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Sedative depth on neurological outcomes in a juvenile rat model of cardiopulmonary resuscitation

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A B S T R A C T

The guidelines for cardiopulmonary resuscitation (CPR) in pediatric advanced life support suggest that midazolam is the preferred agent for sedation in patients with mild hypothermia, whereas children with cardiac arrest (CA) are at a crucial stage regarding their immature nervous system. Studies have shown that midazolam may have a detrimental effect on the developmental of the pediatric nervous system. Our previous study found that midazolam induced neuronal damage after CPR in young rats. It is speculated that: midazolam causes the potential injury of neurons by inhibiting mitochondrial autophagy expression and is an important factor for the poor prognosis in children after successful CPR. This project intends to adopt the modified asphyxiant CPR model in juvenile rats. Survival rate, neurological function and histopathological changes were evaluated to determine the protective effects of appropriate sedation depth on cerebral ischemia-reperfusion injury in juvenile rats after CPR. Combined with cell biology and molecular biology related technologies, the mechanism by which the mitochondrial pinkl-parkin signaling pathway induces autophagy to inhibit neuronal apoptosis may be key factor in the protective effects of sedation depth on the brain. The aim of this study is to provide experimental evidence and elucidate the mechanisms of improvement of cerebral ischemia-reperfusion injury by sedation depth in children after successful CPR and to lay a theoretical and experimental basis for clinical treatment.

Introduction

In past few decades, although cardiopulmonary resuscitation (CPR) performance after cardiac arrest (CA) in children has improved, the survival rate of post-CA patients with good neurological outcomes still pose a challenge. Post-return of spontaneous circulation (ROSC) in-hospital mortality rates in children after non-traumatic out-of-hospital cardiac arrest (OHCA) or in-hospital cardiac arrest (INHCA) were estimated to be 70% [1] and 55% [2–4], respectively. It is well known that therapeutic hypothermia (TH) is an effective treatment to decrease mortality after successful CPR, and the only approach confirmed to improve post-cardiopulmonary resuscitation neurological outcomes [5]. The guidelines for cardiopulmonary resuscitation (CPR) suggest that during TH, for shiver and pain prevention, sedative agents (midazolam) and analgesia agents (fentanyl) are preferred choices [6].

However, studies have shown also that midazolam may have a harmful effect on the developmental nervous system, when children with CA are at a crucial stage regarding their immature nervous system [7]. Studies have shown that anesthetic administration to neonatal rodents is associated with long-term impairments in cognitive function with impaired learning and memory later in life [7].Numerous animal studies have shown that general anesthetics have neurodegenerative effects on the developing brain and cause anesthesia-induced developmental neurotoxicity (AIDN) [8]. Thus, an important question is raised: whether sedative agents such as (midazolam) and analgesia agents (fentanyl) damage the brain cell further, leading to a decreased survival rate or to increased neurological deficits in the pediatric population after successful CPR?

In addition, mild hypothermia alters the pharmacokinetics of fentanyl and midazolam and reduces the systemic clearance of agents in rats after the initial success of CPR [9]. For CA patients who have had a CA undergoing TH, midazolam may be overdosed, accompanied with delaying in awakening, prolonging the duration of mechanical ventilation and other subsequent complications [4]. Therefore, accurate assessment of the sedative depth induced by sedative agents may be a critical step for CA pediatric patients during TH to increase the survival rate. An objective assessment of sedation depth could increase patients comfort by without jeopardizing his/her safety [10]. In the operate room, hypnotic titration to a bispectral index score (BIS) 40–60 is associated with a reduced anesthetic dose and faster recovery time from anesthesia in adults and children [11]. Furthermore, the mitochondrial pinkl-parkin signaling pathway induces autophagy and thereby inhibits

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neuronal apoptosis which is the most important responsible factor regarding the protective effects on heart ischemia-reperfusion injury [12].

In our previous study (unpublished), midazolam induced neuronal damage after cardiopulmonary resuscitation in young rats. The animal survival rate at 3 days after ROSC was not significant difference between midazolam and control groups. The number of TUNEL-positive cells in the hippocampal CA-1 region was significantly higher in the midazolam group compared with the control group ($P = 0.041$). Furthermore, the number of TUNEL-positive cells was signifcantly lower in the control group compared with the midazolam group ($P < 0.05$).

Hypothesis. *From the above data, we hypothesize that midazolam would damage the brain cells further, leading to a decreased survival rate or increased neurological deficits after an initially successful CPR, and the accurate assessment of sedative depth during TH would reduce the detrimental effects of midazolam on brain cells, improving the survival rates of juvenile rats with good neurological outcomes. Combined with cell biology and molecular biology related technologies, the mechanism by which the mitochondrial pinkl-parkin signaling pathway induces autophagy and thereby inhibits neuronal apoptosis is the crucial factor in the protective effects of sedation depth on the brain.*

Rationale for the hypothesis

For healthy children, previous studies have shown in previous studies that peak vulnerability to the apoptogenic action of N-methyl-D-aspartate antagonists and γ-aminobutyric acid mimetic occurs during the synaptogenesis period, also known as the brain growth-spurt period [7]. This is a readily recognized period during which the brain grows at an accelerated rate when newly differentiated neurons throughout the brain are rapidly expanding their dendritic arbors to provide the required surface area to accommodate new synaptic connections [13]. Furthermore, the cerebral pathophysiology of children with post-ROSC ischemic/reperfusion injury is more complicated than one of healthy children [14]. The primary injury of the brain being devoid of nutrient stores leads to consequently neuroglycopenia and a metabolic crisis occurring within minutes after CA [14], leading to cell death. The secondary injury is the additive cerebral injury characterized by an imbalance in post CPR cerebral oxygen delivery and use, ultimately culminating in neuronal death [15]. Finally, after ROSC, microcirculatory perturbations lead to further neuronal dysfunction. For children with post-ROSC syndrome, the status is very critical and the neural cells are more vulnerable. From the aspect of cerebral protection for children after CA, the depth of anesthesia should be regulated and monitored precisely.

During TH, midazolam and fentanyl have been recommended as sedatives and analgesia agents for mechanical ventilation [4]. However, for patients who area post-CPR and are undergoing mild TH, these agents may possibly be overdosed because of altered pharmacokinetics [16]. It would be more beneficial and easy to control for patients who are post-CA if analgesic and sedative drugs are used that have short half-life. Therefore, precisely regulating the concentration of midazolam during TH might be the key step to reducing the detrimental effects on the brain effort of children after initial successful CPR. The Awakening and Breathing Controlled ~~Trail~~ (Trial) demonstrated that the assessment of anesthetic depth could decrease the dose of sedatives agents (50%) [17–18]. Intensive care unit (ICU) severe brain injury outcomes are associated with noninvasive BIS and the BIS on admission to the ICU can be used to predict the post resuscitative outcome of patients with OHCA [19].Patients with acute respiratory distress syndrome without ICU recall had a greater magnitude of cognitive impairments at hospital discharge [20]. For health children in the operating room, monitoring the depth of anesthesia was important to reduce the rate of complications [21]. From the aspect of pharmacokinetic and pharmacodynamics of sedate agency under TH, monitoring the depth of anesthesia was not only necessary, but it is also imperative to improve the neurological outcome of children after initial success of CPR.

For children, midazolam could induce the brain injury. There is potential for ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain [22].Jevtovic-Todorovic et al. demonstrated that a one time exposure to a common anesthetic cocktail of isoflurane, midazolam, and nitrous oxide, also caused neuroapoptosis in the developing rat brain during the period of synaptogenesis [13]. In addition, the mitochondrial pinkl-parkin signaling pathway induces autophagy and thereby inhibits neuronal apoptosis, which maybe the key factor to the protective effects on the heart ischemia-reperfusion injury.

From the information discussed above data, brain cells damaged by sedative agents (midazolam) will lead to a decreased survival rate in pediatric populations who have initially survived CPR. In addition, an objective assessment of sedation depth could increase patients comfort by without jeopardizing safety. Therefore, we propose that during TH, an accurate assessment of the sedative depth is encouraged method to improve neurological outcomes in the pediatric population to adjust the autophagy induced the mitochondrial pinkl-parkin signaling pathway.

Evaluation of the hypothesis

In the situation of experimental laboratory settings, this hypothesis can be investiagated. A prospective, randomized and controlled trial on infant rat models of CPR was performed.

(1) the Sedative Depth on Brain Protection after CPR

The CPR method of rat model was performed as described by Zhang et al4. The present study was approved by the Institutional Animal Care and Use Committee of Sichuan University. All animals were maintained in compliance with the Guide for the Care and Use of Laboratory Animal. Briefly, after injection with pentobarbital, for six minutes of asphyxia-induced CA in an infant rat, followed effective CPR, followed with mechanical ventilation with 100% oxygen (80 breaths/min, tidal volume 10 ml/kg), applying sternal compression (with two fingers) at a rate of 200 times/min and CPR was stopped until ROSC (MAP of 60 mmHg, and maintaining over 10 min). Giving epinephrine (0.02 mg/kg i.v.) was administered and repeated it every 3 min if necessary. The rectal temperature in rats would be adjusted with a lamp, a fan or ice bag.

After ROSC, rats will be divided randomly into five groups: S group (sham-operate group), CPR group (infusion with saline 4 ml/kg for 30 min), (midazolam) group (0.15 mg/kg/h midazolam and 1.5 μg/kg/h fentynal were infused for 6 h), TH group (TH will be maintained at 33 ± 0.5 ˚C with midazolam 0.15 mg/kg/h and fentynal 1.5 ug/kg/h for 6 h) and Narcotrend group (TH will be maintained at 33 ± 0.5 ˚C, the arrange of sedation with (midazolam) and (fentynal) was adjusted by Narcotrend electroencephalogram monitor for 6 h). Survival rate, behavioral evaluations, and histopathologic analysis (Nissl staining of neurons) in each group was evaluated on day seven after CA.

In the present study, the accurate assessment of sedative depth during TH was undertaken by Narcotrend EEG monitor (MonitorTechnik, Bad Bramstedt, Germany) [23]. The Narcotrend EGG monitor uses a classification, based on six EEG stages raging from A(awake) to F(very deep hypnosis), with 15 substages. The Narcotrend algorithm integrates these stages to a numerical scale from 100 to 0, assigning a defined range of index values to each EEG stage A through F (A、B = 0-2、C = 0-2、D = 0-2、E = 0-2、F = 0–1).

According the 2013 American Peripheral Artery Disease guidelines, the principle of early treatment for patients with severe craniocerebral injury should be maintained with deep sedation. Therefore, the sedative depth of the Narcotrend Index in this study should be set at C routine sedation: C0-C2 (79–85).

In this part of study, one would find that an accurate assessment of the sedative depth should closely related to improve brain function after CPR.

(2) the Mechanism of Sedative Depth on brain protection by Mitochondrial autophagy induced pinkl-parkin signaling pathway

Seventy-two wild-type C57BL/6 mice and 72 Parkin gene knockout juvenile C57BL/6 mice were randomly divided into four groups: sham-operated wild-type (SWT), sedative depth adjust model wild-type (SDA), sham-operated knockout (SKO) and sedative depth adjust model knockout. The SDA model was created by the same way with first part of this study. Immunohistochemical staining was performed to measure the expression of cell apoptosis protein cleaved-caspase-3 (CC3) in brain tissues. The terminal deoxynucleotidyl transferase dUTP Nick-End Labeling method was applied to measure cell apoptosis. The immunofluorescence staining and Western blot were utilized to measure the expression of cell autophagy protein LC3、Pink1、Parkin、Mfn1、Mfn2、Nix、Beclin-1 and P62.

Consequences and discussion of hypothesis

Mild TH is a helpful therapeutic modality to decrease myocardial damage and provide protection for the brain after CA. In the present study, if sedative agents (midazolam) and analgesia agents (fentanyl) during TH were confirmed to have additional detrimental effects on the brain cells, leading to a decrease in the survival rate after initially successful CPR, and data demonstrating that the accurate assessment of anesthesia depth during TH would improve the the survival rates of patients after CA with good neurological outcomes in neonatal rats was established, that would put forward an innovative therapeutic concept for pediatric patients after initially successful CPR: adjusting the depth of sedation would be a key factor in increasing the survival rate.

The electroencephalographic Narcotrend Index (NI) and BIS are able to distinguish EEGs from the awake state or near awake state and EEG stages of deep hypnosis, but BIS poorly differentiated between moderate and deep levels of sedation in all age groups in a combined secondary analysis of four independent studies evaluating age- and sedative agent-related differences in BIS in a large sample of children younger than 18 yr [23]. Many studies have shown the usefulness of BIS in monitoring depth of anesthesia. However, data have been limited and conflicting regarding the use of BIS in infants [24].The BIS algorithm cannot automatically be extrapolated to young children [25]. Some investigations suggest that BIS may be valid in children older than 2 yr of age [26].On the other hand, the Narcotrend is an EEG monitor designed to measure the effects of anesthetics and sedatives on the brain in terms of 'depth of hypnosis'[27].The performance of the Narcotrend monitor as a tool for monitoring depth of anesthesia continues to be evaluated both in adults [28,29] and in children [30,31]. During propofol induction in children, the Narcotrend electroencephalogram monitor was capable of following changes in the sedation level of children to some extent and the Narcotrend index indicates age-related changes during propofol induction in children [32].The Narcotrend Index would help to titrate sedative medication during diagnostic and therapeutic procedures in children and may be an objective non-disruptive tool for assessment of hypnotic depth in children under propofol-induced sedation procedures.

According to guidelines for the management of severe traumatic brain injury, in infants, children, and adolescents, moderate or deep levels of sedation should be maintained [33]. High-dose barbiturate therapy may be considered in hemodynamically stable patients with refractory intracranial hypertension despite maximal medical and surgical management [34]. The intent of their use is to prevent secondary brain injury by facilitating and optimising ventilation, reducing cerebral metabolic rate and reducing intracranial pressure [34]. Therefore, in the present study, the index values of Narcotrend EEG monitor was set as moderate or deep levels of sedation:C0-2 (79–85). According to the sedation, the depth of anesthesia was adjusted during TH. In the present study, pentobarbital was used, for the protective effect of pentobarbital on cerebral ischemia seems to be correlated to its action of preventing a decrease in the [3H]IP3 binding sites in ischemic brain [35].

If this hypothesis was established to improve neurological function by our experiment, three significant results could be achieved. First, to our knowledge, this is the first report of the relationship between sedative agents and the survival rate during TH for CA, and the sedative depth during TH on a CA animal model, especially in juvenile model. Secondly, if midazolam is suggested to have an additional detrimental effect on the brain cells, leading to a decreased survival rate after CA, it would establish an innovative therapeutic concept for pediatric patient who suffered from CA. Finally, if accurate assessment of anesthesia depth during TH would improve the the survival rates of CA with good neurological outcomes in infant rats, it would be important evidence for establishing a more effective therapeutic strategy for post-CA bran injury and would supply experimental data for the use of sedative and analgesia agents during TH for pediatric patient who have suffered from CA in the ICU.

Our hypothesis shows an innovative method to surviving pediatric CA through the relationship between sedative depth and the survival rate, and through the precise adjustment of sedative depth during TH. It also demonstrates how to improve the survival rates of CA in infant rats and, provides the promising therapeutic strategies for post-CA brain injury.

The authors have no potential conflicts of interest to disclose.

References

[1] Atkins DL, Everson-Stewart S, Sears GK, et al. Epidemiology and Outcomes From Out-of-Hospital Cardiac Arrest in Children: The Resuscitation Outcomes Consortium Epistry-Cardiac Arrest. Circulation 2009;119:1484–91.

[2] Andersen LW, Raymond TT, Berg RA, et al. Association Between Tracheal Intubation During Pediatric In-Hospital Cardiac Arrest and Survival. JAMA 2016;316:1786–97.

[3] Laver S, Farrow C, Turner D, Nolan J. Mode of death after admission to an intensive care unit following cardiac arrest. Intensive Care Med. 2004;30:2126–8.

[4] Zhang YJ, Wu MJ, Li Y, Yu H. Cardiocerebral protection by emulsified isoflurane during cardiopulmonary resuscitation. Med Hypotheses. 2015;84:20–4.

[5] Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med. 2002;346:549–56.

[6] Niermeyer S, Kattwinkel J, Van Reempts P,et al. International Guidelines for Neonatal Resuscitation: An excerpt from the Guidelines 2000 for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care: International Consensus on Science. Contributors and Reviewers for the Neonatal Resuscitation Guidelines. Pediatrics. 2000;106:E29.

[7] Sanders RD, Davidson A. Anesthetic-induced neurotoxicity of the neonate: time for clinical guidelines?Paediatr. Anaesth. 2009;19:1141–6.

[8] Madathil RJ, Hira RS, Stoeckl M, Sterz F, Elrod JB, Nichol G. Ischemia reperfusion injury as a modifiable therapeutic target for cardioprotection or neuroprotection in patients undergoing cardiopulmonary resuscitation. Resuscitation. 2016;105:85–91.

[9] Empey PE, Miller TM, Philbrick AH, Melick JA, Kochanek PM, Poloyac SM. Mild hypothermia decreases fentanyl and midazolam steady-state clearance in a rat model of cardiac arrest. Crit Care Med. 2012;40:1221–8.

[10] Agrawal D, Feldman HA, Krauss B. Waltzman ML. Bispectral index monitoring quantifies depth of sedation during emergency department rocedural sedation and analgesia in children. Ann Emerg Med. 2004;43:247–55.

[11] Zhang YJ, Wu MJ, Yu H, Liu J. Emulsified isoflurane postconditioning improves survival and neurological outcomes in a rat model of cardiac arrest.Exp Ther. Med. 2017;14:65–72.

[12] Sadoshima J, Kubli DA, Cortez MQ, et al. PINK1 is dispensable for mitochondrial recruitment of parkin and activation of mitophagy in cardiac myocytes. PLoSOne 2015;10:e0130707.

[13] Jevtovic-Todorovic V, Hartman RE, Izumi Y, et al. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci. 2003;23:876–82.

[14] Wagner SR, Lanier WL. Metabolism of glucose, glycogen, and high-energy phosphates during complete cerebral ischemia: a comparison of normoglycemic, chronically hyperglycemic diabetic, and acutely hyperglycemic nondiabetic rats. Anesthesiology 1994;81:1516–26.

[15] Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A Scientific Statement from the

International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Stroke. Resuscitation. 2008;79:350–79.
[16] Wu MJ, Zhang YJ, Yu H, Liu B. Emulsified isoflurane combined with therapeutic hypothermia improves survival and neurological outcomes in a rat model of cardiac arrest.Exp Ther. Med. 2017;13:891–8.
[17] Jackson James C, Timothy D, Long-term, et al. Cognitive and Psychological Outcomes in the Awakening and Breathing Controlled Trial. Am J Respir Crit Care Med. 2010;182:183–91.
[18] Dunham CM, Ransom KJ, McAuley CE, et al. Severe brain injury ICU outcomes are associated with Cranial-Arterial Pressure Index and noninvasive Bispectral Index and transcranial oxygen saturation: a prospective, preliminary study. Crit Care. 2006;10:R159.
[19] Larson MJ, Weaver LK, Hopkins RO. Cognitive sequelae in acute respiratory distress syndrome patients with and without recall of the intensive care unit. J Int Neuropsychol Soc. 2007;13:595–605.
[20] Musialowicz T, Mervaala E, Kälviäinen R, Uusaro A, Ruokonen E, Parviainen I. Can BIS monitoring be used to assess the depth of propofol anesthesia in the treatment of refractory status epilepticus? Epilepsia. 2010;51:1580–6.
[21] Young C, Jevtovic-Todorovic V, Qin YQ, et al. Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain. Br J Pharmacol. 2005;146:189–97.
[22] Chiari PC, Pagel PS, Tanaka K. Intravenous emulsified halogenated anesthetics produce acute and delayed preconditioning against myocardial infarction in rabbits. Anesthesiology 2004;101:1160–6.
[23] McCann ME, Bacsik J, Davidson A, Auble S, Sullivan L, Laussen P. The correlation of bispectral index with endtidal sevoflurane concentration and haemodynamic parameters in preschoolers. Paediatr Anaesth. 2002;12:519–1253.
[24] Degoute CS, Macabeo C, Dubreuil C, Duclaux R, Banssillon V. EEG bispectral index and hypnotic component of anaesthesia induced by sevoflurane: comparison between children and adults. Br J Anaesth. 2001;86:209–12.
[25] Davidson AJ, McCann ME, Devavaram P, et al. The differences in the bispectral index between infants and children during emergence from anesthesia after circumcision surgery. Anesth Analg. 2001;93:326–30.
[26] Bannister CF, Brosius KK, Sigl JC, Meyer BJ, Sebel PS. The effect of bispectral index monitoring on anesthetic use and recovery in children anesthetized with sevoflurane in nitrous oxide. Anesth Analg. 2001;92:877–81.
[27] Kreuer S, Bruhn J, Larsen R, et al. Application of Bispectral Index and Narcotrend index to the measurement of the electroencephalographic effects of isoflurane with and without burst suppression. Anesthesiology 2004;101:847–54.
[28] Schmidt GN, Bischoff P, Standl T, et al. Narcotrend, bispectral index, and classical electroencephalogram variables during emergence from propofol ? remifentanil anesthesia. Anesth Analg. 2002;95:1324–30.
[29] Schmidt GN, Bischoff P, Standl T, et al. Narcotrend and bispectral index monitor are superior to classic electroencephalographic parameters for the assessment of anesthetic states during propofol–remifentanil anesthesia. Anesthesiology 2003;99:1072–7.
[30] Weber F, Pohl F, Hollnberger H, et al. Impact of the Narcotrend Index on propofol consumption and emergence times during total intravenous anaesthesia with propofol and remifentanil in children: a clinical utility study. Eur J Anaesthesiol. 2005;22:741–7.
[31] Weber F, Hollnberger H, Gruber M, et al. The correlation of the Narcotrend Index with endtidal sevolurane concentrations and hemodynamic parameters in children. Pediatr Anesth. 2005;15:727–32.
[32] Weber F, Gruber M, Taeger K. The correlation of the Narcotrend Index and classical electroencephalographic parameters with end-tidal desfurane concentrations and hemodynamic parameters in different age groups. Pediatr Anesth 2005;15:378–84.
[33] Kochanek PM, Carney N, Adelson PD, et al. Guidelines for the acute medical management of severe traumatic brain injury in infants, children, and adolescents-second edition. Pediatr Crit Care Med. 2012;13(Suppl 1):S1–82.
[34] Oddo M, Crippa IA, Mehta S, et al. Optimizing sedation in patients with acute brain injury. Crit Care. 2016;20:128.
[35] Araki T, Kato H, Kogure K. Alteration of second messenger systems after transient cerebral ischemia in gerbils: protective effect of pentobarbital and an autoradiographic analysis. Neurosci Lett. 1991;130:57–60.