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Coadministration of a Na^+-H^+ exchange inhibitor and sodium bicarbonate for the treatment of asphyxia-induced cardiac arrest in piglets

Xinchun Lin[1], Jeffrey A. Kraut[2,3] and Dongmei Wu[1,4]

BACKGROUND: The present study tested the hypothesis that addition of an inhibitor of Na^+/H^+ exchanger (NHE1) to sodium bicarbonate might improve the response to base therapy from prolonged asphyxial cardiac arrest in piglets.

METHODS: Asphyxial cardiac arrest was induced by endotracheal tube clamping. Animals were randomly assigned to four study groups: (i) vehicle control, (ii) administration of sabiporide (NHE1 inhibitor), (iii) administration of sodium bicarbonate, and (iv) administration of sabiporide and sodium bicarbonate.

RESULTS: Administration of sodium bicarbonate alone did not affect survival, hemodynamic measures, and regional blood flow to critical tissues such as brain, heart, kidney, liver, and spleen. In contrast, sabiporide given alone or combined with sodium bicarbonate improved these. Furthermore, treatment with sabiporide reduced accumulation of neutrophils, reduced cytokine production in the lung, and reduced plasma levels of cardiac troponin-I, alanine aminotransferase, aspartate aminotransferase, and urea. In addition, the combined use of sabiporide and sodium bicarbonate had more profound reduction in interleukin (IL)-6 and IL-10, compared to sabiporide alone.

CONCLUSION: These results suggest that addition of sabiporide to the administration of sodium bicarbonate might improve hemodynamic response and dampen the inflammatory cascade noted with cardiac arrest, and therefore being an attractive option in the treatment of cardiac arrest.

The belief that a reduction in the intracellular pH and interstitial pH of the myocardium reduced contractility and predisposed to the development of arrhythmias initially prompted the aggressive administration of base in the immediate treatment of cardiac arrest (1–4). However, evidence that the most common base used sodium bicarbonate was associated with generation of carbon dioxide and a transient paradoxical exacerbation of intracellular acidosis during its administration despite improvement in extracellular pH led to recommendation by the American Heart Association to avoid base therapy (1–5). Little in pharmacological therapy has been introduced in the intervening years to replace base therapy in treatment of cardiac arrest.

Studies by us and others, using various models of acute metabolic acidosis have shown that activation of the ubiquitous Na^+/H^+ exchanger (NHE1) contributes to a reduction in cardiac contractility and predisposition to arrhythmias with acidosis (6–11). Moreover, pretreatment with a selective inhibitor of NHE1 improves cardiac contractility, suppresses inflammation, and most importantly, reduces mortality (8–11). This improvement was attributed to attenuation of the increase in intracellular sodium and calcium, even though theoretically inhibition of the Na^+/H^+ exchanger might worsen intracellular acidosis (6–8).

Based on this evidence, we postulated that coadministration of an NHE1 inhibitor with sodium bicarbonate might allow expression of the positive effects of sodium bicarbonate administration. To examine this possibility, we examined the impact of the coadministration of sabiporide, a selective Na^+-H^+ exchange inhibitor with sodium bicarbonate on hemodynamics, metabolic, and inflammatory responses in the treatment of asphyxia-induced cardiac arrest in piglets. The results indicated that sabiporide given alone or combined with sodium bicarbonate improved systemic blood pressure, heart rate, and regional blood flows to brain, heart, kidney, liver, and spleen. Animals treated with sabiporide had improved cardiac function compared to control animals. Furthermore, treatment with sabiporide reduced accumulation of neutrophils, cytokine production in the lung, plasma levels of cardiac troponin-I, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea. In addition, the combination of sabiporide and sodium bicarbonate caused a great reduction in interleukin (IL)-6 and IL-10, compared to sabiporide alone. These findings could indicate that addition of sabiporide to base treatment of cardiac arrest might be a reasonable option in the treatment of cardiac arrest.

[1]Department of Research, Mount Sinai Medical Center, Miami Beach, Florida; [2]Medical and Research Services and Division of Nephrology, Veterans Administration Greater Los Angeles Healthcare System, Los Angeles, California; [3]David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California; [4]Department of Biotechnology, Information Science, and Nanotechnology (BIN) Fusion Technology, Chonbuk National University, Jeonju, Korea. Correspondence: Dongmei Wu (dongmeiwu@bellsouth.net)

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Table 1. Changes in blood gas data following asphyxial cardiac arrest and resuscitation in pigs

	Baseline	Asphyxia	R30 min	R60 min	R120 min	R180 min	R240 min	R300 min
pH								
Vehicle	7.37 ± 0.04	6.88 ± 0.05**	7.18 ± 0.05**	7.23 ± 0.04**	7.31 ± 0.03	7.28 ± 0.04**	7.32 ± 0.04	7.35 ± 0.06
Sabiporide	7.38 ± 0.04	6.79 ± 0.06**	7.16 ± 0.06**,***	7.19 ± 0.05**	7.25 ± 0.03**	7.25 ± 0.04**	7.28 ± 0.06**	7.33 ± 0.05
Veh+Bic	7.43 ± 0.05	6.82 ± 0.07**	7.43 ± 0.05*	7.45 ± 0.04*,***,****	7.46 ± 0.04*,****	7.48 ± 0.03*,****	7.47 ± 0.03*,****	7.46 ± 0.02*,****
Sab+Bic	7.42 ± 0.05	6.90 ± 0.00**	7.39 ± 0.03*,****	7.35 ± 0.03*,**,****	7.40 ± 0.05*,****	7.42 ± 0.03*,***,****	7.45 ± 0.02*,****	7.46 ± 0.03*,****
pCO2 (mmHg)								
Vehicle	40.3 ± 2.4	83.7 ± 10.5**	46.3 ± 1.9**	38.3 ± 2.5	35.5 ± 2.4**	38.2 ± 2.5	41.1 ± 1.9	42.0 ± 2.4
Sabiporide	37.2 ± 2.9	90.3 ± 12.7**	43.6 ± 2.2*,**	34.3 ± 2.7*	35.5 ± 1.9	37.4 ± 2.1	38.7 ± 2.2	35.8 ± 2.4*
Veh+Bic	41.2 ± 2.5	88.2 ± 14.9**	35.7 ± 2.1*,**,****	41.2 ± 2.0****	38.4 ± 2.3	35.4 ± 2.2**	42.4 ± 1.9****	40.2 ± 2.1
Sab+Bic	38.1 ± 2.1	97.0 ± 15.5**	37.8 ± 1.6****	36.7 ± 1.9***	35.4 ± 2.3	34.1 ± 2.1*,**	39.2 ± 1.8***	42.1 ± 1.5**,****
pO2 (mmHg)								
Vehicle	99.3 ± 9.1	14.2 ± 2.7**	95.5 ± 7.9	98.1 ± 5.0	97.6 ± 4.5	95 ± 8.6	99.8 ± 7.6	101.2 ± 6.6
Sabiporide	105.8 ± 8.7	17.3 ± 4.2**	101.8 ± 8.7	105.0 ± 9.4	104.2 ± 6.7	100.3 ± 4.4	105.3 ± 6.8	99.8 ± 7.1
Veh+Bic	106.7 ± 8.2	16.3 ± 2.1**	96.3 ± 8.9	103.4 ± 9.2	99.5 ± 7.1	98.2 ± 5.6	101.3 ± 5.4	100.5 ± 4.5
Sab+Bic	101.3 ± 7.8	8.45 ± 1.2*,**,***,****	92.1 ± 6.8	100.2 ± 6.9	95 ± 10.5	98.6 ± 7.2	99.3 ± 4.5	104.8 ± 3.2
Bicarbonate (m(mol/l)/l)								
Vehicle	24.1 ± 2.2	14.7 ± 2.0**	20.9 ± 2.9	17.6 ± 2.1**	20.3 ± 2.9**	19.2 ± 2.6	22.7 ± 1.8	25.0 ± 1.8
Sabiporide	25.8 ± 2.7	13.3 ± 1.9**	18.6 ± 2.7**,***,****	19.8 ± 2.2**,***	21.4 ± 2.4**,***	23.6 ± 2.2*,***	25.4 ± 2.1	26.1 ± 2.3
Veh+Bic	24.9 ± 2.9	14.0 ± 2.5**	26.1 ± 2.3*	25.4 ± 2.7*	27.2 ± 2.0*	28.3 ± 2.6*	28.1 ± 2.1*	27.1 ± 2.8
Sab+Bic	26.2 ± 3.4	15.7 ± 2.1**	26.1 ± 2.5*,****	25.2 ± 2.4*,****	27.6 ± 2.0*,****	28.6 ± 2.5*,***,****	27.2 ± 1.8*	26.5 ± 2.7
Mix-venous sO2 (%)								
Vehicle	76.6 ± 8.7	8.0 ± 2.2**	49.9 ± 4.0**	45.3 ± 4.2**	51.8 ± 7.8**	46.7 ± 6.1**	52.3 ± 6.5**	56.4 ± 7.2**
Sabiporide	73.3 ± 6.9	7.2 ± 2.1**	67.3 ± 12.2*	64.2 ± 14.6*	66.4 ± 12.0*,***	52.6 ± 8*,**,***	69.4 ± 7.2*,***	74.5 ± 7.1*,***
Veh+Bic	78.1 ± 7.6	7.9 ± 1.9**	52.4 ± 10.3**	50.1 ± 11.2**	49.6 ± 7.3**	55.1 ± 8.6**	54.5 ± 7.6**	58.1 ± 8.0**
Sab+Bic	77.8 ± 9.5	7.1 ± 2.4**	70.0 ± 8.1*,***	61.0 ± 9.3*,***	69.0 ± 7.5*,***	67.7 ± 4.6*,***	72.3 ± 7.9*,***	79.2 ± 8.3*,***

All values are the mean ± SD, $n = 7–8$.

Asphyxia, at 8 min of asphyxia; Bic, sodium bicarbonate; R30–R300, at 30 to 300 min after return of spontaneous circulation; Sab, sabiporide; Veh, vehicle.

*$P < 0.05$ vs. the vehicle control. **$P < 0.05$ vs. the baseline. ***$P < 0.05$ vs. the vehicle + $NaHCO_3$ group. ****$P < 0.05$ vs. sabiporide group.

RESULTS

Acid–Base Measurements

Table 1 shows changes in acid–base parameters during control, following asphyxia, and after resuscitation in all groups. Acid–base parameters were similar in all groups prior to development of asphyxia. Asphyxia resulted in severe metabolic acidosis with a blood pH < 6.9 and a mean plasma bicarbonate level that ranged from 13.3 to 15.7 m(mol/l)/l). No differences were found between the groups. Blood pH and plasma bicarbonate in the groups receiving the vehicle or sabiporide alone increased slowly throughout the study reflecting administration of resuscitation fluids. By contrast, in the groups receiving bicarbonate alone or bicarbonate and sabiporide, plasma bicarbonate concentration increased rapidly (by 30 min) to 26 ± 2.1 m(mol/l)/l. Blood pH also normalized within this period. Both values were relatively constant during the remaining of the resuscitation period. However, animals receiving sabiporide alone or combined with sodium bicarbonate had significant improvement in blood oxygenation as evidenced by increased mixed venous blood oxygen saturation, compared to vehicle control animals or animals received sodium bicarbonate alone (Table 1), reflecting from improved cardiac function and tissue perfusion in those animals.

Hemodynamics and Cardiac Function

Compared to the vehicle control group, acid–base correction with sodium bicarbonate alone did not affect the hemodynamics or cardiac performance. However, animals that received sabiporide alone or combined with sodium bicarbonate had higher blood pressure, heart rate, coronary perfusion pressure, and cardiac output during the prolonged postresuscitation period (Figure 1). This increase in cardiac output in sabiporide groups is attributed in part to an improved cardiac performance (Table 2), and is also attributed to the increased heart rate, possibly through an acute compensatory mechanism. Combination of sabiporide and acid–base correction with sodium bicarbonate had more profound improvement in cardiac output during the prolonged postresuscitation period, compared to administration of sabiporide alone (Figure 1). Postresuscitation myocardial stunning was present in vehicle control animals, as evidenced by impaired left ventricular ejection fraction, fractional shortening, and wall motion score index (Table 2), and treatment with sabiporide significantly attenuated the myocardial stunning developed during postresuscitation.

Regional Blood Flows

Figure 2 depicts changes in regional blood flow of vital organs following asphyxial cardiac arrest and with resuscitation.

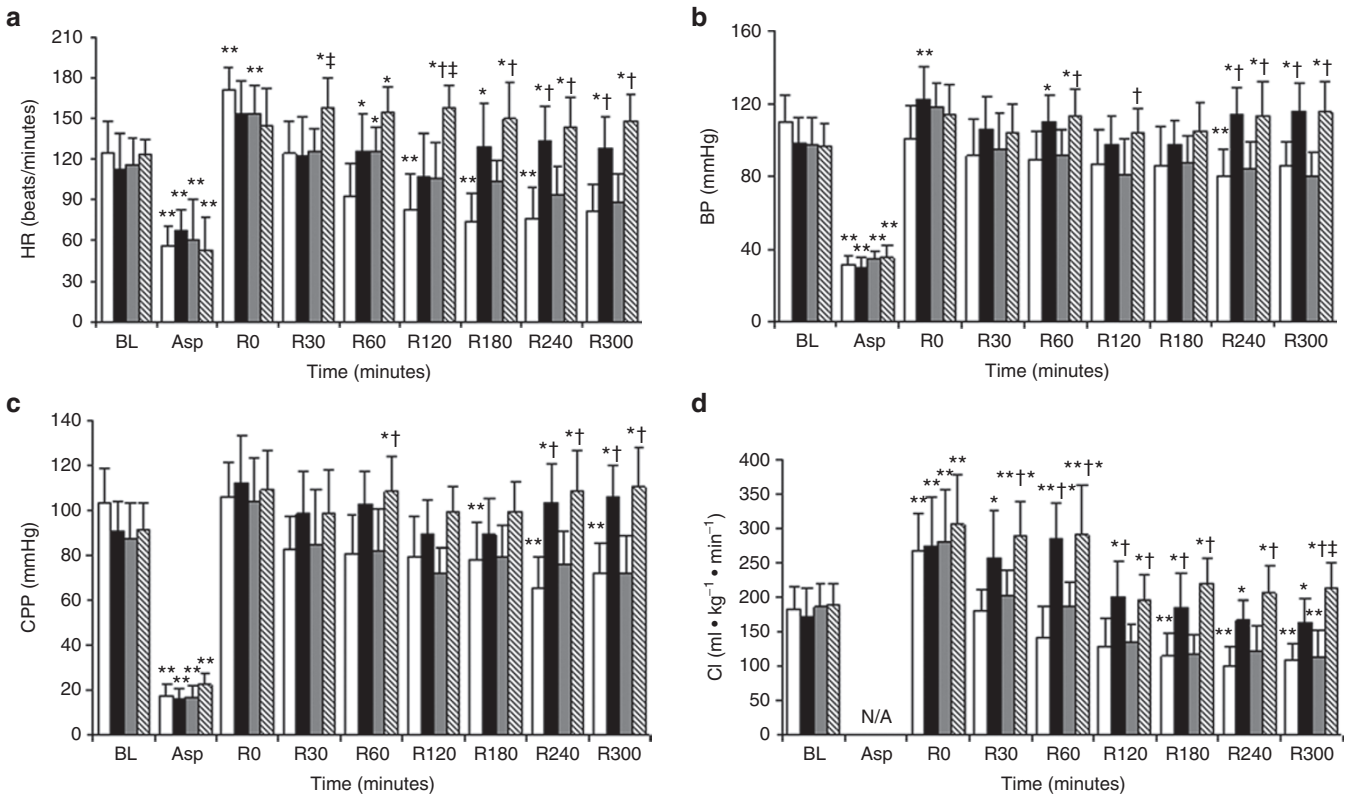

Figure 1. Hemodynamic changes following asphyxia-induced cardiac arrest. Administration of sabiporide alone or combined with sodium bicarbonate improved (**a**) heart rate (HR), (**b**) systemic blood pressure (BP), (**c**) coronary perfusion pressure (CPP), and (**d**) cardiac index (CI) following asphyxia-induced cardiac arrest and resuscitation. All values are the mean ± SD. $N = 7–8$, $^{**}P < 0.05$ vs. baseline; $^{*}P < 0.05$ vs. the vehicle controls; and $^{\dagger}P < 0.05$ vs. vehicle + sabiporide; and $^{\ddagger}P < 0.05$ vs. the sabiporide group. Asp, at 8 min of asphyxia; BL, at baseline; R30–R300, at 30–300 min after return of spontaneous circulation. Vehicle (white column), sabiporide (black column), vehicle + bicarbonate (gray column), and sabiporide + bicarbonate (hatched column).

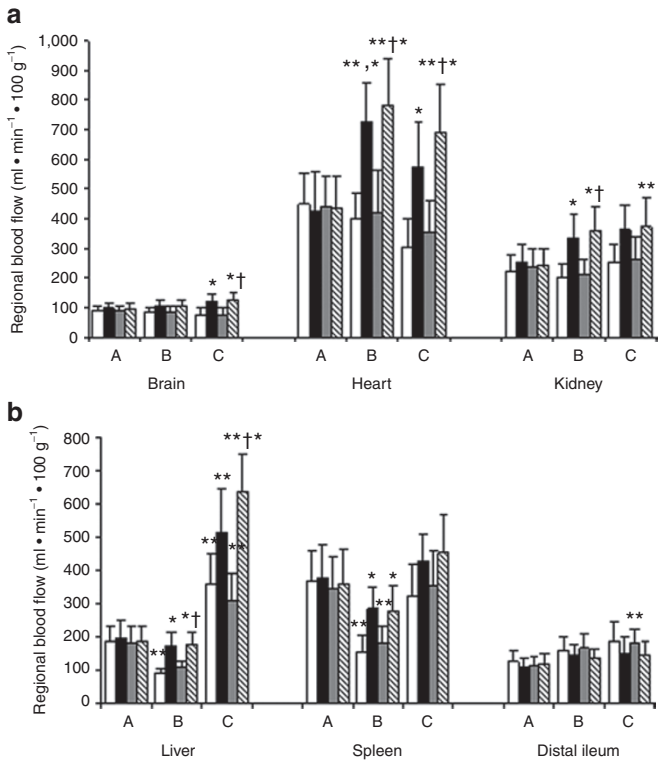

Figure 2. Regional blood flows measured by the colored microsphere technique following asphyxia-induced cardiac arrest and resuscitation in pigs. Panel **a**: brain, heart, and kidney; panel **b**: liver, spleen, and distal ileum. The values in each group are as (**a**) baseline, (**b**) 30 min after return of spontaneous circulation (ROSC), (**c**) 240 min after ROSC. Vehicle (white column), sabiporide (black column), vehicle + bicarbonate (gray column), and sabiporide + bicarbonate (hatched column). Data are presented as mean ± SD, $n = 5–7$, $^{**}P < 0.05$ vs. baseline; $^{*}P < 0.05$ vs. the vehicle control group; and $^{\dagger}P < 0.05$ vs. vehicle + sabiporide.

During the first 30 min after return of spontaneous circulation (ROSC), blood flows were fairly maintained in vital organs in brain and heart by compensatory mechanisms, but decreased over prolonged postresuscitation period. However, sabiporide groups had higher blood flows in the brain, heart, kidney, liver, and spleen. At 4 h after ROSC, the blood flows were higher in the brain (by 65.3%), heart (by 126%), and liver (by 78.4%) in the sabiporide groups compared to vehicle control animals or animals received only sodium bicarbonate alone (Figure 2). There was no significant blood flow difference in distal ileum mucosa between control and sabiporide group.

Biochemical Markers

Figure 3 depicts level of proinflammatory cytokines tumor necrosis factor (TNF)-α, IL-6, and anti-inflammatory cytokine IL-10 in lung tissues following asphyxial cardiac arrest and resuscitation (Figure 3). Administration of sodium bicarbonate alone did not affect the level of proinflammatory cytokines despite correction of the acidosis. On the other hand, the level of proinflammatory cytokines fell with sabiporide treatment and this reduction was greater when bicarbonate and sabiporide were combined (Figure 3). Myeloperoxidase activity, a marker of neutrophil accumulation was also reduced in the lung tissues in the sabiporide groups compared to the vehicle control animals or animals received sodium bicarbonate alone (Figure 3).

Plasma levels of cardiac-specific troponin-I were significantly increased postresuscitation, indicating myocardial damage (Table 3). Administration of sodium bicarbonate alone did not affect the troponin-I levels compared to vehicle controls. However, troponin-I release was significantly reduced in animals receiving sabiporide treatment. There was also marked

Table 2. Echocardiography data

	Vehicle	Sabiporide	Vehicle + $NaHCO_3$	Sabiporide + $NaHCO_3$
Body weight (kg)	14.9 ± 3.7	15.1 ± 3.5	14.1 ± 4.3	13.7 ± 3.9
Ejection fraction (%)				
Baseline	63.5 ± 4.1	61.2 ± 3.2	66.8 ± 3.9	64.3 ± 4.1
R30min	32.8 ± 3.5**	45.9 ± 3.0*,**,***	34.1 ± 4.1**	48.2 ± 3.6*,**,***
R300min	34.5 ± 3.2**	47.8 ± 3.5*,**,***	36.4 ± 4.5**	49.2 ± 3.4*,**,***
Fractional shortening (%)				
Baseline	37.1 ± 2.8	38.4 ± 3.1	35.6 ± 3.3	37.9 ± 2.7
R30min	16.4 ± 2.0**	25.2 ± 2.3*,**,***	18.6 ± 2.7**	27.4 ± 2.4*,**,***
R300min	16.9 ± 2.6**	26.4 ± 2.0*,**,***	19.4 ± 2.7**	28.6 ± 2.3*,**,***
Wall motion score index				
Baseline	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
R30min	2.6 ± 0.12**	1.7 ± 0.13*,**,***	2.5 ± 0.23**	1.8 ± 0.22*,**,***
R300min	2.7 ± 0.21**	1.9 ± 0.18*,**,***	2.3 ± 0.18**	1.8 ± 0.19*,**,***

Administration of sabiporide alone or as adjunct therapy to acid–base correction with sodium bicarbonate improved left ventricular ejection fraction, fractional shortening, and wall motion score index following asphyxial cardiac arrest and resuscitation. All values are the mean ± SD, $n = 7–9$.
R30min/R300min, at 30/300 min after return of spontaneous circulation.
$^{*}P < 0.05$ vs. the vehicle control. $^{**}P < 0.05$ vs. the baseline. $^{***}P < 0.05$ vs. the vehicle + $NaHCO_3$ group.

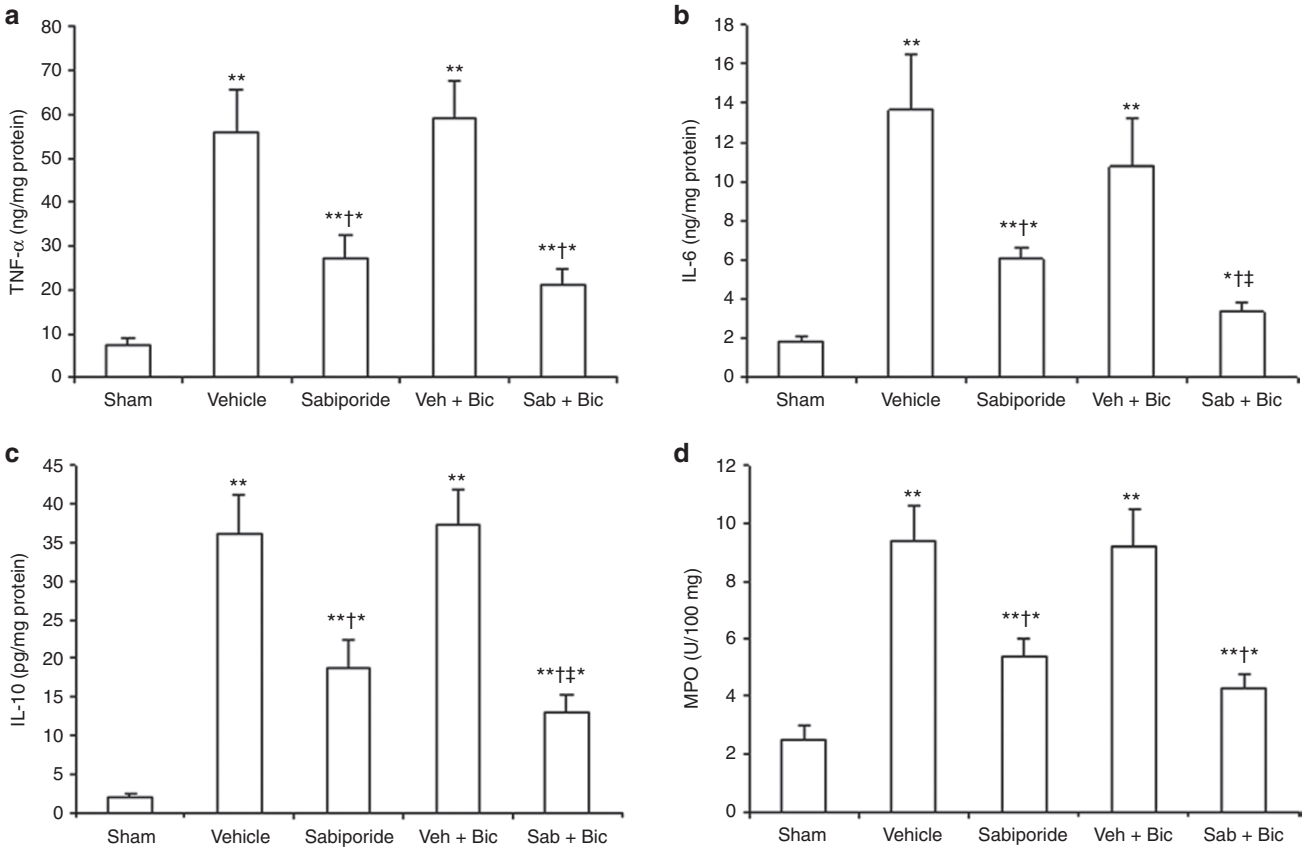

Figure 3. Inflammatory mediators following asphyxia-induced cardiac arrest. Levels of (**a**) tumor necrosis factor (TNF)-α, (**b**) interleukin (IL)-6, (**c**) IL-10, and (**d**) myeloperoxidase (MPO) activity in lung tissues following asphyxia-induced cardiac arrest and resuscitation. All values are the mean ± SD, $n = 5–8$. $^{**}P < 0.05$ vs. sham, $^{*}P < 0.05$ vs. vehicle, $^{\dagger}P < 0.05$ vs. vehicle + sabiporide, and $^{\ddagger}P < 0.05$ vs. sabiporide group. Bic, sodium bicarbonate; Sab, sabiporide; Veh, vehicle.

Table 3. Changes in plasma levels of cardiac troponin-I, ALT, AST, and urea

	Vehicle	Sabiporide	Vehicle + $NaHCO_3$	Sabiporide + $NaHCO_3$
Troponin-I (ng/ml)				
Baseline	0.09 ± 0.01	0.11 ± 0.01	0.10 ± 0.02	0.10 ± 0.02
R30min	0.72 ± 0.13	0.34 ± 0.59	0.61 ± 0.10	0.41 ± 0.05
R300min	14.2 ± 3.5**	8.1 ± 2.1*,**,***	12.8 ± 2.3**	5.7 ± 1.1*,**,***
ALT (U/l)				
Baseline	13.2 ± 2.9	11.4 ± 2.7	12.8 ± 3.1	11.9 ± 2.8
R30min	19.4 ± 4.3	15.8 ± 3.4	17.5 ± 3.5	16.0 ± 3.8
R300min	62.3 ± 11.1**	40.5 ± 6.4*,**,***	63.7 ± 10.4**	32.4 ± 7.1*,**,***
AST (U/l)				
Baseline	90.4 ± 15.4	81.5 ± 21.4	85.5 ± 20.1	83.4 ± 15.8
R30min	129.4 ± 37.1	110.1 ± 30.2	121.3 ± 27.5	112.5 ± 31.3
R300min	319.4 ± 58.0**	190.0 ± 28.4*,**,***	295.1 ± 63.4**	174.6 ± 41.0*,**,***
Urea (mg/dl)				
Baseline	55.1 ± 14.8	49.7 ± 9.5	53.1 ± 12.0	57.9 ± 14.8
R30min	62.3 ± 14.8	58.4 ± 11.5	57.2 ± 13.7	65.4 ± 17.1
R300min	205.7 ± 49.4**	132.7 ± 28.2*,**,***	187.9 ± 34.7**	118.7 ± 31.5*,**,***

Administration of sabiporide alone or as adjunct therapy to acid–base correction with sodium bicarbonate reduced plasma levels of troponin-I, ALT, AST, and urea following asphyxial cardiac arrest and resuscitation. All values are the mean ± SD, $n = 7–9$.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; R30min/R300min, at 30/300 min after return of spontaneous circulation.
$^{**}P < 0.05$ vs. the baseline. $^{*}P < 0.05$ vs. the vehicle control. $^{***}P < 0.05$ vs. the vehicle + $NaHCO_3$ group.

increase in plasma levels of ALT and AST (markers of liver injury) at postresuscitation (**Table 3**), indicating liver injury. Treatment with sabiporide significantly reduced plasma levels of ALT and AST compared to vehicle control animals or animals that received sodium bicarbonate alone. Plasma levels of urea were significantly elevated following asphyxial cardiac arrest and resuscitation, and this elevation of urea was attenuated by sabiporide treatment (**Table 3**).

DISCUSSION

Cardiac arrest whether due to ventricular fibrillation, marked hypoperfusion, or severe hypoxia is associated with a very high mortality (12–15). The acid–base disturbances associated with cardiac arrest often include both metabolic acidosis and respiratory acidosis with tissue pH decreasing as low as 6.6, local concentration of bicarbonate decreasing below 10 mmol/l, and tissue CO_2 concentration increasing as high as >150 mmHg (12). The severe systemic metabolic acidosis and myocardial acidosis found was seen as an impediment to successful resuscitation, and therefore, aggressive administration of bicarbonate was recommended. The mechanisms underlying the deleterious effects of acidosis included a decrease in interstitial pH with impaired binding of important hormones such as catecholamines and insulin to their cognate receptors (1) as well as the decrease in intracellular pH. Administration of bicarbonate had the potential to improve interstitial pH, but also to increase CO_2 concentration during the buffering process and theoretically worsen intracellular acidosis. This latter effect could further depress cardiac function during arrest or delay or prevent myocardial recovery during resuscitation (1,16–18). Therefore, the administration of sodium bicarbonate was removed from the American Heart Association recommendation of pharmacological treatment of cardiac arrest.

Cardiac dysfunction or cardiac failure is a frequent complication associated with cardiopulmonary resuscitation and is an important factor contributing to the high mortality associated with multiorgan failure (19). The intracellular acidosis seen with cardiac arrest has been shown to be associated with activation of the ubiquitous Na^+/H^+ exchanger, NHE1. This activation leads to marked increments of cellular sodium and calcium with resultant depression of cardiac contractility and a predisposition to cardiac arrhythmias (6–8). Inhibition of NHE1 reduces the accumulation of cellular sodium and calcium, improves cardiac output, reduces the level of proinflammatory cytokines, and decreases mortality (6–11). This effect is observed even though inhibition of NHE1 will theoretically worsen intracellular acidosis by paralyzing one of the mechanisms contributing to pH regulation of the myocardial cell.

These results suggested that the depression of cardiac function with cardiac arrest is not only due to acidification of the myocardial cell, but could be due in part to NHE1-dependent accumulation of sodium and calcium. As a corollary, inhibition of NHE1 during the administration of sodium bicarbonate might lessen the potential deleterious effect of bicarbonate therapy and allow the positive effects to be expressed. To examine this possibility, we compared the effects of sodium bicarbonate alone and sodium bicarbonate with an NHE1 inhibitor sabiporide on acid–base parameters, hemodynamics, regional blood flows, and the level of proinflammatory cytokines.

NHE1 is ubiquitously expressed in all mammalian cells, and it represents the most important mechanism for the regulation of intracellular pH (pH_i) (6–8). At the onset of ischemia/hypoxia, pH_i decreases due to anaerobic metabolism and production of lactic acid, leading to an activation of NHE1, resulting in increased intracellular Na^+ and Ca^{2+} (6–8). With subsequent acid–base correction, a rapid change to extracellular pH reactivates NHE1, leading to an unfavorably fast restoration of pH_i and again to Na^+_i and Ca^{2+}_i overload (6–8). High Ca^{2+}_i is assumed to be one of the main reasons for ischemic and reperfusion injury including arrhythmias, myocardial contractile dysfuntion, stunning, and eventually necrosis (6–8). Pharmacological inhibitors of NHE1 have been demonstrated to mitigate ischemia–reperfusion damage in the brain, heart, and other organ systems (6–11,20–22). Thus, NHE1 inhibition as adjunct therapy to acid–base correction with sodium bicarbonate provides a novel strategy for the treatment of severe metabolic acidosis-related diseases. In the present study, the combined use of sabiporide and sodium bicarbonate rapidly restored acid–base status, increased mixed venous oxygen saturation, and significantly improved hemodynamics and myocardial performance. A key to the potential mechanism was our finding that the combined use of sabiporide and sodium bicarbonate significantly improved regional blood flows to vital organs and attenuated tissue inflammatory mediators. Data from the present study support the hypothesis that NHE1 inhibitor as adjunct therapy to acid–base correction with sodium bicarbonate may provide novel approach to improve cardiovascular and metabolic outcomes of resuscitation from asphyxia-induced cardiac arrest.

Following cardiopulmonary resuscitation, clinical manifestations of ischemic–reperfusion injury in the cardiovascular system consist of ventricular arrhythmias, left ventricular dysfunction, reduced sensitivity of blood vessels toward vasoconstrictor stimuli, and failure of vascular function to maintain systemic blood pressure (19,23). We and others have previously shown that NHE1 inhibition protects against myocardial ischemic injury, attenuates ischemic hypercontracture, reduces arrhythmias and myocardial infarction, and prevents ventricular fibrillation and death (6–11). In the present experimental model of asphyxial cardiac arrest, postresuscitation myocardial and metabolic dysfunction was present in vehicle control animals, as evidenced by impaired left ventricular ejection fraction, fraction shortening, and ventricular wall motion. Animals received sodium bicarbonate alone had no effect on cardiovascular function compared to vehicle controls. In contrast, administration of sabiporide alone or as adjunct therapy to acid–base correction with sodium bicarbonate improved cardiac performance with an increase in left ventricular ejection fraction, fractional shortening, and a decrease in wall motion abnormality following asphyxial cardiac arrest and resuscitation. Furthermore, sabiporide treatment enhanced hemodynamic stability following prolonged resuscitation

period. Combination of sabiporide and acid–base correction with sodium bicarbonate had more profound improvement in cardiac output during the prolonged postresuscitation period, compared to administration of sabiporide alone. This increase in cardiac output in sabiporide groups is attributed in part to an improved cardiac performance, and is also attributed to the increased heart rate, possibly through an acute compensatory mechanism.

A unifying definition of ischemia at the cellular level is inadequate oxygen consumption by the cell for its metabolic needs. The negative consequence is the accumulation of an oxygen debt with eventual cellular dysfunction and death (12,13). The severity of the asphyxial cardiac arrest state utilized in the present study is reflected by poor peripheral perfusion, greatly reduced blood oxygenation as evidenced by decreased mixed venous oxygen saturation, and excessive acidosis. Given the fact that acidosis impairs oxygen-binding capability of hemoglobin, acid–base correction with sodium bicarbonate to restore acid–base status was considered a reasoned approach in critical care medicine. However, in the present study, administration of sodium bicarbonate alone, did not affect tissue perfusion or blood oxygenation. In contrast, sabiporide given alone or as adjunct therapy to acid–base correction with sodium bicarbonate significantly improved tissue perfusion in brain, heart, liver, and kidney following prolonged asphyxial cardiac arrest and resuscitation, leading to improved tissue oxygenation reflecting from increased mixed venous oxygen saturation.

Previous studies have shown that organ blood flow after VF cardiac arrest is biphasic in nature, with global hyperemia present for 15–30 min after the ROSC, followed by delayed hypoperfusion (24,25). Few studies have characterized organ blood flows after asphyxia. In a rat model of asphyxial cardiac arrest, Manole *et al.* (26) examined cerebral blood flow after resuscitation from three increasing durations of cardiac arrest. They found that postresuscitation, cerebral blood flow is insult duration dependent: shorter insults result in hyperemia and resolution of baseline cerebral blood flow, whereas longer insults result in hypoperfusion without hyperemia. Although early hyperemia is regarded as beneficial and hypoperfusion as detrimental (26,27), the optimal reperfusion pattern after cardiac arrest remains unknown. In the present study, sabiporide increased blood flows to the brain, heart, kidney, liver, and spleen after ROSC from asphyxial cardiac arrest, and this increase in tissue perfusion was associated with an increase in mixed venous oxygen saturation and decreased ALT, AST, and troponins, suggesting a reduced organ injury resulting from improved tissue perfusion with sabiporide treatment. Therefore, it is possible that hyperemia could be a vital sign that reflects the physiological compensatory response.

Hypoxia and reoxygenation contributes to multiorgan failure in asphyxiated neonates, including cardiac and systemic complications (28). With the reestablishment of blood flow, the function of a number of metabolic and inflammation pathways are accelerated within organ systems. The postresuscitation inflammatory processes are characterized by the upregulation of cytokine expression and accumulation of neutrophils in a variety of tissues. The lung is a primary site of injury as studies have suggested that ischemia–reperfusion primes circulating neutrophils, mediating neurophil-dependent lung injury once the neutrophils are sequestered in the lung (29,30). Over hours, these processes may lead to worsening heart, brain, and other organ injury, leading to persistent myocardial dysfunction or multiple organ failure (31,32). There is strong evidence suggests that NHE1, a predominant isoform in neutrophils, regulates inflammatory processes (33,34). NHE1 inhibitors have been shown to inhibit neutrophil accumulation, chemokine production, and NF-κB activation; to attenuate leukocyte-endothelial cell interactions; and to improve endothelial dysfunction induced by ischemic–reperfusion (11,35,36). In the present study, prolonged asphyxial cardiac arrest and resuscitation resulting in a significant increase of accumulation of neutrophils and cytokine in lung tissues. Sabiporide treatment attenuated accumulation of neutrophils and reduced cytokine production in the lung. Furthermore, the combined use of sabiporide and sodium bicarbonate had more profound reduction in IL-6 and IL-10, compared to sabiporide alone. The collective effects of sabiporide in improving cardiovascular and metabolic performance result in improved resuscitation outcomes, reflecting from decreased markers for multiorgan dysfunction, including cardiac troponin-I, ALT, AST, and urea.

It should be noted that IL-10 is a cytokine with anti-inflammatory properties. IL-10 increase is associated with inflammatory states (37). IL-10 induction involves ERK1/2, p38, and NFkB signaling and transcriptional activation via promoter binding of the transcription factors NFkB and AP-1 (37). NHE1 inhibitors have been shown to inhibit ERK1/2, p38, and NFkB signaling, and inhibit neutrophil activation (10,11,33–36). Therefore, it can suggest that the lower levels of IL-10, TNF-α, and IL-6 in sabiporide-treated groups reflect from the lower inflammatory response, compared to vehicle controls. However, we cannot rule out that the possibility that sabiporid-induced reduction in IL-10 may be deleterious. It should also be noted that VF-induced cardiac arrest is uncommon in children (2), while VF was present in asphyxia-induced cardiac arrest in the present study.

In conclusion, this study shows that NHE1 inhibitor as adjunct therapy to acid–base correction with sodium bicarbonate may provide a novel approach to improve resuscitation outcome from prolonged asphyxia-induced cardiac arrest by improving cardiovascular and metabolic function, improving blood flows to vital organs, and attenuating proinflammatory responses.

METHODS

Animal Preparation

All animal studies were approved by the Institutional Animal Care and Use Committee and were in compliance with the US Animal Welfare Act. Animals were housed at a freestanding animal care facility on the campus of Mount Sinai Medical Center. Forty-seven Yorkshire piglets (14.3 ± 4.1 kg) of either gender were used in the present study. The animals were initially anesthetized with ketamine, 10 mg/kg, intramuscularly, and maintained in a surgical plane of anesthesia with intravenous propofol. Ventilation was provided on room air with a

volume-controlled ventilator (PB 7200 Ventilatory System; Puritan-Bennett, Carlsbad, CA) set to deliver a tidal volume of 10 ml/kg. The respiratory rate was adjusted to ensure a $Paco_2$ from 35 to 45 mmHg. The left external jugular vein was cannulated for the administration of fluids and drugs. After administration of heparin, 100 IU/kg, a catheter was inserted via the right carotid artery into the left ventricle for the measurement of left ventricular pressure, and for the injection of microspheres. Another catheter was placed into the abdominal aorta via femoral artery for reference blood sampling and for the measurement of arterial blood pressure. An 5.5 F balloon-tipped flow-directed thermodilution pulmonary arterial catheter (Abbot Laboratories, Chicago, IL) was inserted via the right jugular vein and floated into the pulmonary artery under direct pressure monitoring for measurements of pulmonary arterial pressure and cardiac output. A standard ECG 3-lead harness was placed on the chest to monitor ECG, and a pair of electrodes (Kendall-LTP, Chicopee, MA) were placed across the chest to produce defibrillation by connecting the electrodes to a LifePak 8 defibrillator (Physio-Control, Redmond, WA). Catheters were connected to pressure transducers (ADInstruments, Colorado Springs, CO). All blood pressures were continuously recorded with a Powerlab data acquisition system (ADInstruments). Arterial and mixed venous blood gases were measured at various intervals during the experiments using a blood gas analyzer (Rapidlab 855; Bayer Corporation, New York, NY). Body temperature was maintained between 37 and 39 °C.

Experimental Protocol

Following surgery, the animals were allowed to equilibrate for 30 min to achieve stable resting levels. The first colored microspheres were injected to determine the baseline regional blood flow. All animals were paralyzed with 0.1 mg/kg pancuronium bromide. Asphyxia was induced by endotracheal tube clamping. Cardiac arrest was defined as the point of loss of aortic pulsations (aortic pulse pressure is <5 mmHg, occurred at 8–11 min after endotracheal tube clamping). The animals remained untreated for 3 min, followed by cardiopulmonary resuscitation: ventilation, chest compression. The animals were intravenously given epinephrine (1:10,000) 0.1 ml/kg, and then followed by defibrillation attempts. Defibrillation attempts were given after 1–2 min of chest compression, and repeated up to 10 attempts. Thirty-four of 47 pigs had ROSC and were randomly assigned to four study groups. Staff technicians were blinded to the treatment (vehicle/sabiporide). Fifteen minutes after ROSC, the animals received a bolus infusion of: (i) vehicle (saline, $n = 9$); (ii) 3 mg/kg sabiporide (NHE1 inhibitor, $n = 9$), (iii) vehicle + $NaHCO_3$ ($n = 8$), and (iv) 3 mg/kg sabiporide + $NaHCO_3$ ($n = 8$). In our preliminary study, administration of sabiporide to control animals did not affect mean blood pressure, heart rate, and arterial blood gases in pigs (see **Supplementary Table S1** online). The blood pH and plasma HCO_3^- were closely monitored to ensure that their levels did not exceed the normal range. The target for both parameters were: a blood pH, between 7.35 and 7.40 and a plasma HCO_3^- between 22 and 25 m(mol/l)/l. Different colored microspheres were injected at 30 min and 4 h after ROSC. All animals were continuously monitored for 5 h after ROSC, and provided with maintenance intravenous fluids (Ringer's lactate, 10 ml/kg/h for the first hour, followed by 5 ml/kg/h for 2 h). All animals that had returned of spontaneous circulation survived the entire experimental protocol. At the end of 5 h resuscitation, the animals were humanely euthanized while still under anesthesia with 10 ml of sodium pentobarbital, a method that is consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association. The tissues were harvested for determining microsphere numbers.

Echocardiography

Echocardiography was performed with the use of a Hewlett-Packard echocardiographic system SONOS 2000 with a 3.5/2.7 MHz transducer and recorded on VCR tapes. In each animal, two-dimensional short-axis view was taken at midpapillary muscle level to obtain left ventricular ejection fraction. Linear dimensions were measured from two-dimensionally guided M-mode tracing. An ECG tracing was recorded simultaneously with the echocardiogram. The measurements were made after the recommendation of the American Society of Echocardiography (38). The measurements were made both on line and off line. All measurements were repeated three times, and the results were averaged. Researcher responsible for obtaining and processing the echocardiography data was blinded to group allocation. Echocardiographic data were obtained at: baseline, 30 min and 5 h of ROSC.

Biochemical Analysis

Enzyme immunoassay kits for porcine cardiac troponin I (Life Diagnostics, West Chester, PA), TNF-α, IL-6, and IL-10 (R & D Systems, Minneapolis, MN) were used to determine the concentrations of these mediators in the plasma or tissues. Plasma levels of ALT and AST (Biotron Diagnostics, Hemet, CA) and plasma levels of urea (Bioassay System, Hayward, CA) were determined by using assay kits according to the manufacturer's instructions. Neutrophil accumulation in the lung was measured by determining myeloperoxidase activity according to previously published methods (39).

Regional Blood Flow Measurement by Using Colored Microspheres

A bolus of 6×10^6 colored microspheres (Dye-Trak; Triton Technologies, San Diego, CA) suspended in 3 ml of a carrier solution of 0.9% NaCl and 0.01% Tween-80 (Sigma, St Louis, MO) was injected into the left ventricle. The reference blood sample was collected from the abdominal aorta by a withdrawal pump started 20 s prior to the injection of the microspheres at a withdrawal rate of 5 ml/min and continued for 2 min after the injection of microspheres. The blood sample was then centrifuged for 20 min at 2,500 rpm. The supernatant was discarded, and the sample was included in the tissue processing routine described below.

Microspheres extraction, purification, and quantification were performed according to the manufacturer's instructions and were described previously (24). Blood flow was calculated as the tissue-derived microsphere numbers to reference arterial blood–derived microsphere numbers times the reference arterial blood withdrawal rate normalized per gram of tissue weight.

Statistical Analysis

All data were reported as means ± SD. Hemodynamic, blood gas, and blood flow data were analyzed by one-way ANOVA and Bonferroni's postcomparison test for repeated measures using GraphPad InStat (GraphPad Software, San Diego, CA). For echocardiographic and biochemical assay data, group differences were evaluated with a one-way ANOVA, followed by Bonferroni's post-test analysis using GraphPad Prism 5 (GraphPad Software). P values < 0.05 were considered to be statistically significant.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

STATEMENT OF FINANCIAL SUPPORT

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