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BRIEF COMMUNICATION

Increased Survival Time With SS-31 After Prolonged Cardiac Arrest in Rats

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Background Cardiac arrest is one of the leading causes of death with a very high mortality rate. No therapeutic drug that can be administered during resuscitation has been reported. Mitochondrial dysfunction is believed to play an important role for the pathogenesis of cardiac arrest. SS-31, a tetra-peptide, has been shown to protect mitochondria from ischaemia/reperfusion injury. Therefore, we tested whether SS-31 improves rat survival after prolonged cardiac arrest.

Methods Rats were randomised into two groups. After 25 minutes of asphyxia-induced cardiac arrest, rats were resuscitated with or without SS-31 using cardiopulmonary bypass resuscitation. Rat survival was followed for additional 4.5 hours using haemodynamic monitoring. The blood gas was analysed for surviving rats at multiple time points.

Results and Conclusions After 5 hours, 5 of 10 rats survived in the SS-31 group whereas only 1 of 10 rats survived in the control group ($p = 0.026$). At 90 minutes after resuscitation, the blood lactate level in the SS-31 treated rats (4.29 ± 2.5 mmol/L) was significantly lower than in control rats (7.36 ± 3.1 mmol/L, $p = 0.026$), suggesting mitochondrial aerobic respiration was improved with SS-31 treatment. Overall, our data show the potential of SS-31 as a novel therapeutic in cardiac arrest.

Keywords Mitochondria-targeted therapeutics • Anaerobic metabolism • Haemodynamics • Resuscitation • Physiology

Introduction

Cardiac arrest (CA) is one of the leading causes of death worldwide [1]. However, the search for mechanism-driven drugs is limited. Moreover, drugs that can be tested during resuscitation have not been reported. Previous studies showed that mitochondria play important roles for the pathogenesis of CA [2]. Therefore, drugs directed at mitochondria may serve as novel therapeutics in CA. SS-31, a tetra-peptide, is a mitochondria targeting drug currently in clinical trials [3]. The protective effect of SS-31 has been demonstrated in various tissues using several animal models of ischaemia [4–6]. As well as its wide application, different protective mechanisms were proposed depending on disease models, such as scavenging ROS [7], inhibiting cytochrome c peroxidase activity [8], and inhibiting proinflammaotry cytokines [9]. Regardless of its protective mechanism, the wide spectrum of protective action makes SS-31 a promising therapeutic agent in CA, where multiple organs are affected.

Previously, we developed a rat model of asphyxial CA-cardiopulmonary bypass (CPB) resuscitation. Because asphyxia induces highly reproducible CA, it is the most commonly used

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CA model in small rodents [10]. Customised CPB resuscitation, which models extracorporeal membrane oxygenation, was used to resuscitate animals. Cardiopulmonary bypass is an emerging resuscitation method that multiple studies showed improved outcomes with CPB [11,12], providing support for its potential as a treatment for CA. For this study, compared to conventional chest compression resuscitation, blood flow aided by the CPB pump provides more consistent reperfusion and delivery of SS-31 to individual organs, allowing careful assessment of the therapeutic potential of SS-31 in CA. Using this established CA model, we test whether SS-31 is protective in CA. The data show that SS-31 significantly increases rat survival after 25 minutes prolonged CA, supporting the potential of SS-31 as a novel therapeutic in CA.

Materials and Methods

Asphyxial CA and CPB Resuscitation

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania (#804889). The detailed surgical procedures were provided in supplementary materials [13]. Male adult Sprague–Dawley rats were used. Cardiac arrest was induced by stopping the ventilator. After 25 minutes CA, resuscitation was started with the initiation of CPB flow and resumption of ventilation. Cardiopulmonary bypass resuscitation was continued for 30 minutes. SS-31, dissolved in 0.8% sodium bicarbonate, was administered in the CPB fluid during resuscitation and by IV infusion after resuscitation at 0.05 mg/kg/hour. Previous studies tested SS-31 using different dosages in various diseases [3] and 0.05 mg/kg/h is a commonly used dosage for continuous administration with consistent effectiveness [6,14]. Control rats were treated with 0.8% sodium bicarbonate. There was no difference in the time to reach CA between the two groups (188 sec for control and 179 sec for SS-31 groups). Survival was monitored using haemodynamic monitoring for 5 hours. Death was defined by mean arterial pressure (MAP) below 30 mmHg lasting for 3 minutes.

Haemodynamics and Blood Gas Analysis

Mean arterial pressure, heart rate, and pulse pressure was measured using haemodynamic monitoring. Blood samples (0.15 mL) were drawn from the left femoral catheter at designated times for blood gas analysis. Urine production was measured by measuring the volume of urine withdrawn by bladder puncture. The log-rank test was used to compare survival between the two groups. Data from haemodynamic monitoring and blood gas analyses were presented as mean ± standard deviation and the significance determined using student's t-test. A p value < 0.05 was considered statistically significant.

Results

In the control group, 7 of 10 rats died before 210 minutes after the initiation of resuscitation and only one rat survived for 5 hours (Figure 1). In the SS-31 group, all rats survived for a minimum of 3 hours post-CA, and half survived to the experimental endpoint of 5 hours ($p = 0.026$). This result shows that SS-31 significantly increased short-term survival of rats in this model. The average time to achieve return of spontaneous circulation (ROSC) was 463 seconds for the control group and 319 seconds ($p = 0.281$) for the SS-31 group, showing SS-31 has minial effect on achieving ROSC.

SS-31 treatment also decreased the blood level of lactate in surviving rats from 90 minutes after resuscitation (Table 1). Consistent with the decreased lactate level, pH was higher in rats in the SS-31 group. No significant difference was found in haemodynamic parameters (Table 1) or urine output (Table S1) between the two groups. These data suggest SS-31 treatment has no impact on heart function and kidney function at this early stage of recovery. In the SS-31 group, 7 of 10 surviving rats developed recurrent seizures, whereas only two rats in the control group (Table S1). These seizures were clearly detectable by abrupt increases in MAP accompanied with body movements. The average frequency of the seizures observed in surviving rats was ~30 times per hour. No rats in either group responded to stimuli, such as toe pinching or corneal irritation, showing these rats have limited brain function.

Discussion

This study focusses on the survival benefit of SS-31, the most important outcome in CA. The pathology in CA is complicated due to multi organ involvement. In addition to the pathological condition, the timing and quality of resuscitation is also important for survival of patients. Because of this complexity, we used asphyxial CA and CPB resuscitation to reduce potential variables in the ischaemic time, reperfusion efficiency, and drug delivery to each organ, by the consistent induction of asphyxial CA and blood flow of CPB resuscitation. Although involving elaborate surgical techniques and

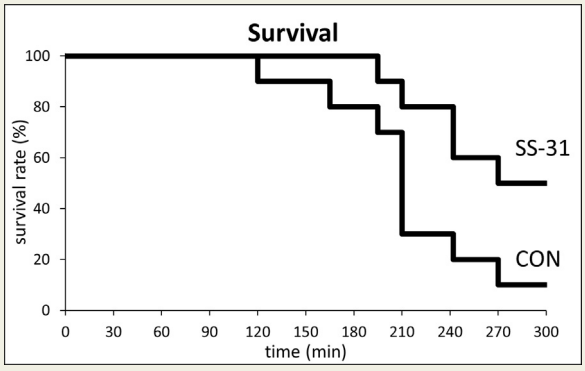

Figure 1 Kaplan–Meier survival curve following 25 min of CA and 30 min of CPB resuscitation. The rats treated with SS-31 have significantly higher survival rates than animals in the control group ($p = 0.026$).
Abbreviations: CA, cardiac arrest; CPB, cardiopulmonary bypass.

Table 1 Haemodynamic and blood gas analysis for surviving rats.

	baseline	after the beginning of resuscitation			
		30 min	90 min	150 min	210 min
Control Group					
number of rats	10	10	10	9	6
MAP[1] (mmHg)	78.9 ± 4.9	57.0 ± 29.8	67.8 ± 13.4	52.8 ± 18.2	34.8 ± 3.8
PP[2] (mmHg)	51.0 ± 6.8	29.3 ± 17.5	51.7 ± 9.5	47.8 ± 14.3	39.5 ± 13.1
HR[3] (bpm)	329 ± 29.1	282 ± 43.2	279 ± 71.2	289 ± 83.8	256 ± 46.4
Haematocrit (%)	38.8 ± 1.0	26.3 ± 1.5	28.6 ± 3.7*	29.6 ± 3.8	27.3 ± 3.3*
Lactate (mmol/L)	1.61 ± 0.4	10.46 ± 2.9	7.36 ± 3.1*	8.15 ± 2.7*	12.34 ± 3.8*
Glucose (mg/dL)	210 ± 51	165 ± 38	143 ± 50	153 ± 64	156 ± 64
pH[4]	7.44 ± 0.04	–	–	7.28 ± 0.12*	7.26 ± 0.05*
SS-31 Group					
number of rats	10	10	10	10	9
MAP (mmHg)	81.4 ± 1.8	51.5 ± 7.7	88.1 ± 28.0	58.8 ± 19.2	53.6 ± 19.4
PP (mmHg)	43.2 ± 8.6	22.4 ± 7.7	46.4 ± 17.4	42.4 ± 13.8	43.8 ± 10.3
HR (bpm)	316 ± 14.8	275 ± 19.7	273 ± 45.8	278 ± 61.0	291 ± 48.3
Haematocrit (%)	37.8 ± 1.4	27.2 ± 1.4	32.2 ± 2.4	32.2 ± 2.4	31.7 ± 10.3
Lactate (mmol/L)	1.69 ± 0.8	9.87 ± 1.5	4.29 ± 2.5	6.05 ± 2.7	7.79 ± 2.8
Glucose (mg/dL)	228 ± 41	194 ± 64	154 ± 62	144 ± 29	163 ± 45
pH	7.42 ± 0.03	–	–	7.42 ± 0.07	7.37 ± 0.07

Abbreviations: CA, cardiac arrest; CPB, cardiopulmonary bypass; MAP, mean arterial pressure, PP, pulse pressure; HR, heart rate; ROSC, return of spontaneous circulation.

[1]mean arterial pressure.

[2]pulse pressure.

[3]heart rate.

[4]sodium bicarbonate added in the resuscitation fluid interferes with blood pH at 30 and 90 min.

*$p < 0.03$.

prolonged preparation time, this model provides a compelling platform for this initial test. Here, we clearly showed that SS-31 increases rat survival after CA, when the pharmaceutical dosage is delivered in a clinically relevant manner, supporting the potential of SS-31 as a novel drug in CA.

Although this study was not designed to directly address the mechanism of the protective action of SS-31, our blood gas analysis data is supportive of the capacity for SS-31 to protect mitochondrial function against cardiac arrest mitochondrial injury and thus contribute to relatively improved survival. During normal conditions, cells primarily depend on mitochondrial oxidative phosphorylation for the production of ATP. However, during CA, the limited supply of oxygen causes a shift away from mitochondrial oxidative phosphorylation to anaerobic glycolysis, resulting in the accumulation of lactate [15]. Multiple pathways are responsible for lactate clearance and the decreased lactate level may not be a direct indication of improved mitochondrial activity. However, improved mitochondria function will restore the aerobic metabolism, decreasing the generation of lactate. Therefore, the decreased lactate level found in rats treated with SS-31 is consistent with the proposed mechanisms that SS-31 protects mitochondria from reperfusion injury [3,7].

A major limitation of the study remains that no markers of mitochondrial dysfunction or oxidative modification were assessed in this specific model. However, we previously showed CA time-dependent alterations in lipid metabolism and mitochondria respiration, particularly in the brain and heart using the same rat model [16,17]. Therefore, we need to follow how cardiac and brain mitochondria function is specifically affected by SS-31 treatment in this severe model of CA. Correlations between mitochondrial function and survival will provide insight into the role of mitochondria in CA and help to design more effective therapeutic drugs targeting mitochondria.

In conclusion, we have demonstrated that SS-31, adminstered from the beginning of resuscitation, significantly improves rat survival following 25 minutes of CA. This result requires further investigation of SS-31's underlying protective mechanisms as well as its effect on long-term survival.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.hlc.2018.01.008.

References

[1] Rivera NT, Kumar SL, Bhandari RK, Kumar SD. Disparities in survival with bystander CPR following cardiopulmonary arrest based on neighborhood characteristics. Emerg Med Int 2016;2016:6983750.
[2] Kim J, Perales Villarroel JP, Zhang W, Yin T, Shinozaki K, Hong A, et al. The responses of tissues from the brain, heart, kidney, and liver to resuscitation following prolonged cardiac arrest by examining mitochondrial respiration in rats. Oxid Med Cell Longev 2016;2016:7463407.
[3] Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. Br J Pharmacol 2014;171:2029–50.
[4] Wu J, Zhang M, Hao S, Jia M, Ji M, Qiu L, et al. Mitochondria-targeted peptide reverses mitochondrial dysfunction and cognitive deficits in sepsis-associated encephalopathy. Mol Neurobiol 2015;52:783–91.
[5] Eirin A, Ebrahimi B, Zhang X, Zhu XY, Woollard JR, He Q, et al. Mitochondrial protection restores renal function in swine atherosclerotic renovascular disease. Cardiovasc Res 2014;103:461–72.
[6] Kloner RA, Hale SL, Dai W, Gorman RC, Shuto T, Koomalsingh KJ, et al. Reduction of ischemia/reperfusion injury with bendavia, a mitochondria-targeting cytoprotective Peptide. J Am Heart Assoc 2012;1:e001644.
[7] Brown DA, Hale SL, Baines CP, del Rio CL, Hamlin RL, Yueyama Y, et al. Reduction of early reperfusion injury with the mitochondria-targeting peptide bendavia. J Cardiovasc Pharmacol Ther 2014;19:121–32.
[8] Birk AV, Liu S, Soong Y, Mills W, Singh P, Warren JD, et al. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. J Am Soc Nephrol 2013;24:1250–61.
[9] Li G, Wu J, Li R, Yuan D, Fan Y, Yang J, et al. Protective effects of antioxidant peptide SS-31 against multiple organ dysfunctions during endotoxemia. Inflammation 2016;39:54–64.
[10] Vognsen M, Fabian-Jessing BK, Secher N, Lofgren B, Dezfulian C, Andersen LW, et al. Contemporary animal models of cardiac arrest: a systematic review. Resuscitation 2017;113:115–23.
[11] Stub D, Bernard S, Pellegrino V, Smith K, Walker T, Sheldrake J, et al. Refractory cardiac arrest treated with mechanical CPR, hypothermia, ECMO and early reperfusion (the CHEER trial). Resuscitation 2015;86:88–94.
[12] Yannopoulos D, Bartos JA, Martin C, Raveendran G, Missov E, Conterato M, et al. Minnesota resuscitation consortium's advanced perfusion and reperfusion cardiac life support strategy for out-of-hospital refractory ventricular fibrillation. J Am Heart Assoc 2016;5.
[13] Kim J, Yin T, Shinozaki K, Lampe JW, Becker LB. DHA-supplemented diet increases the survival of rats following asphyxia-induced cardiac arrest and cardiopulmonary bypass resuscitation. Sci Rep 2016;6:36545.
[14] Eirin A, Li Z, Zhang X, Krier JD, Woollard JR, Zhu XY, et al. A mitochondrial permeability transition pore inhibitor improves renal outcomes after revascularization in experimental atherosclerotic renal artery stenosis. Hypertension 2012;60:1242–9.
[15] Wang CH, Huang CH, Chang WT, Tsai MS, Yu PH, Wu YW, et al. Monitoring of serum lactate level during cardiopulmonary resuscitation in adult in-hospital cardiac arrest. Crit Care 2015;19:344.
[16] Kim J, Lampe JW, Yin T, Shinozaki K, Becker LB. Phospholipid alterations in the brain and heart in a rat model of asphyxia-induced cardiac arrest and cardiopulmonary bypass resuscitation. Mol Cell Biochem 2015;408:273–81.
[17] Kim J, Villarroel JP, Zhang W, Yin T, Shinozaki K, Hong A, et al. The responses of tissues from the brain, heart, kidney, and liver to resuscitation following prolonged cardiac arrest by examining mitochondrial respiration in rats. Oxid Med Cell Longev 2016;2016:7463407.