The effect of oxygenation by continuous low-pressure oxygen flow through thyrocricocentesis cannulation on asphyxia-induced injury in canine.

Fengyi Huang
Fujian Provincial Hospital
Feng Chen
Fujian Provincial Hospital
Xiaochun Zheng (zhengxiaochun7766@163.com)
Fujian Provincial Hospital https://orcid.org/0000-0002-6213-0789
Fei Gao
Fujian Provincial Hospital

Research article

Keywords: Continuous low-pressure oxygen; venous catheter needle; cricothyroid membrane; continuous oxygenton; PaO2; brain; heart; prognosis

Posted Date: February 26th, 2019

DOI: https://doi.org/10.21203/rs.2.408/v1

Abstract

Background To observe the oxygenation efficacy using a continuous low-pressure oxygen flow through thyrocricoid emergency airway by venous catheter needle in canine model of asphyxia. Methods Eighteen healthy male canine were randomly assigned to three group A (control), group B (continuous low-pressure oxygen flow insufflation), and group C (continuous low-pressure oxygen flow insufflation with chest compressions). After thyrocricocentesis cannulation and intubation achieved, ventilation was stopped for 40 minutes to establish asphyxia model. Electrocardiogram (ECG), arterial blood pressure (ABP), oxygen saturation (SpO2), heart rate (HR), arterial blood gas analysis was recorded in duration. Concentration of S100β protein, cardiac troponin I (cTnI), creatine kinase isoenzyme (CK-MB) and moisture in brain tissue were measured at before asphyxia, 2h and 4h after resuscitation. Results Compared with the control group, the pace of decline in SpO2, PaO2, pH value and the raise of PaCO2, LAC were slower in the other two groups. Similarly, a delay in increase of concentration of S100β, cTnI was observed, moreover, cerebral edema in group B and group C was significantly alleviated. Compared with Group B, the level of serum S100β, cTnI and brain tissue moisture content was lower in Group C ($p<0.05$). Conclusions The implication of continuous low-pressure oxygen insufflation through venous catheter needle after thyrocricoid puncture with assisted chest compression can ameliorate oxygenation in vivo, slow the increasing of PaCO2 and reduce the accumulation of H+. Consequently, it could attenuate the injury of vital organs, and finally improve prognosis. So it can be a promising approach for rescuing patients with asphyxia in clinic.

Background

As in the practice of clinical anesthesia, first aid and resuscitation, in case of failure in exposure by laryngoscope, failure or difficult in mask ventilation, which is known as difficult airway. It is a critical incident that clinicians may be confronted with in daily work. Once difficult airway occurred in clinic, it was often desperate and improper treatment can lead to asphyxia in few minutes, even cardiac arrest (1). It is reported that the incidence of difficult airway may be from 1% to 4% during the administration of anesthesia (2). Due to the low incidence, it is difficult for anesthesiologists to have the opportunity to accumulate enough experience to handle it.

At present, the best solution to difficult airway is fiberoptic bronchoscope, however, because of its high price, it is unusual to popularize, especially in the primary hospital. In case of rescuing the unpredictable difficult airway, time is the most important factor. However, tracheal incision and the cricothyroid membrane incision often waste a lot of time for specialized medical personnel and special surgical instruments. Thyrocricocentesis only takes a short period of time to set up emergency airway, so it plays an important role in the emergency case (3).

But the way for ventilation after successful thyrocricocentesis is hard to choose. The aim to find an effectual ventilation with the most simplest, safely, and effective approach in this emergency circumstance, we conveyed this study. In this study, we stopped ventilation to establish asphyxia model

in canine and then we evaluated the efficacy of oxygenation using continuous low-pressure oxygen flow through thyrocricoid emergency airway with venous catheter needle. Meanwhile we observed and analyzed the changes by hypercapnia in vital organs, such as heart and brain. The main contents were in follows.

Methods

This prospective, randomized, laboratory study was conducted with Ethical Approval of Fujian Medical University Institutional Animal Care Committee and the Fujian Provincial Hospital Animal Care and Use Committee. All animals received treatments in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals.

Animal preparation

The study was performed on 18 healthy adult male canine and weighing 10 to 15kg. All animals were provided by Animal Center of Fujian Medical University. Before operation, all animals fasted for 12h and were banned from drinking for 4h (4). The animals were placed in a suitable temperature and humidity and kept in a clean environment. Then they were randomized into five groups in a blinded manner with the sealed envelope indicating the animals assignment to group A (control group, *n*=6), group B (continuous low-pressure oxygen flow insufflations upon 40-min asphyxia), group C (LPOFI-CP2continuous low-pressure oxygen flow insufflations accompany by chest compression upon 40-min asphyxia).

Operation was performed as previously described (5-7). Sedation: Intramuscular injection of ketamine 10 mg/kg. Induction: propofol 1.0 mg/kg, sufentanyl 0.4 μg/kg and cistracurium 0.3mg/kg was injected through ear vein as our previous experiment. Fixing: Supine, fixed the limb, and pulled the tongue out to prevent suffocation. Monitoring: Femoral artery puncture measured arterial pressure (normal value: 180 + / 20/136 + / 15mm Hg), ecg (HR: 125 + / 20 BPM), respiration (23 + / 5bpm), SpO2 (sandwiched between the tongue), airway pressure (Paw). Intubation: The anesthetized animals were then intubated and placed on a ventilator with volume-controlled mode with a FiO_2 of 1.0. Maintenance: Continuous infusion of propofol (1.0 mg/kg per hour), sufentanyl (0.4 μg/kg per hour) and cistracurium (0.3mg/kg per hour). Thyrocricocentesis: a venous catheter needle was uesd and then stylet was withdrawn and then immobilize the catheter. The correct location of the catheter was confirmed by the airflow through the catheter. And after completion of the medical study, animals were euthanized by injection of potassium chloride in deep anesthesia with propofol.

Experimental protocol

After intubation, the animals were allowed to equilibrate for 30 minutes to achieve a stable level (the range of systolic blood pressure was 120 mmHg and the heart rate was 130 bpm), and the baseline data

were collected. In the control group, hypoxia was induced by extubation and stopping ventilation without any rescue measures; in group B, 40-min hypoxia was performed accompany by continuous 5L/min fresh oxygen insufflation through thyrocricocentesis cannulation with airway pressure lower than 35cmH_2O, and then the intubation and ventilation was continued; in group C, 40-min hypoxia were performed respectively accompany by insufflations and chest compression (20 times per minute)

Measurement

Arterial blood gas analysis

Arterial blood samples for gas analysis including SpO_2, PaO_2, $PaCO_2$, pH and lactic acid (LAC) were drawn prior to experiments for baseline control, 5, 10, 15, 20, 25, 30, 35, 40 minutes after hypoxia. Arterial blood gas analysis was performed by using automatic blood analysis device (GEM PREMIER3500).

S100-βprotein, cTNI, and CK-MB measurement

Blood samples for S100-βprotein、cTNI, and CK-MB measurement were drawn prior hypoxia and 2, 4 hours after ventilation resumption. S100-βprotein was measured by using enzyme-linked immuno sorbent assay (ELISA) (MR, USA), and chemiluminescent immunoassay (CLIA) was applied for detecting cTnI, CK-MB by using automatic chemiluminescence immunoassay analyzer (ADVIA Centaur CP, SIEMENS).

Determination of moisture ratio in brain tissue

After the animals were sacrificed at 4 hours after intubation in group B and C, several pieces of brain tissues, each of 1 cubic millimeters, were taken from two halves of the parietal cortex. Moisture ratio in brain tissue was determined by dry wet weight method. Gross specimens from both hemispheres, dry on the filter paper, and measured by Precision Electronic Balances of wet weight. Then put into drying oven at 110℃ for 24h to constant weight, and measured the dry weight, difference of two measurements was less than 0.5g.

Calculated as follows: (8)

Statistical analysis

Data analysis: statistical analysis was performed using SPSS 11.5 software, Measurement data were described as mean ± SD, One-way analysis of variance (ANOVA) or paired *t* test was used to determine differences over time within groups. $P<0.05$ was considered statistically significant.

Results

Comparisons of blood gases and lactate

Canines in control group were dead at about 15 minutes after hypoxia. Correspondingly, the SpO_2 was too low to be measured at 5 minutes after hypoxia in this group. The SpO_2 in group B and C decreased over

time. However, the SpO_2 in group B was lower at varying time points from 30 to 40 minutes after hypoxiathan that in group C (*p<0.05*). (Fig. 1A)

The PaO_2 decreased to 5.36 ± 2.10mmHg. By contrast, either in group B or group C, the fresh oxygen insufflation maintained the PaO_2 effectively after hypoxia. In group B, raised the PaO_2 continuously in a time-dependent manner, and similarly, the PaO_2 increased slightly after hypoxia and then remained at around normal range in group C. However, difference between two groups in statistic was significant (*p<0.05*). (Fig. 1B)

$PaCO_2$ in three groups increased upon varying time points. However, $PaCO_2$ in group C were statistical significant lower upon varying time points than that in group B (*p<0.05*). (Fig. 1C)

In three groups, pH value decreased in a time-dependent manner. However, pH value declined more mildly in both group B and C than that in control group (*p<0.05*). (Fig. 1D)

LAC increased sharply after hypoxia in group A, which reached 3.89 mM at 15 min after asyphsia. On the contrary ,the level of serum LAC slightly elevated after hypoxia carried out for 10 to 20 minutes in group B and C (*p<0.05*), and then slowly and constantly dropped to normal range at 25 min after hypoxia (*p□0.05*). (Fig. 1E)

Comparisons of serum S-100 β protein, cTnI and CK-MB

Canines in control group were dead at about 15 minutes after stopped ventilation, the data of serum S-100 β protein, cTnI and CK-MB in this group therefore were not measured. Serum S-100β protein and cTnI increased significantly in a time-dependent manner in both group B and C. The serum S100β and cTnI were significantly lower in group C than that in group B after 2, 4 hours after ventilation resumption (*p<0.05*), suggesting that continuous low-pressure oxygen insufflation with combined chest compression has superior therapeutic effect. Besides, level of CK-MB remained stable, and no statistically significant difference were observed (*p>0.05*). (Fig. 2A-C)

Moisture rate measurement of brain tissue

3 canines without any treatment were randomly selected to be sacrificed for moisture rate measurement of parietal cortex tissue, which taken as control. Moisture rate in brain tissues significantly raised after hypoxia in both groups (*p<0.05*), however, the moisture rate at same time point was significantly higher in group B than that in group C, suggesting that continuous low-pressure oxygen insufflation with combined chest compression has superior therapeutic effect, which difference was significant (*p<0.05*). (Fig. 3) And the data of histology of brain edema in each group was showed in Fig. 4(A-F).

Discussion

In this study, the major findings of this study are as follows. First, the implication of continuous low-pressure oxygen insufflation through venous catheter needle after thyrocricoid puncture with assisted chest compression can ameliorate oxygenation in vivo. Second, it could inhibit the increasing of $PaCO_2$ and reduce the accumulation of H^+. It is important that heart and brain can be protected from asphyxia and eventually improved prognosis. Together, these data identify a most simplest, safely, and effective approach for rescuing patients with asphyxia in clinic.

Difficult airway, which has a potential risk of being turned to “emergency airway” of which management has been closely related to the safety and quality of anesthesia. More than 30% of serious complications, the major cause of anesthesia-related morbidity and mortality, can be attributed to the improper airway management, which turns “difficult airway” to “emergency airway” and results in hypoxia and asphyxia (9). Emergency airway may occur when anesthesia induction, maintenance, extubation, recovery and postoperative stages, etc. with a variety of influential factors. Readily accessible emergency airway facilities, which maintaining adequate oxygenation while emergency airway happening, is therefore essential to buy time as effective artificial airway established.

As a minimal invasive and effective technique, administration of thyrocricocentesis by needle catheter can establish temporary airway in a short time, and usually the entire procedure can be accomplished within 60s (10). However, whether fresh oxygen insufflation through thyrocricocentesis cannulation provides sufficient oxygen supplement, and avoids asphyxia, is still unclear. Therefore, we investigated the impact of oxygen insufflation through thyrocricocentesis cannulation on blood gases, lactate of canine without effective ventilation. We further measured serum S100β protein, cTnI, CK-MB and moisture rate in brain tissue of subject in this study to demonstrate whether oxygen insufflation protects canine from damage of asphyxia.

Since oxygen exchange in alveolus follows the concentration diffusion theory, the alveolar-arterial oxygen difference is the primary factor to determine the direction of oxygen exchange. However, the amount of oxygen exchange is mostly influenced by the areas for oxygen exchange. Alveolus, the units for pulmonary gas exchange, have a total area of nearly 130 square meters (11). In this study, we provided positive airway pressure by using fresh oxygen insufflation through thyrocricocentesis cannulation, this positive pressure prevents alveolus collapse. And more important, the pressure gradient improves the oxygen absorption on diffusion areas when oxygen flow through the alveolus surface, promotes oxygen exchange to enhance oxygen content in serum, that increase the oxygen reserve and alleviate the hypoxia status. In this study, hypoxia canine received continuous low-pressure oxygen insufflation can mostly survive for more than 40 minutes, demonstrated that for urgent cases, oxygen insufflation through thyrocricocentesis cannulation is helpful for buying time when artificial airway is difficult to achieve.

However, since effective ventilation is not able to be achieved, such an approach will result in carbon dioxide accumulation. Chest compression suspects to reduces this accumulation by thoracic movement and subsequent passive pulmonary ventilation. Therefore, continuous oxygen insufflated in combined with chest compression is performed to investigate whether this method supplies more sufficient

oxygenation and reduces carbon dioxide accumulation. The physiological parameters of canine including SpO_2, PaO_2, $PaCO_2$, pH value and serum LAC were detected. In our study, we found that oxygen insufflation with or without chest compression raise the oxygen partial pressure in circulation. As for their effect, continuous low-pressure oxygen insufflation was more prominent to improve the oxygen partial pressure, while oxygenation in combined with chest compression more likely to keep oxygen partial pressure very close to normal status. These two methods can both slow down the increase of carbon-dioxidepartial pressure, and the disturbance of acid-base balance, in which, the effect of that with chest compression was particularly evident.

Hypoxia always results in vital organs injury, including heart and brain. In our study, we further detected S100β protein, cTnI, CK-MB and moisture rate in brain tissue of subject suffered different treatmentto investigate the different influence that two treatments have had on damage and alteration in these organs.

As one of the acid calcium binding protein, serum S100β protein has small molecular weight, and its concentration may not be interfered by age, hemorrhage, temperature, heparin, and so on. Of which, the β subunit be regarded highly specific for CNS, that, the specific biochemical marker of brain injury (12). Normally, S100β protein could not pass through the blood-brain-barrier (BBB), unless brain tissue was damaged (13). Then the S100β protein in cerebrospinal fluid can easily get through the BBB, and be detected in the blood, which concentration has positive correlation with the severity of injury in center nerve system (14). Chaparro-Huerta etc (15)also demonstrated in their research on neonates with asphyxia that, the amount of serum S100β protein, can reflect the severity of brain injury, and can be prediction of its progression, which has closely relation to the condition and prognosis secondary to the hypoxia and ischemia of brain. In this study, serum S100β protein of hypoxia canine in Group B and Group C were relatively high compared with the controlled group, suggested that hypoxia and hypercapnia can cause damage to BBB. However, in the same hypoxia condition, the level of serum S100β protein of Group C was lower than that of Group B, which indicated that the supplemental chest compression can further relieve the hypercapnia induced brain damage.

Brain edema can, to some extent, reflect the severity of hypoxia, parameters to evaluate brain edema were mostly the moisture rate in brain tissue (16). Hypoxia may lead to cerebral autoregulation dysfunctional, once exceeds the tolerable limit, it can cause brain edema, intracranial hypertension, even cerebral hemorrhage (17). According to the results, hypoxia can apparently aggravate the moisture rate of brain tissue in compared with the controlled group. As for Group B and Group C, with the same duration of resuscitation after hypoxia, moisture rate of brain tissue was significantly higher in Group B than that of the Group C, which suggested that continuous low-pressure oxygen insufflation in combined with chest compression exhibit remarkable curative effect. Besides, as shown by Group C, moisture rate of brain tissue have no significant increase, when compared with the results of 2h or 4h after resuscitation, some even decrease. That indicated the supplemental chest compression can, to great extent, alleviate hypercapnia induced brain damage, or even reverse the brain damage secondary to hypoxia, to improve

the prognosis of patients with hypoxia, to provide valuable option for rescuing patient with hypoxia in emergency department.

cTnI, one of the components of the cardiac troponin complex, be expressed only in myocardium, disease for elsewhere, including skeletal muscle injury (18), will not increase its baseline level. Normally, it can't penetrate the cell membrane of cardiac muscle, so that it could hardly be detected in plasma. When myocardiac hypoxia and ischemia occur, and followed with secondary degeneration and necrosis, the highly specific biochemical marker of myocardiac, cTnI, can enter the interstitial tissue, and early present in circulation. Serum concentration of cTnI above 5g/L can be regarded as myocardial lesion (19). Our study revealed that, compared with the controlled group, serum level of cTnI were significantly elevated in Group B and Group C. Moreover, under the same hypoxia duration, serum level of cTnI was higher, and more markedly with Group C. Which indicates that, supplemental chest compression can alleviate damage to myocardia during hypoxia, and improve the tolerance limit of survival for myocardia in hypercapnia. As for CK-MB, another biomarker for myocardial injury, can be released into circulation after myocardial injury occurred 6h (20). However, its fluctuation seems beyond our observation period that, except for significant increase of serum CK-MB in few subjects, most of which could not obtain the anticipated result, due to the time of detection.

Several limitations need to be considered when interpreting our findings. First, our study is an animal experiment after all, so the data and results can only be provided as clinical reference. Second, experiments were restricted by the amount of samples and lack of repeats, so the obtained results are correspondingly short of conviction. Third, the kidneys are sensitive to acute hypoxia like the heart and brain, and we will further investigate kidney damage in such conditions.

Taking these data together, thyrocricocentesis cannulation can quickly establish temporary airway for ventilation, which can postpone the symptoms of hypoxia, and attenuate the subsequent injury of vital organs.

Conclusions

Fresh oxygen insufflation through thyrocricocentesis cannulation accompany by chest compression may be an appropriate choice for severe acute upper respiratory tract obstruction which has difficulty in urgent tracheal intubation or tracheotomy.

Abbreviations

ECG Electrocardiogram

ABP arterial blood pressure

SpO_2 oxygen saturation

HR heart rate

LAC lactic acid

cTnI cardiac troponin I

CK-MB creatine kinase isoenzyme

ELISA enzyme-linked immuno sorbent assay

CLIA chemiluminescent immunoassay

ANOVA One-way analysis of variance

Declarations

Ethics approval and consent to participate: This study was conducted with Ethical Approval of Fujian Medical University Institutional Animal Care Committee and the Fujian Provincial Hospital Animal Care and Use Committee. All animals received treatments in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals.

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Funding: This study was supported by Medical Education Branch of the Chinese Medical Association and the Medical Education Committee of the Chinese Higher Education Society (2016B-LC033), Natural science foundation of Fujian province (2018J01246), and Medical innovation project of Fujian province (2018-CX-2). They provided funding for this research and did not influence the design of the study, data collection and analysis, interpretation of data and the writing of the manuscript.

Authors' contributions: FH carried out the studies and drafted the manuscript. FC participated in the design of the study and helped to carry out the studies. XZ conceived the study, participated in its design and coordination, and helped to draft the manuscript. FG was responsible for data collection and performed the statistical analysis. All authors read and approved the final manuscript.

Acknowledgements: Not applicable.

References

1. Vasdev GM, Harrison BA, Keegan MT, Burkle CM. Management of the difficult and failed airway in obstetric anesthesia. J Anesth. 2008;22(1):38-48.

2. Caplan RA, Benumof JL, Berry FA, Blitt CD, Bode RH, Cheney FW, et al. Practice guidelines for management of the difficult airway. Anesthesiology. 2003;98(1269-1277):2.
3. El-Orbany M, Woehlck HJ, Difficult mask ventilation. Anesth Analg,2009, 109：6：: 1870-1880.
4. Liang L-Y. Injection anesthesia for dogs and cats [J], Animal medicine, 2009,30 (1) : 123-126.
5. Wang X-M, Gao Q-J, Sun F-W. Observation on the anesthetic effect of the combination of ketamine hydrochloride and diazepam [J].Health profession education, 2010,28 (22) : 37
6. Wang H, Zhou X-H. Clinical application anatomy of peritoneum puncture and incision [J].Shandong pharmaceutical, 2008,48 (18) : 39.40.
7. Liu Y，Zhang X-F，Wang B-Q，Development and experimental study of disposable thyrocricoid puncture cannula. Department of Otolaryngology and and Neck Surgery，Weihai Hospital，Affdiated to Medical College ofQingdao University，Weihai，Shandong，264200，China.
8. Schoch HJ, Fischer S, Marti HH. Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain. Brain. 2002;125(11):2549-57.
9. Peterson GN, Domino KB, Caplan RA, Posner KL, Lee LA, Cheney FW. Management of the Difficult Airway A Closed Claims Analysis. The Journal of the American Society of Anesthesiologists. 2005;103(1):33-9.
10. Wong D-T, Lai K, Chung F-F, Ho R-Y. Cannot intubate-cannot ventilate and difficult intubation strategies: results of a Canadian national survey. Anesth Analg. 2005;100(5):1439-46, table of contents.
11. Hsia CC, Hyde DM, Weibel ER. Lung Structure and the Intrinsic Challenges of Gas Exchange. Compr Physiol. 2016;6(2):827-95.
12. Shirasaki Y, Edo N, Sato T. Serum S-100b protein as a biomarker for the assessment of neuroprotectants. Brain Res. 2004;1021(2):159-66.
13. Huang S-H, Wang L, Chi F, Wu C-H, Cao H, Zhang A, et al. Circulating brain microvascular endothelial cells (cBMECs) as potential biomarkers of the blood–brain barrier disorders caused by microbial and non-microbial factors. PloS one. 2013;8(4):e62164.
14. Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, Janigro D. Peripheral markers of blood-brain barrier damage. Clin Chim Acta. 2004;342(1-2):1-12.
15. Chaparro-Huerta V, Flores-Soto ME, Merin Sigala ME, Barrera de Leon JC, Lemus-Varela ML, Torres-Mendoza BM, et al. Proinflammatory Cytokines, Enolase and S-100 as Early Biochemical Indicators of Hypoxic-Ischemic Encephalopathy Following Perinatal Asphyxia in Newborns. Pediatr Neonatol. 2017;58(1):70-6.
16. Sarada SK, Titto M, Himadri P, Saumya S, Vijayalakshmi V. Curcumin prophylaxis mitigates the incidence of hypobaric hypoxia-induced altered ion channels expression and impaired tight junction proteins integrity in rat brain. J Neuroinflammation. 2015;12:113.
17. Beaumont A, Marmarou A, Fatouros P, Corwin F. Secondary insults worsen blood brain barrier dysfunction assessed by MRI in cerebral contusion. Intracranial Pressure and Brain Biochemical

Monitoring: Springer; 2002. p. 217-9.
18. Bodor GS, Porterfield D, Voss EM, Smith S, Apple FS. Cardiac troponin-I is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue. Clinical Chemistry. 1995;41(12):1710-5.
19. Maynard S, Menown I, Adgey A. Troponin T or troponin I as cardiac markers in ischaemic heart disease. BMJ Publishing Group Ltd and British Cardiovascular Society; 2000.
20. Rajappa M, Sharma A. Biomarkers of cardiac injury: an update. Angiology. 2005;56(6):677-91.

Figures

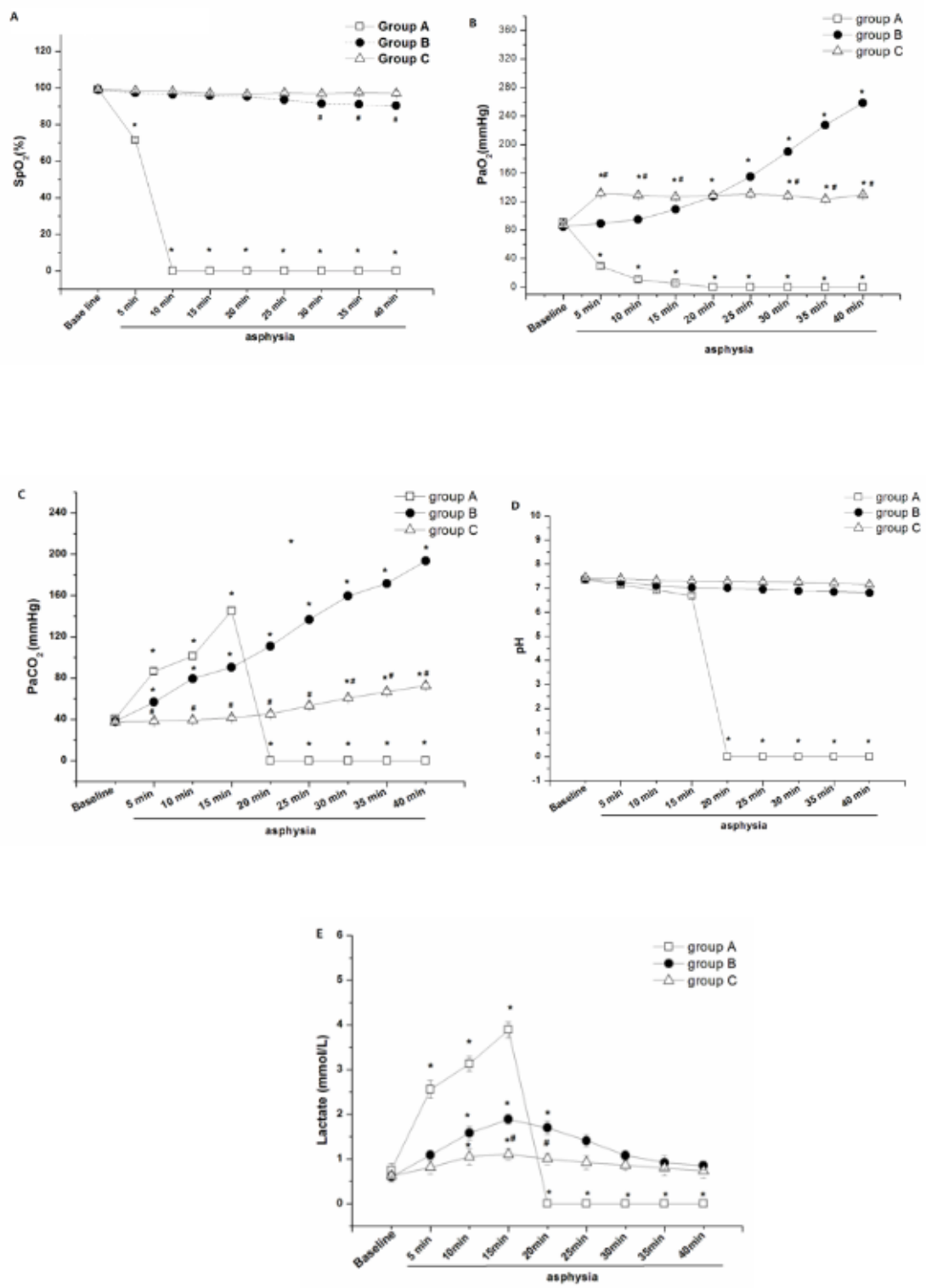

Figure 1

Comparison of arterial blood gas analysis parameters among group A, B and C. The measurement were made at baseline and varying time points up to 40 minutes post-hypoxia. A. SpO2; B. PaO2; C. PaCO2; D. pH; E. Lactate. The values are presented as mean ± SD. *p□0.05 vs. baseline; #p□0.05 vs. group B.

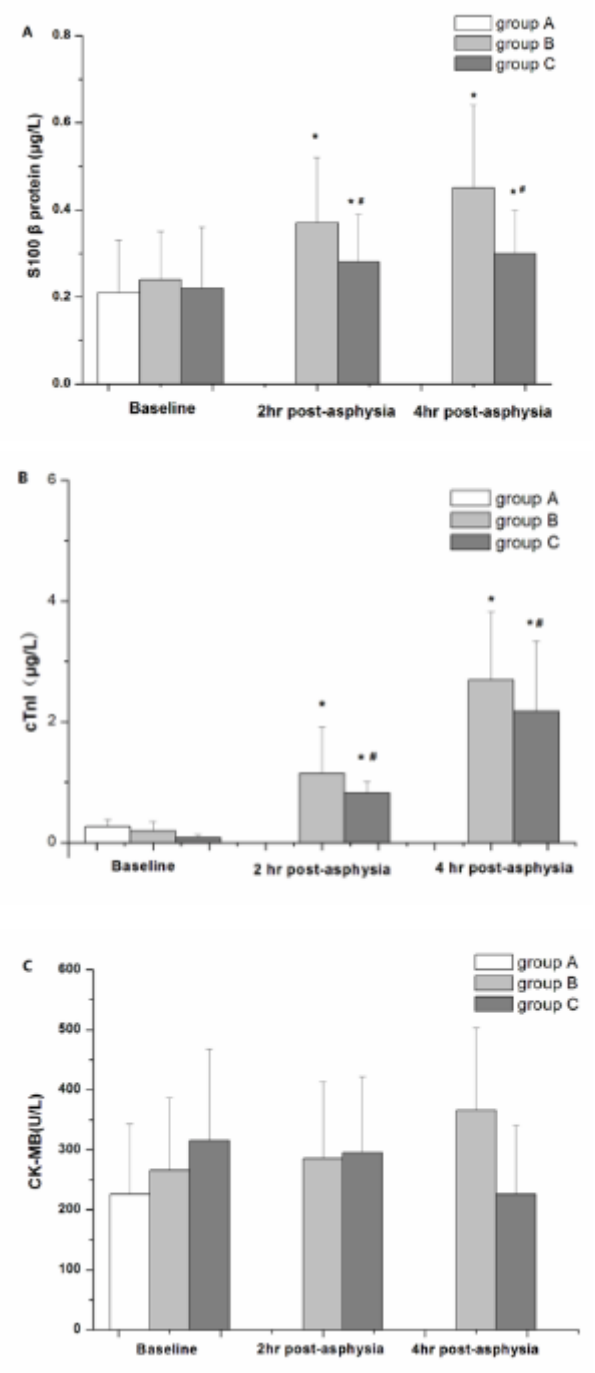

Figure 2

Comparison of serum S100-β protein, cTNI, and CK-MB among group A, B and C. The measurement were made at baseline and varying time points of 2 and 4 hours post-hypoxia. A. S100-β protein; B. cTNI; C. CK-MB. The values are presented as mean ± SD. *p□0.05 vs. baseline; #p□0.05 vs. group B.

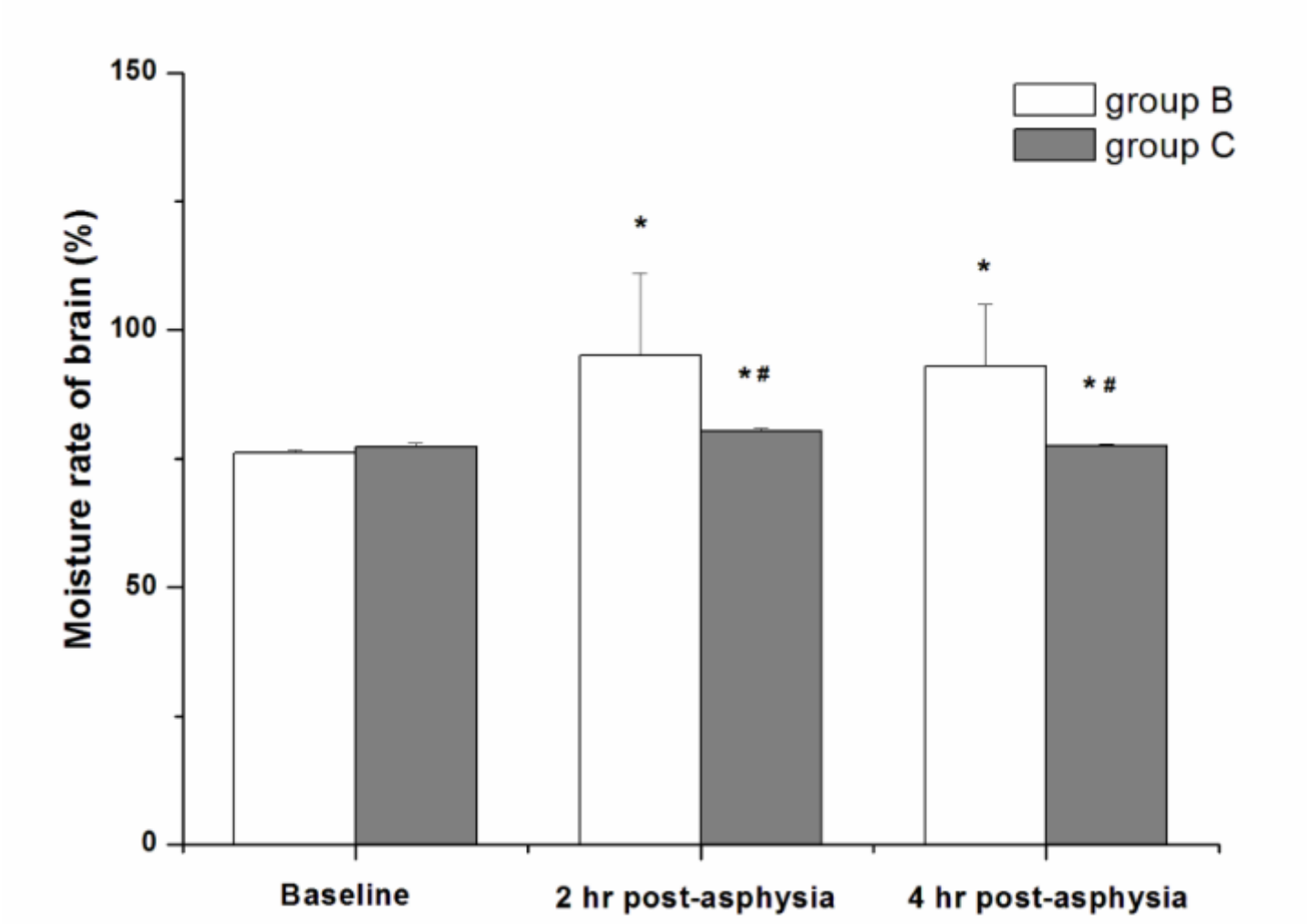

Figure 3

Comparison of moisture ratio in brain tissue among group A, B and C. The measurement were made at baseline and varying time points of 2 and 4 hours post-hypoxia. The values are presented as mean ± SD. *p0.05 vs. baseline; #p0.05 vs. group B.

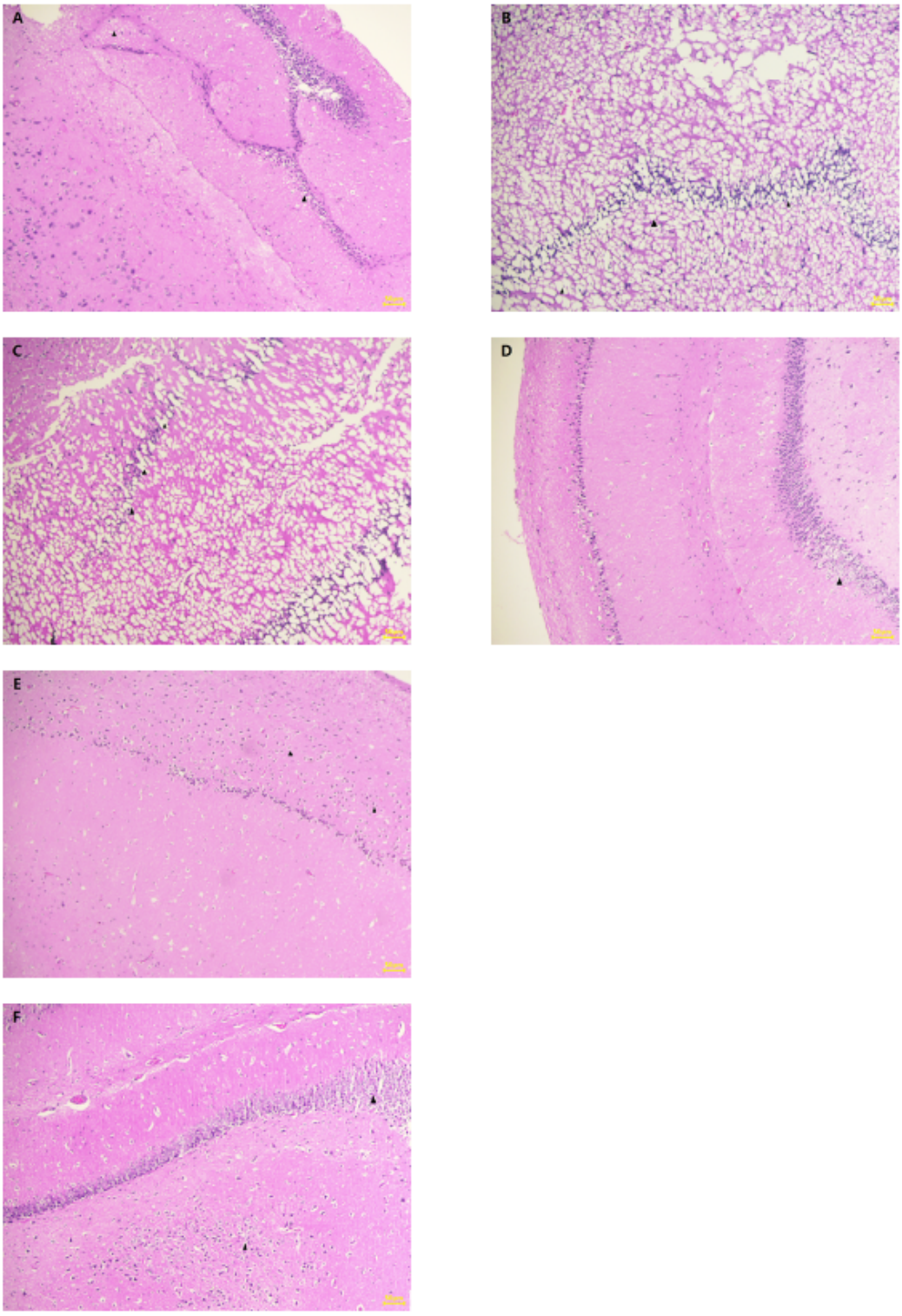

Figure 4

The histology of brain edema in group B and group C. group B Under the microscope，The brain parenchyma was no hemorrhage and could see vacuoles of different sizes. The tissue was loosely and the neurons and perivascular space were enlarged. The neurons were degenerated and necrotic, and could see neutrophils and microglia. (black arrows，fig 4A，Baseline，fig 4B，2hr post-asphyxia; fig 4C，4hr post-asphyxia) group C Under the microscope，The brain parenchyma was no hemorrhage and could see

a small number of vacuoles of different sizes. The tissue was tightly and the neurons and perivascular space were enlarged. The neurons were degenerated and necrotic mildly, and could see a few neutrophils and microglia. (black arrows：fig 4D：Baseline：fig 4E：2hr post-asphyxia; fig 4F：4hr post-asphyxia)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.docx