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Nitrite elicits divergent NO-dependent signaling that associates with outcome in out of hospital cardiac arrest

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A B S T R A C T

Brain and heart injury cause most out-of-hospital cardiac arrest deaths but limited pharmacotherapy exists to protect these tissues. Nitrite is a nitric oxide precursor that is protective in pre-clinical models of ischemic injury and safe in Phase I testing. Protection may occur by cGMP generation via the sGC pathway or through S-nitrosothiol and nitrated conjugated linoleic acid (NO_2-CLA) formation. We hypothesized that nitrite provided during CPR signals through multiple pathways and that activation of signals is associated with OHCA outcome. To this end, we performed a secondary analysis of a phase 1 study of intravenous nitrite administration during resuscitation in adult out-of-hospital cardiac arrest. Associations between whole blood nitrite and derived plasma signals (cGMP and NO_2-CLA) with patient characteristics and outcomes were defined using Chi-square or t-tests and multiple logistic regression. Whole blood nitrite levels correlated inversely with plasma NO_2-CLA ($p = 0.039$) but not with cGMP. Patients with shockable rhythms had higher cGMP ($p = 0.027$), NO_2-CLA ($p < 0.0001$) and trended towards lower nitrite ($p = 0.077$). Importantly, plasma cGMP and NO_2-CLA levels were higher in survivors ($p = 0.033$ and 0.019) and in those with good neurological outcome ($p = 0.046$ and 0.021). Nitrite was lower in patients with good neurologic outcome ($p = 0.029$). cGMP (OR 4.02; 95% CI 1.04–15.54; $p = 0.044$) and NO_2-CLA (OR 3.74; 95% CI 1.11–12.65; $p = 0.034$) were associated with survival. Nitrite (OR 0.20; 95% CI 0.05–0.08; $p = 0.026$) and NO_2-CLA (OR 3.96; 95% CI 1.01–15.60; $p = 0.049$) were associated with favorable neurologic outcome. In summary, nitrite administration was associated with increased plasma cGMP and NO_2-CLA formation in selected OHCA patients. Furthermore, patients with the highest levels of cGMP and NO_2-CLA were more likely to survive and experience better neurological outcomes.

1. Introduction

In 2018, out-of-hospital cardiac arrest (OHCA) resulted in 356,000 deaths in the U.S.A. mostly due to brain or heart injury [1]. Improvements in OHCA outcomes appear to be the result of better resuscitation (e.g. cardiopulmonary resuscitation [CPR] quality) rather than post-resuscitation care [2] with limited pharmacotherapy available to protect the heart and the brain [3]. In the past two decades, a novel role has emerged for nitrite as a vascular endocrine reservoir for nitric oxide (NO) and as source of bioactive nitrated and nitrosated derivatives [4,5]. Numerous studies have documented the role of nitrite in mediating protection after ischemia-reperfusion [6–10]. Nitrite has been demonstrated to be a promising therapy in experimental models of heart [7,10] and brain [8,11] ischemia-reperfusion (IR) as well as cardiac arrest, where it prevented delayed neuronal death and improved neurological function [12–14]. Having demonstrated safety in phase 1 testing [13,15,16], NHLBI has funded an ongoing phase 2 randomized clinical trial (RCT; NCT03452917) investigating the role of intravenous (IV) nitrite provided during CPR after OHCA.

Nitrite administration offers potential advantages over forms of NO therapy such as inhaled NO (iNO) in terms of drug delivery and signaling. Upon inhalation, iNO diffuses into the bloodstream and instantly reacts with oxyhemoglobin to generate nitrate and methemoglobin while smaller fractions of inhaled NO are either oxidized to nitrite or react with deoxyhemoglobin to generate nitrosylhemoglobin [17,18]. As a result, while the acute effects of iNO are restricted to the pulmonary vasculature, peripheral effects are ascribed to the actions of more stable intravascular species such as S-nitrosothiols or nitrite [19]. In contrast to iNO, nitrite is only reduced to NO under the conditions of hypoxia and acidosis specifically found within ischemic tissue beds, thus providing site-specific NO delivery [4,5]. These considerations, together with its stability, low-cost and safety profile [13,15,16] make

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nitrite an ideal candidate for therapeutic use in out-of-hospital emergency settings. Notably, *in vitro* data suggest that nitrite-mediated neuroprotection is in part soluble guanylate cyclase (sGC) independent [14]. In addition to engaging the canonical sGC-cGMP pathway [20,21], nitrite can also participate in S-nitrosothiol formation [14] and mediate the generation of nitrated fatty acids [22]. Nitrated fatty acids are formed endogenously under normal gastric conditions; are increased during inflammation and have been shown to be generated in a reperfusion-dependent manner in preclinical models of focal cardiac infarction [23–25]. Nitrated conjugated linoleic acid (NO_2-CLA) is the most abundant nitrated fatty acid formed in humans [23,26]. Upon formation, NO_2-CLA is gradually metabolized by β-oxidation [27] and prostaglandin reductase-1 catalyzed nitroalkene saturation to dihydro derivatives [28] among other pathways [29]. NO_2-CLA activates Nrf2-dependent antioxidant gene expression and inhibits NF-κB regulated pro-inflammatory signaling [25]. As a result, nitrated fatty acids are protective in a wide range of animal models of cardiovascular disease including *ex vivo* and *in vivo* models of cardiac ischemia-reperfusion injury [24,30–32].

Taken together, nitrite is a pluripotent signaling molecule potentially capable of providing acute IR protection through hypoxia-activated NO-sGC signaling plus S-nitrosothiol generation, and sustained anti-inflammatory protection via the generation of longer-lived nitrated fatty acids such as NO_2-CLA [4,22]. We tested the hypothesis that nitrite provided IV during CPR could signal through multiple pathways and that activation of these forms of signaling would be associated with outcome in a secondary analysis of a previously reported phase 1 randomized trial [16]. We measured whole blood nitrite levels following acute dosing during CPR after OHCA, and assessed their relationship with simultaneous plasma cGMP and NO_2-CLA concentrations. Since NO_2-CLA metabolism has only recently been reported in humans [26,33], we measured its upstream and downstream metabolites. We examined associations between these levels and important clinical characteristics and outcomes after OHCA.

2. Methods

2.1. Reagents

(9Z,11E)-octadecadienoic acid (CLA, 90%) was from Nu-Check Prep, Inc. All other reagents were from Sigma-Aldrich. The term NO_2-CLA refers to a mixture of the 9- and 12-nitro-octadeca-9,11-dienoic acid positional isomers. Dinor- and tetranor-NO_2-CLA are the products of one and two rounds of β-oxidation. DH-NO_2-CLA is a reduced NO_2-CLA derivative bearing a single unsaturation at the gamma position with respect to the nitro group. Chemical structures are provided in Supplemental Fig. 1.

2.2. Clinical trial description

Sodium nitrite was administered as part of a published phase 1 study (NCT02987088) under Exception from Informed Consent [16]. Nitrite (25 or 60 mg) was given as an IV push in 2.5 mL during CPR in all adult patients undergoing emergency medical services treatment for OHCA who did not have a pre-existing do not resuscitate order. Nitrite was provided under an investigational new drug exception.

2.3. Blood sampling

Blood was obtained at the time of hospital arrival or immediately upon ceasing resuscitation efforts. 4 mL of blood were injected into a specimen tube containing 1 mL nitrite preservation solution [12]. An additional 5 mL blood were collected in heparin. Tubes were stored at 4 °C for up to 6 h followed by centrifugation (2000 G for 10 min) to isolate plasma. All samples were maintained in preservation solutions at −80 °C.

2.4. Blood and plasma measurements

A subset of 82 subjects from whom blood and plasma samples were available were used out of a total of 120 participants [16]. Whole blood nitrite was measured using reductive chemiluminescence [12]. Plasma cGMP was measured using a kit according to manufacturer's instructions (Cayman, Ann Arbor, MI). Plasma nitrated and non-nitrated fatty acids were measured by LC-MS/MS as described [22,28]. Complete methodological details are provided in Supplemental Materials.

2.5. Clinical variables and outcomes

Data were collected on patient age, sex, race, weight, whether the OHCA was witnessed and whether bystander CPR was provided, rhythm presentation, duration of no flow and low flow (CPR). We recorded the time from nitrite dosing until blood sampling. Post-resuscitation outcomes are survival to hospital discharge and favorable neurological outcome based on a Cerebral Performance Category of 1 or 2 at the time of discharge. A complete report of phase 1 study variables has been published [16].

2.6. Statistics

We report continuous variables as mean ± SD or median and interquartile range (IQR) as appropriate. Categorical variables are reported as number and proportion. Correlations between continuous variables were evaluated with the nonparametric Spearman rank correlation statistic. We used univariable logistic regression to assess the association between biochemical measurements (nitrite, cGMP, NO_2-CLA after log transformation) and two outcomes at hospital discharge: survival and good neurologic function. We report odds ratios (OR) and 95% confidence intervals (CI). Independent associations between biochemical measurements and outcome were examined by fitting a model using each of the biochemical measurements and clinical variables associated with outcome in multivariable logistic regression ($p < 0.05$). Prism 8.0 and SPSS Version 19.0 were used for analysis.

2.7. Study approval

The study was approved by the University of Washington Institutional Review Board and was performed under Exception from Informed Consent (EFIC).

3. Results

We previously reported the results of our phase 1 study [16]. Rates of return of spontaneous circulation (ROSC) and hemodynamics were similar to historical controls [16]. In our prior report (n = 120), whole blood nitrite levels were higher in patients receiving the higher nitrite dose (60 vs. 25 mg) and decreased with time from dose to sampling, which roughly followed the pharmacokinetics predicted from lung transplant patients. The time of blood sampling relative to nitrite dosing was shorter in patients without ROSC than in those who attained ROSC [16]. For the present secondary analysis, plasma was available from 82/120 (69%) patients from the phase 1 study but due to limited quantities only 74/82 (90%) had cGMP levels measured along with nitrite and NO_2-CLA (n = 82). These plasma samples were obtained at a mean 29.3 ± 15.8 min after nitrite dosing, with 39 out of the total 82 patients (48%) receiving the lower nitrite dose (25 mg). Demographics and OHCA characteristics for these patients are provided in Table 1.

In the present sample subset, whole blood nitrite levels were not associated with dose (Suppl Fig 2), sampling time, low or no flow ischemic time (Suppl Table 1). Likewise, cGMP, NO_2-CLA and NO_2-CLA metabolites levels in plasma were unaffected by nitrite dose (Suppl Fig 2) or sampling/ischemic time (Suppl Table 1). Interestingly, whole blood nitrite levels were inversely correlated to plasma NO_2-CLA

Table 1
Characteristics for patients (n = 82).

Characteristic	Mean ± SD/Median (IQR) or N (%)
Age (years)	63 ± 16
Women	28 (34%)
Weight (kg)	81 ± 21
VF/pVT	22 (27%)
Time from call to arrival (minutes)	4.8 ± 2.4
Time from dose until blood collection (minutes)	29 ± 16
Witnessed arrest	32 (39%)
Bystander CPR	47 (57%)
No flow time (min)	8.6 (8.5)
Low flow time (min)	23.8 ± 11.3
Pressors in first 24 h	36 (44%)
Positive troponin	45 (55%)
Any ROSC	44 (54%)
Rearrest	9 (11%)
Survival to hospital discharge	18 (22%)
CPC 1, 2 at hospital discharge	14 (17%)
Whole blood nitrite (μM)	2.60 (5.45)
Plasma cGMP (nM)	16.3 (23.1)
Plasma 9,11 CLA (nM)	317 (296)
Plasma NO_2-CLA (nM)	1.66 (2.95)
Plasma DH-NO_2-CLA (nM)	0.000 (0.674)

($\rho = -0.229$; $p = 0.039$) and to the one-round NO_2-CLA β-oxidation metabolite Dinor-NO_2-CLA ($\rho = -0.283$; $p = 0.010$) but not to plasma cGMP ($p = 0.945$). In turn, NO_2-CLA strongly correlated with its plasma metabolites (Dihydroxy [DH]-NO_2-CLA, Dinor-NO_2-CLA, Tetranor-NO_2-CLA) and with its precursor 9, 11-CLA (Table 2; all $p < 0.001$) but was not correlated with cGMP ($p = 0.99$). Patients presenting with shockable rhythms (ventricular fibrillation or pulseless ventricular tachycardia [VF/pVT]) trended towards lower levels of whole blood nitrite ($p = 0.077$) and had higher levels of both cGMP ($p = 0.027$) and NO_2-CLA ($p < 0.0001$) (Fig. 1A–C). Age was positively correlated with nitrite and inversely correlated with NO_2-CLA but not with cGMP (Fig. 2A–C). Weight was correlated with NO_2-CLA ($\rho = -0.224$; $p = 0.043$) but not with nitrite or cGMP.

Regarding clinical outcomes, nitrite levels did not differ between patients who survived versus those who died in-hospital (Fig. 3A) but plasma levels of cGMP and NO_2-CLA were significantly higher in patients who survived to discharge (Fig. 3B–C). Notably, whole blood nitrite levels were significantly lower in patients that were discharged with good neurologic function (Fig. 4A), while both cGMP and NO_2-CLA levels in plasma were elevated in patients who had favorable neurological outcome (Fig. 4B and C). In univariable logistic regression, cGMP (OR 4.02; 95% CI 1.04–15.54; $p = 0.044$) and NO_2-CLA (OR 3.74; 95% CI 1.11–12.65; $p = 0.034$) were associated with survival. Nitrite (OR 0.20; 95% CI 0.05–0.08; $p = 0.026$) and NO_2-CLA (OR 3.96; 95% CI 1.01–15.60; $p = 0.049$) were associated with favorable neurologic outcome whereas cGMP only showed a trend ($p = 0.055$). These results remained essentially unchanged when the analysis was restricted to patients that achieved ROSC for both whole blood nitrite and plasma NO_2-CLA levels (Suppl Fig. 3). None of the demographic variables were associated with outcome whereas low flow time, bystander CPR and shockable rhythm were associated with both survival and neurological outcomes. In multivariable regressions, only low flow time and the presence of a shockable rhythm were independently associated with outcome (Suppl Table 2).

4. Discussion

This study demonstrates significant biochemical heterogeneity in a cohort of patients with OHCA who were treated with IV nitrite as part of a completed phase 1 study [16]. Whole blood nitrite, cGMP and NO_2-CLA levels varied substantially within patients receiving the same nitrite dose and these differences could not be explained by sampling time, patient weight or ischemic time. NO_2-CLA levels increased concomitantly with nitrite consumption as indicated by the negative correlation observed between whole blood nitrite and plasma NO_2-CLA. In addition, plasma NO_2-CLA levels were strongly correlated with downstream metabolites (DH-, Dinor- and Tetranor-NO_2-CLA) and were also directly proportional to the concentration of precursor 9,11-CLA. Interestingly, neither whole blood nitrite levels nor plasma NO_2-CLA concentrations correlated with cGMP, but both NO_2-CLA and cGMP were associated with improved discharge outcomes. Elevated levels of plasma cGMP and NO_2-CLA were observed in patients presenting with a shockable rhythm (VF/pVT) and correlated with outcomes, whereas age and weight were correlated with NO_2-CLA. Consistent with many prior reports we found low flow time, shockable rhythm and presence of bystander CPR to be associated with outcomes. In multivariable regression, only these cardiac arrest factors but not the plasma levels of cGMP and NO_2-CLA were independently associated with outcome.

These results must be interpreted within the context of nitrite reactivity and biological signaling. The one-electron reduction of nitrite by metalloproteins allows NO to be generated under hypoxic conditions in which NO synthases are inhibited [4,5]. Thus, hypoxic nitrite can activate cGMP synthesis through the canonical NO/sGC pathway. However, nitrite can also give rise to the formation of additional cGMP-independent signaling mediators such S-nitrosothiols and nitrated fatty acids [6,7,9,22,34–36]. The reliable determination of circulating S-nitrosothiols requires immediate processing and freezing of blood samples, which was incompatible with this pre-hospital protocol [37]. In our prior study examining IV nitrite given in-hospital with rapid blood sampling and processing, we demonstrated increases in plasma S-nitrosothiols after nitrite dosing with no correlation to plasma cGMP [15]. In addition, we previously demonstrated that similar mechanistic pathways are involved in the formation of S-nitrosothiols and NO_2-CLA such that the measured increases in NO_2-CLA may correspond to unmeasured increases in S-nitrosothiols [22]. Nitrite-derived NO_2-CLA formation has been reported to occur as consequence of intragastric acidification during normal digestion and also as a result of acute inflammation, with nitro fatty acid levels shown to increase in cardiac ischemia reperfusion models [22–25,33,38,39]. Herein, the negative

Table 2
Plasma species correlations.

Spearman ρ	NO_2^-	cGMP	NO_2-CLA	DH-NO_2-CLA	Dinor-NO_2-CLA	Tetranor-NO_2-CLA	9,11 CLA	Age	Weight
NO_2^-	–	–	−0.229*	–	−0.283*	–	–	0.343**	–
cGMP	–	–	–	–	–	–	–	–	–
NO_2-CLA	−0.229*	–	–	0.699***	0.780***	0.793***	0.380***	−0.227*	0.224*
DH-NO_2-CLA	–	–	0.699***	–	0.566***	0.592***	0.422***	−0.241*	–
Dinor-NO_2-CLA	−0.283	–	0.780***	0.566***	–	0.750***	0.340**	−0.259*	0.238*
Tetranor-NO_2-CLA	–	–	0.793***	0.592***	0.750***	–	0.258*	−0.287**	–
9,11 CLA	–	–	0.380***	0.422***	0.340**	0.258*	–	–	–
Age	0.343**	–	−0.227*	−0.241*	−0.259*	−0.287**	–	–	–
Weight	–	–	0.224*	–	0.238*	–	–	–	–

*p < 0.05, **p < 0.01, ***p < 0.0001.

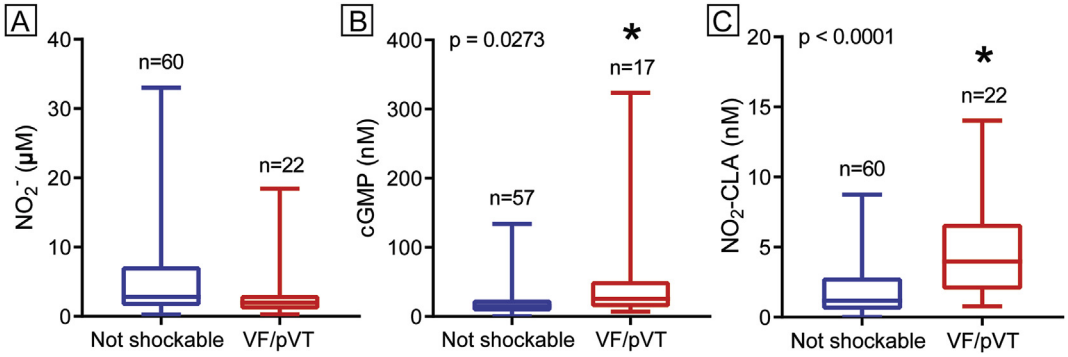

Fig. 1. Nitrite, cGMP and NO_2-CLA levels differ based on presentation with a shockable rhythm. Median, interquartile range (IQR; box) and range (whiskers) for circulating levels of the indicated species grouped by presentation with shockable rhythm with comparison by Mann-Whitney *U* test and significance set at *p < 0.05.

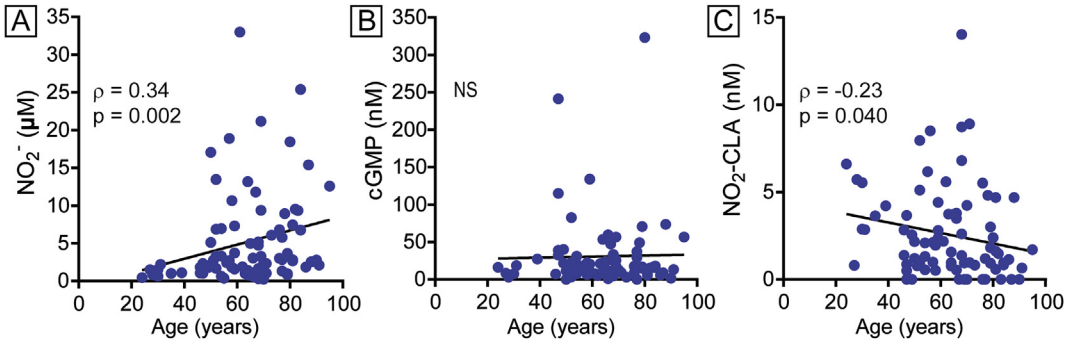

Fig. 2. Correlations between age and circulating levels of Nitrite, cGMP and NO_2-CLA. Spearman correlation between the indicated species and patient age in years. NS = not significant.

correlation observed between whole blood nitrite and plasma NO_2-CLA levels is consistent with the hypothesis that at least part of IV administered nitrite reacts with endogenous CLA to generate the corresponding nitrated derivative. In this regard, the strong correlation of NO_2-CLA levels with multiple downstream metabolites and with its 9,11-CLA precursor provides further support to the endogenous nature of NO_2-CLA formation in this patient population. Nevertheless, given the that NO_2-CLA is normally present in humans, pre-arrest levels of this metabolite may also affect survival to discharge and neurological outcome in OHCA patients [23,26,33].

Unlike NO_2-CLA and S-nitrosothiol formation, cGMP is generated by NO-dependent activation of intracellular soluble guanylate cyclase [20]. In line with a prior report that plasma cGMP levels do not reflect the physiologic actions of NO [40], we failed to detect significant associations between plasma cGMP and whole blood nitrite levels. This lack of association might be attributed to a combination of factors including poor membrane permeability for intracellularly-generated cGMP and differential contributions of phosphodiesterase activities as well as other nitrite-consuming pathways that do not result in NO formation such as reaction with oxygenated hemoglobin and myoglobin [5,20]. Interestingly, basal plasma levels of cGMP are typically below 10 nM [40,41] which was in many cases exceeded in OHCA patients receiving IV nitrite. Thus, while we cannot be certain that nitrite failed to form NO and downstream cGMP in patients presenting with plasma cGMP levels within the normal range, it is very likely that those patients with high plasma cGMP had substantial NO-dependent sGC activation. With these caveats in mind, our results show that IV nitrite results in the formation of substantial quantities of NO_2-CLA and potentially cGMP after human OHCA but this process is highly patient-dependent.

We noted the highest levels of cGMP and NO_2-CLA were measured in patients presenting with shockable rhythms which is a marker for cardiac etiology OHCA, generally due to coronary atherosclerosis or cardiomyopathy [42]. OHCA patients with vascular disease, which often impairs endothelial NO formation, may have a greater response to

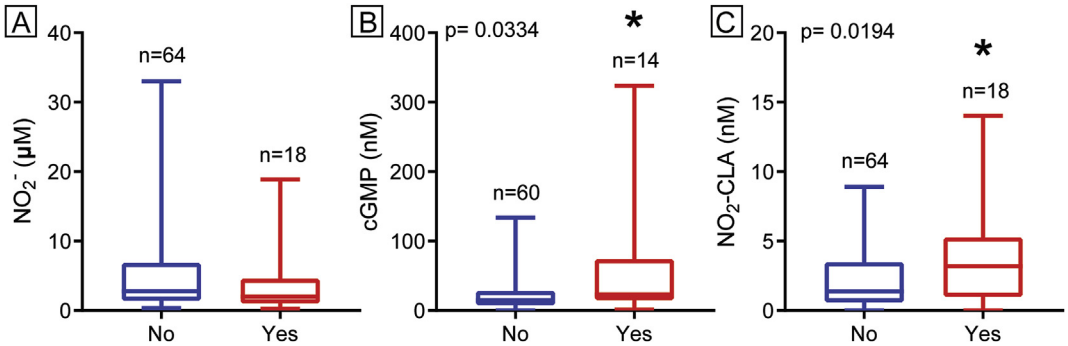

Fig. 3. Nitrite, cGMP and NO_2-CLA levels differ based on survival to discharge. Median, interquartile range (IQR; box) and range (whiskers) for circulating levels of the indicated species grouped by survival to discharge with comparison by Mann-Whitney *U* test and significance set at *p < 0.05.

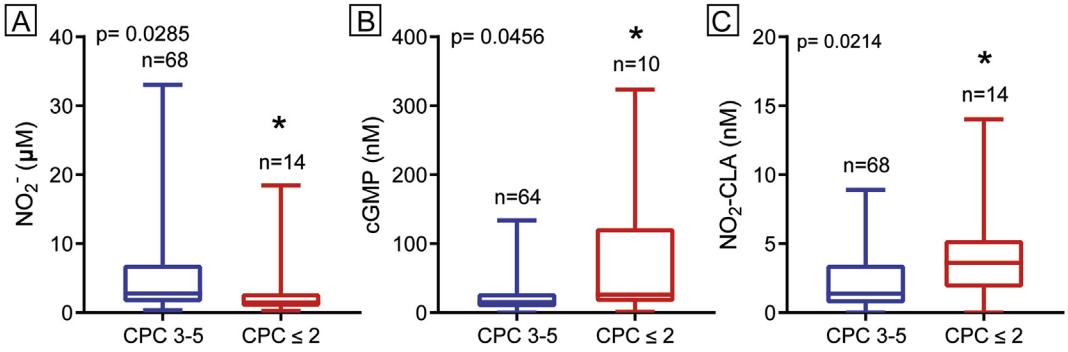

Fig. 4. Nitrite, cGMP and NO_2-CLA levels are different depending on neurological outcome. Median, interquartile range (IQR; box) and range (whiskers) for circulating levels of the indicated species grouped by CPC score with comparison by Mann-Whitney *U* test and significance set at *p < 0.05.

an exogenous NO source such as nitrite and thereby may derive the greatest therapeutic benefit. The associations between higher cGMP and NO_2-CLA levels with better outcomes certainly are consistent with this hypothesis, but the fact that OHCA patients with shockable rhythms are known to have better outcomes creates a significant confounding relationship that limits our ability to draw definitive conclusions [1]. In fact, the powerful association between shockable rhythm and outcome combined with the limited number of patients with this presentation (22/82, Table 1) determines that our study is not sufficiently powered to independently assess the effect of individual plasma metabolites levels on clinical outcomes. Additional noteworthy correlations were the increases in nitrite levels observed with increasing age, implying less nitrite utilization in older individuals, which is further corroborated by an inverse correlation between age and NO_2-CLA. Weight was also associated with an increase in NO_2-CLA which could not be explained by 9,11 CLA precursor availability, as the levels of this fatty acid were not affected by either weight or age. Finally, it is interesting that the dose of nitrite had no impact on the levels of cGMP and NO_2-CLA. This implies that other biological factors are more important in nitrite-mediated signaling than mere precursor availability, and may explain the broad therapeutic range reported in prior nitrite preclinical studies [7,8].

The lack of effective neuroprotective drugs in the setting of OHCA coupled with numerous neutral trial results [43–46] highlight the importance of understanding mechanism and heterogeneity within this patient population. Our finding of improved outcomes in OHCA patients with the highest NO_2-CLA levels is novel. Nitrated fatty acids such as NO_2-CLA preserve cardiovascular function and reduce cell death after ischemia, attenuate immune cell recruitment and mediate both broad inhibition of NF-κB dependent pro-inflammatory signaling and activation of Nrf2-dependent antioxidant enzyme synthesis [24,30,31,39,47]. Moreover, nitrated fatty acids cross the blood brain barrier and persist in tissues for several days following a single administration [48,49]. These preclinical findings combined with our present results provide rationale to consider NO_2-CLA itself as a potential therapeutic modality after OHCA. The availability of precursor 9,11-CLA is a critical factor in NO_2-CLA formation [33], however whereas free 9,11-CLA directly correlates with levels of NO_2-CLA and its downstream metabolites, 9,11-CLA alone did not associate with either survival to discharge or neurological outcome (Suppl Fig. 4). This suggests that whereas 9,11-CLA is necessary, the ability to direct nitrite consumption towards nitration is the main determinant for nitrated fatty acid formation in OHCA patients.

Our study has a number of limitations. We have already noted the issues around plasma cGMP measurement as an indicator for intracellular sGC activation and acknowledged our inability to measure S-nitrosothiols, which may also play a significant role in the mechanism of nitrite mediated protection after cardiac arrest [14]. Blood sampling in this phase 1 study was done prehospital with variable times to sample processing and storage. Thus, we cannot compare our levels to those obtained from immediate sampling done on healthy subjects nor did this phase 1 study incorporate a placebo control for comparison. The present study is observational rather than experimental so the associations we note do not prove causality. Nevertheless, our results suggest that nitrite utilization via cGMP and NO_2-CLA formation may be important in determining outcome. Furthermore, the present findings provide support for hypotheses that are consistent with existing mechanistic data in animals. Ultimately, understanding which patient- and disease-specific characteristics correspond to nitrite signaling along different pathways, and whether these associations in turn correspond to a heterogeneity in treatment effect, will require a placebo control group. Since our phase 1 study enrolled subjects prehospital under exception from informed consent, only limited data was collected on these subjects and factors such as medical comorbidities, which may contribute to nitrite signaling, cannot be assessed. Collectively our data underscore the need to study mechanism in humans: ideally within a randomized experimental design to understand heterogeneity and better target drugs to avoid continued therapeutic failures [43–46].

In conclusion, we observe that intravenous nitrite administration after OHCA results in increases in cGMP and NO_2-CLA levels with considerable heterogeneity between patients, which may be correlated to factors such as weight, age and other co-morbidities. Patients with the highest levels of cGMP and NO_2-CLA experienced the best clinical outcomes though neither marker was independently associated with outcome after controlling for clinical variables. Future placebo-controlled clinical studies will be necessary to elucidate whether nitrite, NO_2-CLA and cGMP dictate outcome independently from arrest presentation as well as to establish the potential contributions of pre-existing co-morbidities to the heterogeneity of our population.

Author contributions

DAV, CD prepared the manuscript, designed, performed and analyzed experiments. MO, NK collected samples and performed experiments. ACS, PJK, GN, MS and FK contributed to the overall concept, experimental design, data interpretation and writing.

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Declaration of competing interest

DAV is an *ad hoc* consultant for Complexa Inc.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101463.

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