clinical contexts (e.g., chronic iron overload), and with some of them using a route of administration that is different from the IV route. For these reasons, the parameters presented may not be entirely reliable for use in our setting of interest.

4.3.3 Safety

It has been reported that rapid administration of DFO can cause hypotension and tachycardia within 15 min in patients with chronic iron overload [90]; nevertheless, this effect is not expected to occur when the infusion rate is less than 15 mg/kg/h [92].

4.4 Combined Antioxidant Therapy

To understand the rationale of effectiveness demonstrated by the clinical trials cited above, a summary of the underlying mechanisms is presented here. Studies suggest that VC has a role in: direct ROS scavenging; recycling the reduced form of some antioxidants, such as GSH, vitamin E, among others; modulating enzyme reactions involved in maintaining redox balance; and other long-term functions that help reduce oxidative stress [16], as shown in Fig. 2. However, given its reduction potential, VC also reduces Fe^{3+} to Fe^{2+}, which can further contribute to Fe^{2+} overload by an ascorbate-driven cyclic Fenton reaction with a subsequent ROS generation [93]. Furthermore, as previously mentioned, VC recycling is dependent on GSH oxidation, so a decrease in GSH levels when VC is being oxidized should be expected [29, 50, 94].

The mechanism of antioxidant action of NAC is neither unique nor entirely elucidated [21, 70]. On the one hand, there is an important role of NAC in the filling of intracellular levels of GSH [70]; on the other hand, it has a direct antioxidant effect, provided by the sulfur atom, which allows

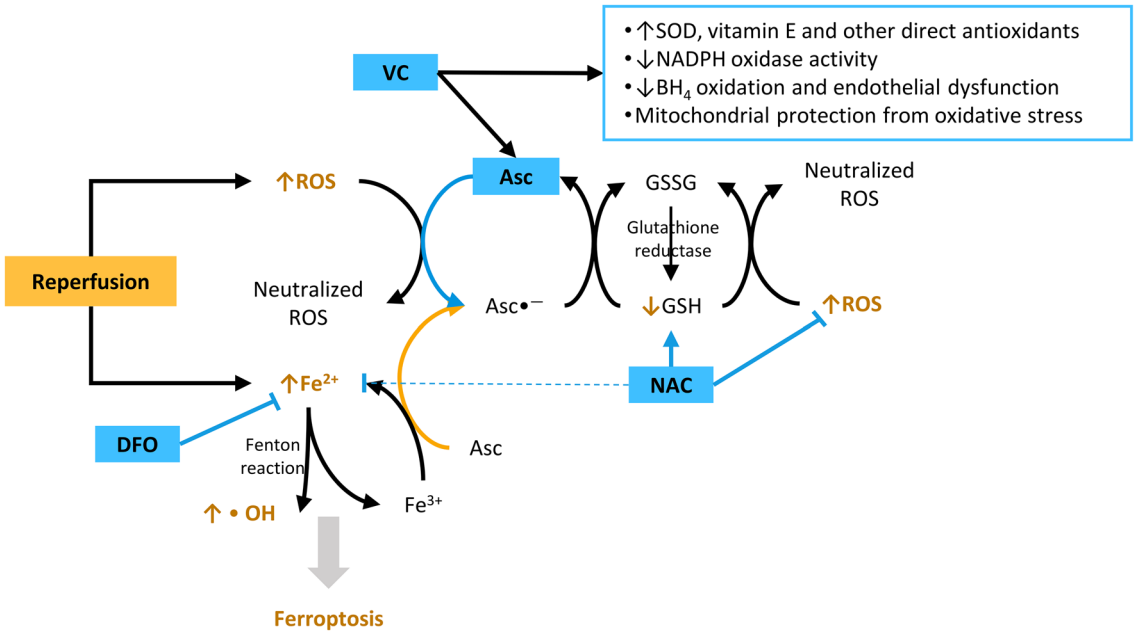

Fig. 3 VC, NAC and DFO mechanism of action in myocardial RI. Orange-colored processes represent the harmful processes induced by reperfusion injury. Blue-colored processes represent the mechanisms of action for each drug reviewed in this article. After reperfusion, ROS (and RNS, not illustrated here) and iron levels increase. ROS and RNS should be neutralized by antioxidants VC, NAC, and GSH. Since GSH is one of the main physiological antioxidants being also responsible for recycling VC, its decrease could be restored by NAC. However, this reaction takes time. Iron overload could be further increased by VC oxidation. This iron overload can be neutralized by DFO. *Asc* ascorbate, $Asc^{\cdot-}$ reduced ascorbate, *BH4* tetrahydrobiopterin, *DFO* deferoxamine, Fe^{2+} ferrous iron, Fe^{3+} ferric iron, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *NAC* *N*-acetylcysteine, *·OH* hydroxyl radical, *ROS* reactive oxygen species, *SOD* superoxide dismutase; *VC* vitamin C

the oxidized form to remain stable, and the ability to quickly form disulfide bridges, which also contributes to this direct effect [18].

DFO is an iron chelator that binds to free plasma iron [85], thus decreasing the bioavailability of the metal to generate the hydroxyl radical through the Fenton reaction [14, 21]. In addition, it has been seen that it can act as a scavenger of oxygen free radicals; however, these actions have been seen at different concentrations of the drug: small concentrations of DFO (< 10 µM) are able to act as an iron chelator and inhibit the formation of iron-dependent oxygen free radicals, but higher concentrations are required to act as a scavenger [95]. The achieved concentrations of the drug, approximately 20 µM [95, 96] in in vivo studies, are not sufficient for the latter [95]. However, in a multitarget protocol, this action could be carried out by other pharmacological agents [13].

Since the proposed mechanism of action of the three drugs is different, it might be expected that the coadministration of DFO, VC, and NAC could result in a different degree of effectiveness compared to using them individually. In agreement with this, Gao et al. found in a rat heart ischemia-reperfusion model a possible synergistic effect following the coadministration of GSH and VC [97]. Another preclinical study performed in sheep showed that combination of DFO and VC prevent the onset of arrhythmias following an ischemia-reperfusion episode [98].

Recently, another preclinical study by Parra-Flores et al. [14] described the efficacy and synergy of coadministration of VC, NAC, and DFO in rat heart tissue submitted to ischemia and reperfusion. This study group compared the impact on viability of cardiac fibroblasts that different concentrations of the three drugs had by themselves and together. They managed to demonstrate that each drug had a beneficial effect at high concentrations (100 µM or more), except for VC, which was deleterious at a concentration of 10 mM. Despite this, when exposed to the three drugs together at low concentrations (10 µM each), at which each drug alone was shown to be ineffective, a series of beneficial effects were seen [14]. Therefore, it should be expected that lower concentrations than the ones achieved on the clinical trials that showed beneficial effects with monotherapy (estimated and illustrated graphically in Fig. 1) could still have a role in cardioprotection when these drugs are administered together. According to the mechanisms mentioned before, Fig. 3 illustrates a possible cooperative mechanism of the three drugs in myocardial reperfusion injury.

As previously mentioned, one complicating factor of developing a pharmacologic strategy for the setting of AMI is due to the short period of time before reperfusion, given that any intervention should not delay the reperfusion onset. Accordingly, it is reasonable to consider an intervention time window of 20–30 min. The emergency setting also implies that it is difficult to implement an administration scheme with different infusion rates for each of the three drugs in a clinical setting. As a result, to implement this strategy in a simpler way, an infusion of a single solution containing the three drugs could be used to reach approximate target concentrations.

To plan this strategy, a strong knowledge of PK properties for each drug could be useful, with CPK models being more helpful to predict plasma drug concentrations over time. This plasma concentration would reflect myocardial concentrations of the drugs as it is highly perfused. As has been mentioned, results from PK studies for each drug are different, making it hard to establish accurate conclusions. Furthermore, none of the studies have analyzed the behavior of these properties when the drugs are administered together in the setting of AMI. Nevertheless, these data could be useful to provide a general idea about how drugs plasma concentrations would behave. In this sense, it should be noted that VC seems to follow a one-compartment model. DFO, due to its high peripheric V_d, to simplify calculations could also be modeled as a one-compartment model in an acute phase with a V_d equal to the sum of peripheric and central V_d. Finally, NAC is the drug that would decrease faster, considering it has an initial half-life of about 1.5 min, but this faster drop would only last for about 7.5 min (five times the initial half-life). This is because, theoretically, NAC appears to follow a three-compartment model, with a high CL from the first to the second compartment. Thus, the second compartment should reach an equilibrium concentration with the central compartment within the period before the onset of reperfusion to have stable concentrations at the onset of reperfusion, which would help obtain more accurate conclusions with respect to the effective doses of the drugs. Figure 4 illustrates these fundamental CPK models aspects. Finally, all the three drugs have a good safety profile in humans

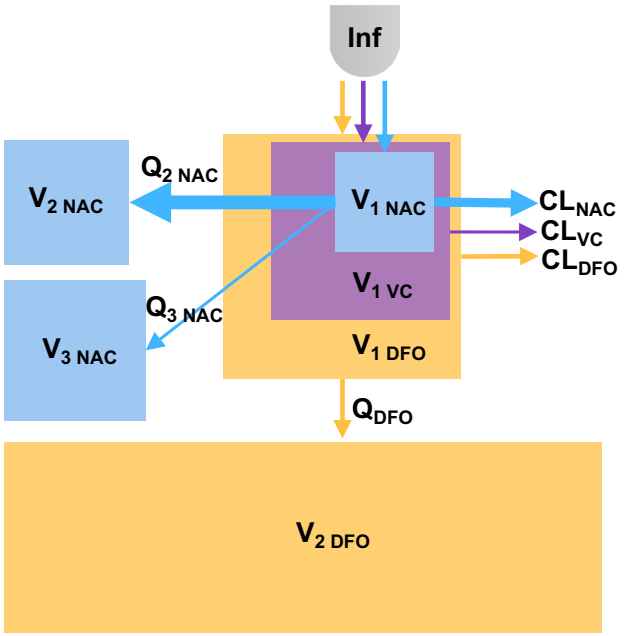

Fig. 4 Pharmacokinetic compartment model for coadministration of the three drugs. Color code alludes to each drug: purple, VC; light blue, NAC; yellow, DFO. Boxes represent V_d and arrows represent the clearance for each compartment. Overlapped center boxes illustrate the central compartment and the rest illustrate peripheral compartments (the two smaller for NAC and the larger one for DFO). Larger size of the boxes represents higher V_d and smaller size represents lower volumes; the thicker arrow for Q_{2NAC} and CL_{NAC} represents the higher clearance from central compartment to third compartment and outside of the body. *Inf* infusion solution, *V* volume of distribution, *Q* intercompartimental clearance, *CL* elimination clearance

5 Conclusion

Preclinical models of reperfusion injury intervention with VC, NAC, and DFO have shown beneficial effects and have contributed to a better understanding of their mechanisms of action. However, this benefit has not been translated into the clinical setting. Also, due to the different administration schemes and outcomes measured in clinical trials that assessed monotherapy for each drug, it is difficult to compare their results and establish a consensus for an optimal therapeutic strategy.

CPK models presented here to estimate the mean tendency of drug concentrations allowed us to better visualize the magnitude of differences in plasma concentrations during the period of ROS burst. This, together with data reviewed in this paper, can help interpret the findings of the studies in terms of the concentrations achieved by each administration scheme. As Parra et al. suggest, the simultaneous administration of the three drugs could have major efficacy at low concentrations that may not be beneficial in monotherapy. Therefore, data gathered here about the efficacy of different administration schemes and drug PK parameters support the hypothesis that combined therapy is a feasible, safe, and effective strategy in reducing RI due to PTCA. Moreover, this view should help in designing a cardioprotective pharmacologic strategy.

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Declarations

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Conflict of interest Daniel San-Martín-Martínez, Dayanara Serrano-Lemus, Vicente Cornejo, Abraham IJ. Gajardo, and Ramón Rodrigo declare that they have no potential conflicts of interest that might be relevant to the contents of this article.

Ethics approval Not applicable

Consent to participate Not applicable

Consent for publication Not applicable

Availability of data and material Datasets and explanation of code generated to establish comparisons between trials and to design Figs. 1 and 2 are available in the FIGSHARE repository, https://doi.org/10.6084/m9.figshare.18551417.v3.

Code availability Code generated to establish comparisons between trials and to design Figs. 1 and 2 are available in the FIGSHARE repository, https://doi.org/10.6084/m9.figshare.18551417.v3.

Authors contributions R.R. and A.I.J.G contributed to conceptualization, supervision, validation and writing review and editing; D.S.-M.-M., D.S.-L., and V.C. contributed to investigation collecting evidence, data curation, writing the original draft, and visualization; D.S.-M.-M. contributed to software code development.

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