Protective effect of haemopexin in rats subjected to focal cerebral ischaemia–reperfusion injury

Z. Zhishen1, D. Beibei1,2, W. Guolin1,2 and Y. Yonghao2

1Department of Anesthesiology, General Hospital of Tianjin Medical University, Tianjin, China, and 2Tianjin Institute of Anesthesiology, Tianjin, China

Stroke has become one of the most severe diseases worldwide. Haemopexin (HPX) has been shown to provide neuroprotection in rats, but its mechanism of action is not yet clear. In this study, we investigated the potential neuroprotective role of haemopexin and explored its underlying mechanism in rats subjected to focal cerebral ischaemia–reperfusion injury.

A model of focal cerebral ischaemia–reperfusion injury was produced by middle cerebral artery occlusion (MCAO). With ethical committee approval, male Sprague–Dawley rats, 7–8 weeks old, weighing 250–280 g, were randomly divided into the following groups (20 per group): a sham-operated group, a MCAO group, a vehicle control group and an HPX treatment group. Both vehicle and HPX were administered immediately after reperfusion by a single intracerebroventricular injection. Measurement of the neurological functional outcome, determined by the modified neurological severity score (mNSS), was carried out before ischaemia and on days 1, 3, 7, 21, and 28 after ischaemia–reperfusion. Assessment of learning and memory abilities was carried out by Morris water maze assay at 3–8 days after ischaemia–reperfusion. Six rats per group were killed at 2, 6, 12, and 24 h and 7 days after reperfusion to obtain the ipsilateral ischaemic penumbra brain tissues. Real-time polymerase chain reaction (EPC) count, and the peripheral blood EPCs were counted by flow cytometry. Angiogenesis was assessed by the density of new vessels in both the dentate gyrus and the cortex, which was determined by immunofluorescence.

Compared with the sham-operated group, in the MCAO group the mNSS increased dramatically (P<0.05), the escape latency at days 3–8 after reperfusion was significantly reduced (P<0.05), the percentage of time spent in the target quadrant and the times for crossing the platform decreased significantly (P<0.05). There was also a substantial decrease of mRNA and protein expression of HO-1 at 2, 6, 12, and 24 h and 7 days after reperfusion (P<0.05). The circulating EPC count increased significantly at days 3, 5, 7, and 14 after operation (P<0.05). Meanwhile, the density of new vessels in both the dentate gyrus and the cortex increased drastically (P<0.05). However, there was no statistical difference between the sham-operated group and the vehicle control group in terms of all experimental indexes (P>0.05).

In the HPX treatment group, compared with the vehicle control group, the mNSS decreased drastically (P<0.05), the escape latency at days 3–8 after reperfusion was significantly reduced (P<0.05), and the percentage of time spent in the target quadrant and the times of crossing the platform increased dramatically (P<0.05). Accordingly, the mRNA and protein expression of HO-1 increased markedly (P<0.05). The circulating EPC count increased significantly at days 3, 5, 7, 14, 21, and 28 after operation (P<0.05). Meanwhile, the density of new vessels in both the dentate gyrus and the cortex increased drastically (P<0.05).

Haemopexin can alleviate the impairment in neurological function and improve learning and memory abilities after focal cerebral ischaemia–reperfusion injury in rats. The underlying molecular mechanism may be associated with the upregulation of HO-1, which promotes the mobilization of bone marrow–derived EPCs to the periphery to participate in the repair of damaged vessels and angiogenesis.

Reference

Remifentanil preconditioning confers cardioprotection via glycogen synthase kinase-3β associated with ERK and JNK pathways in rats with heart failure

S-Y. Jin1, S-F. He1, H. Wu1, B. Wang1, Y-X. Wu2, S-J. Zhang2 and Y. Zhang2

1Department of Anesthesiology, The Second Affiliated Hospital of Anhui Medical University, Hefei, China, and 2Department of Anesthesiology, Hefei Children’s Hospital, Anhui Medical University, Hefei, China

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Remifentanil preconditioning has been demonstrated to exert protective effects in normal heart, but its effects are still not known in heart failure. The aim of the present investigation was to evaluate remifentanil preconditioning against ischaemia–reperfusion (IR) injury in rats with chronic heart failure and its underlying mechanisms involving mitogen-activated protein kinases (MAPKs) and glycogen synthase kinase-3β (GSK-3β) pathways.

The rats were confirmed to have chronic heart failure through echocardiography and were randomly divided into nine groups as follows: sham group (n=9); IR group (IR, n=9); remifentanil preconditioning group (RPC, n=9); ERK inhibitor PD98059 + RPC group (RPD, n=9); p38 inhibitor SB203580 + RPC group (RSP, n=9); JNK inhibitor SP600125 + RPC group (RSP, n=9); and the inhibitor control groups PD (n=6), SB (n=6), and SP (n=6). All hearts were subjected to 30 min ischaemia and 120 min reperfusion (IR) except for the sham group. Remifentanil preconditioning (RPC) was induced by three cycles of 5 min remifentanil and 5 min drug-free perfusion before IR. PD98059 (10 µmol litre−1), SB203580 (5 µmol litre−1), and SP600125 (10 µmol litre−1) were perfused for a period of 10 min before RPC until 10 min after the end of RPC. The coronary effluent was collected to detect the activity of lactate dehydrogenase at baseline, 5, and 10 min after reperfusion. Infarct size and area at risk (IR) were measured by 2,3,5-triphenyl-tetrazolium staining at the end of reperfusion. The activity of lactate dehydrogenase at baseline, 5, and 10 min after reperfusion. Infarct size and area at risk were determined by myocardial IR injury. However, SP600125 almost completely abolished the protective effects of RPC, as evidenced by the increased value of infarct size/area at risk and the high activity of lactate dehydrogenase. In addition, PD98059 also partly blocked the protective effects in normal heart,12 but its effects are still not known in heart failure. The aim of the present investigation was to evaluate remifentanil preconditioning against ischaemia–reperfusion (IR) injury in rats with chronic heart failure and its underlying mechanisms involving mitogen-activated protein kinases (MAPKs) and glycogen synthase kinase-3β (GSK-3β) pathways.

Remifentanil preconditioning significantly reduced the infarct size and the elevated lactate dehydrogenase activity caused by myocardial IR injury. However, SP600125 almost completely abolished the protective effects of RPC, as evidenced by the increased value of infarct size/area at risk and the high activity of lactate dehydrogenase. In addition, PD98059 also partly blocked the effects of RPC, while SB203580 showed no influence on RPC. Remifentanil preconditioning markedly elevated the phosphorylation of JNK, ERK, and the downstream GSK-3β, but failed to increase P38 phosphorylation. SP600125 and PD98059 suppressed the phosphorylation of JNK, ERK, and GSK-3β induced by RPC. In contrast, SB203580 did not affect the phosphorylation of GSK-3β induced by RPC. According to the results of quantitative RT-PCR, RPC not only increased Bcl-2 mRNA, but also decreased Bax mRNA, leading to a much higher Bcl-2/Bax ratio than in the IR group; these effects were abolished by the addition of SP600125 and PD98059, but not by SB203580. Remifentanil preconditioning confers cardioprotective effects in failing rat hearts, and its underlying mechanisms involve the activation of JNK/ERK-GSK-3β pathway independent of P38, resulting in regulation of apoptotic genes.

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**References**


**References**


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**Protective effects of hydrogen-rich medium on high-glucose-induced oxidative stress and poly(ADP-ribose)polymerase-1-dependent cell death (parthanatos) in rat Schwann cells in vitro**

Y. Yu, K.-L. Xie, Y. X. Bian, G. L. Wang and Y.-H. Yu

Department of Anesthesiology, General Hospital of Tianjin Medical University, Tianjin, China

Diabetic peripheral neuropathy is considered to be one of the most common and dangerous microvascular diabetic complications, with no effective therapies in existence today. Previous studies have pointed out that oxidative stress is the common pathway of all possible hypotheses that can induce diabetic peripheral neuropathy, and poly(ADP-ribose)polymerase-1-dependent cell death (parthanatos) is the major key to the pathogenic mechanisms of neurodegenerative disease. The aim of this study was to investigate the protective effects and the relative mechanisms of hydrogen-rich medium (HM) on high-glucose (HG)-induced oxidative stress and parthanatos in primary rat Schwann cells in vitro.

Primary rat Schwann cells were purchased from ScienCell Corporation. All the Schwann cells were divided randomly into the following five groups: rat Schwann cells that were treated with primary low-glucose Dulbecco’s modified Eagle’s medium (DMEM; 5.6 mM of glucose) as the control group, with saturated hydrogen-rich medium (0.6 mM hydrogen) as the hydrogen group, with 44.4 mM glucose plus primary low-glucose DMEM (50 mM glucose in the complete DMEM) as the high-glucose group (HG), with high-glucose DMEM (50 mM glucose) accompanied by saturated hydrogen (0.6 mM hydrogen) as the treatment group, and in order to eliminate osmotic interference, we added 44.4 mM mannitol (almost as the same osmotic pressure as 44.4 mM of glucose) to low-glucose DMEM (5.6 mM of glucose) as the high-osmotic control group. We treated all the Schwann cells in different groups for 48 h and used a cell counting kit-8 assay and a lactate dehydrogenase assay to detect cell viability and cytotoxicity, respectively. Intracellular OH− concentrations were measured by dichoro-dihydro-flour-escein diacetate (DCFH-DA) assay. Concentrations of ONOO− and 8-hydroxydeoxyguanosine (8-OHdG) were evaluated by enzyme-linked immunosorbent assay. Relative proteins of parthanatos (poly ADP-ribose (PAR), nuclear apoptosis inducing factor (AIF), and total AIF) were tested by western blot, and immunofluorescence was used to determine the nuclear translocation of AIF.

We found that, after 48 h of treatment, HG could induce severe oxidative stress and promote serious parthanatos in rat Schwann cells. Treatment with HM could inhibit the HG-induced oxidative stress by reducing OH− and ONOO− production and could suppress the parthanatos by downregulating 8-OHdG concentrations, PAR expression, and AIF translocation to the nucleus. Treatment with HM improved the cell viability while inhibiting the cytotoxicity in the HG conditions.

Our results indicated that HM can effectively reduce the oxidative stress induced by HG in rat Schwann cells and can protect them against parthanatos. This may be a new type of drug for treatment of diabetic peripheral neuropathy.

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**References**

Effect of c-Src kinase-mediated ventilator-induced lung injury in rats

T. Zhao, Y. L. Wang, C. P. Gu, M. J. Liu, D. Wang and Y. Z. Gong

Department of Anesthesiology, Qianfoshan Hospital, Shandong University, Ji’nan, Shandong, China

Ventilator-induced lung injury is a common iatrogenic clinical problem in intensive care with anaesthesia and is characterized by increased alveolar permeability, leading to pulmonary oedema. The tyrosine kinase, c-Src, is involved in ventilator-induced lung injury, but its role has not been elucidated fully. This study examined the relationship between c-Src activation and occludin concentrations in ventilator-induced lung injury in vivo.

Thirty Wistar rats were randomly divided into five groups: control (Group C), low tidal volume (Group L), low tidal volume + c-Src inhibitor (PP2; Group L+P); high tidal volume (Group H); and high tidal volume + c-Src inhibitor (PP2; Group H+P). Rats in all groups but Group C underwent mechanical ventilation for 4 h. Ventilation parameters were set as follows: a tidal volume of 7 ml kg⁻¹, a respiratory rate of 120 bpm, and PEEP=0 in Group L and Group L+P; or tidal volume of 20 ml kg⁻¹, a respiratory rate of 40 bpm, and PEEP=5 in Group H and Group H+P. Rats in Group L+P and Group H+P were pretreated with PP2 1 µg kg⁻¹ for 1 h before anesthesia.

After ventilation, rats were killed by exsanguination of arterial blood. Lungs were removed to record scores of pathological damage in lung tissue, calculate the pulmonary wet-to-dry ratio to quantify the magnitude of pulmonary oedema, observe the histological changes with Haematoxylin and Eosin staining, and measure the expressions of total and phosphorylated c-Src and occludin by western blotting.

Mechanical ventilation increased the expression of total and phosphorylated c-Src and the degradation of occludin in Group H (P<0.05) compared with Groups C and L, as seen by western blotting. The expression of occludin was higher and c-Src lower in Group H+P compared with Group H (P<0.05). These results suggest that mechanical ventilation with a high tidal volume can activate c-Src and decrease occludin concentrations. Haematoxylin and Eosin staining, wet-to-dry ratio, and scores of pathological damage showed that mechanical ventilation with a high tidal volume could cause alveolar congestion, haemorrhage, infiltration or aggregation of neutrophils in the airspace or the vessel wall, and increase the thickness of the alveolar wall or induce hyaline membrane formation, leading to pulmonary oedema.

Ventilation with a low tidal volume could reduce the damage. Ventilation with a high tidal volume could activate c-Src kinase by the phosphorylation of c-Src to reduce the concentration of occludin; therefore, an c-Src kinase inhibitor could alleviate pulmonary oedema.

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References


Protective effects of hydrogen on intestinal epithelial barrier against lipopolysaccharide in vitro and the regulation of Rho kinase

X. Y. Ma, Y. Yu, H. T. Zhang, D. Q. Liu, K. L. Xie and Y. H. Yu

1Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin, China, 2Department of Anatomy and Histology, School of Basic Medicine, Tianjin Medical University, Tianjin, China, 3Department of Pharmacology, Institute of Acute Abdominal Diseases, Tianjin Nankai Hospital, Tianjin, China, and 4Tianjin Institute of Anesthesiology, Tianjin, China

Sepsis is defined as systemic inflammation induced by various infections, and often turns out to be lethal. Hydrogen has been proved to be protective against sepsis in multiple organs, including intestine. Our study aimed to exploring whether Rho kinase takes part in the protective effect of hydrogen on the intestinal epithelial barrier against sepsis.

Caco-2 cells were cultured routinely and divided randomly into five groups, as follows: a control group; a hydrogen-rich medium (0.6 mmol litre⁻¹) group; a lipopolysaccharide (LPS, 100 µg ml⁻¹) treatment group; a hydrogen+LPS treatment group; and a Y-27632 (Rho kinase inhibitor, 25 µmol litre⁻¹)+LPS treatment group. After the Caco-2 monolayer models were established, the transepithelial electrical resistance (TEER) values were measured regularly. When the TEER values reached 800 Ω cm², the treatments were administered. The TEER values were measured at 6, 12, and 24 h, and fluorescein isothiocyanate (FITC)–dextran permeability was detected at 24 h. An MTT assay was also performed to test the amount of cell apoptosis at 24 h. Real-time polymerase chain reaction was conducted to assess mRNA expressions of zona occludens-1 (ZO-1) protein and Rho kinase.

An immunofluorescence technique was used to investigate ZO-1 protein distribution. Protein expression of ZO-1 and Rho kinase increased (all P<0.05). Although the amount of cell apoptosis did not change significantly (P>0.05), mRNA expression of ZO-1 decreased, mRNA and protein expression of Rho kinase increased (all P<0.05). The distribution of ZO-1 on the cell membrane was interrupted and the protein accumulated in cytoplasm, the addition of Y-27632 ameliorated the change induced by LPS, the TEER values increased significantly, and FITC–dextran permeability decreased (all P<0.05).

Hydrogen can protect intestinal barrier function against sepsis, retaining the integrity and permeability of intestinal epithelium, and increasing the expression of tight junction proteins. The suppression of the Rho kinase overexpression induced by LPS may be involved in these protective effects of hydrogen.
Dexmedetomidine, a highly selective α₂-adrenergic agonist with sedative and analgesic properties which has been widely used in the intensive care unit and anaesthesia, is mainly cleared by the liver. The characteristics of drug elimination in patients with end-stage renal failure and secondary hyperparathyroidism might be different from the normal ones. Given that the pharmacokinetics and pharmacodynamics among patients with end-stage renal failure and secondary hyperparathyroidism have not been investigated, the primary objective of this study was to characterize the pharmacokinetics and pharmacodynamics in this special type of patients and compare them with those in normal patients.

Fifteen patients with end-stage renal failure and secondary hyperparathyroidism and five patients with normal renal function received dexmedetomidine at 3.6 µg kg⁻¹ h⁻¹ for 10 min before induction of anaesthesia. Arterial blood samples for analysis of the plasma concentration of dexmedetomidine were obtained at 1, 5, and 7 min and at 1, 3, 7, 20, 45, and 75 min and 1.5, 2, 4, and 8 h after the infusion stopped. The pharmacokinetics and pharmacodynamics were analysed using a non-linear mixed-effect model.

### Pharmacokinetics and pharmacodynamics of dexmedetomidine applied to patients with end-stage renal failure and secondary hyperparathyroidism undergoing general anaesthesia

W. Zhong¹, M. Z. Zhang², X. H. Huang³, Y. Li¹, R. Li¹, Q. W. Liu⁴ and Y. Zhang¹

¹Department of Anaesthesiology, The Second Affiliated Hospital of Anhui Medical University, Hefei, China, ²Department of Anaesthesiology, Shanghai Children’s Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, ³School of Pharmacy, Inner Anhui Medical University, Hefei, China, and ⁴Center for Instrumental Analysis, China Pharmaceutical University, Nanjing, China

Dexmedetomidine, a highly selective α₂-adrenergic agonist with sedative and analgesic properties which has been widely used in the intensive care unit and anaesthesia, is mainly cleared by the liver. The characteristics of drug elimination in patients with end-stage renal failure and secondary hyperparathyroidism might be different from the normal ones. Given that the pharmacokinetics and pharmacodynamics among patients with end-stage renal failure and secondary hyperparathyroidism have not been investigated, the primary objective of this study was to characterize the pharmacokinetics and pharmacodynamics in this special type of patients and compare them with those in normal patients.

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### Table 1

Dexmedetomidine population pharmacokinetic parameters for the final model. The estimated parameters are those characterized by the mixed-effect model of NONMEM. The percentage coefficient of interindividual variability (%CV) is the square root of the variance, η (=ω²). 95% CI, 95% confidence interval of the population estimate or bootstrap mean; Cl₁, systemic clearance; Cl₂, rapid distributional clearance; OBJ, objective function value; RES, population estimate standard error/population estimate × 100; RSE, relative standard error; σ², residual variability of variance; V₁, volume of the central compartment; V₂, volume of the rapid compartment

<table>
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<tr>
<th>Parameter</th>
<th>Population estimate (Typical [%RSE])</th>
<th>95% CI</th>
<th>Interindividual variability (%CV)</th>
<th>Bootstrap mean (95% CI)</th>
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<tr>
<td>V₁ (litres)</td>
<td>60.6 (13.6%)</td>
<td>44.4, 76.8</td>
<td>45.7%</td>
<td>60.7 (47.7, 73.8)</td>
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<tr>
<td>V₂ (litres)</td>
<td>222 (12.8%)</td>
<td>166, 278</td>
<td>47.3%</td>
<td>222.8 (171.8, 273.8)</td>
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<tr>
<td>Cl₁ (litres min⁻¹)</td>
<td>0.825 (9.8%)</td>
<td>0.67, 0.98</td>
<td>33.2%</td>
<td>0.826 (0.676, 0.979)</td>
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<td>Cl₂ (litres min⁻¹)</td>
<td>4.48 (14.6%)</td>
<td>3.20, 5.76</td>
<td>51.7%</td>
<td>4.50 (3.25, 5.75)</td>
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<tr>
<td>σ²</td>
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<td>OBJ</td>
<td>– 205.090</td>
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### References


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Fig 1 After the treatment with lipopolysaccharide (LPS), the distribution of zona occludens-1 (ZO-1) protein on the cell membrane decreased dramatically in the LPS group, whereas it increased in the cytosol (c). Either treatment with hydrogen (b) or the inhibition of Rho kinase (e) ameliorated these changes. Groups are as follows: (a) control group; (b) hydrogen-rich medium group; (c) LPS treatment group; (d) hydrogen+LPS treatment group; and (e) Y-27632 + LPS treatment group.
A non-linear mixed-effects model with NONMEM software was used to analyse the pharmacokinetics. The statistical significance of a covariate was examined using the objective function (−2 log likelihood). In the forward inclusion and backward deletion, the covariates such as age, weight, gender, height, lean body mass, body surface area, BMI, and grouping factor (renal failure or not) were tested for significant effects on pharmacokinetic parameters. The validity of our population model was also evaluated using the bootstrap simulations.

The dexmedetomidine concentration–time curves were found to be fitted best with two-compartmental pharmacokinetic models in principle. There was no covariate of systemic clearance that could improve the model further. Results are presented as mean (SEM). The final values of the pharmacokinetic parameters were as follows: $V_1=60.6$ litres, $V_2=222$ litres, $Cl_1=0.825$ litres min$^{-1}$, and $Cl_2=4.48$ litres min$^{-1}$ (see Table 1 for details). Although the dosage of propofol was significantly less in Group R (81.68 (18.08) vs 63.07 (13.45) µg kg$^{-1}$ min$^{-1}$, respectively; $P<0.05$), the context-sensitive half-life and the revival time from anaesthesia showed no differences between the two groups.

This study validated that there was no influence of age, weight, gender, height, lean body mass, body surface area, BMI, and grouping factor (renal failure or not) on pharmacokinetic parameters, and the context-sensitive half-life showed no difference between the two groups. The reduced dose of propofol indicates increased sensitivity to dexmedetomidine. This may be due to increased drug sensitivity or secondary hyperparathyroidism. The dosage of dexmedetomidine should be lessened accordingly, and the time of administration should be prolonged properly.

**Reference**


**Role of microRNA-133b-5p in cardioprotection mediated by morphine preconditioning in H9C2 myocardial cells**

Z. Y. Han, S. F. He, J. Cheng, S. J. Xu, W. Yang and Y. Zhang

Department of Anesthesiology, The Second Affiliated Hospital of Anhui Medical University, Hefei, China

MicroRNAs (miRNAs) have been implicated in the process of myocardial ischaemia–reperfusion injury and preconditioning-induced cardioprotection. ¹ Our recent study screened out a number of differentially expressed miRNAs induced by morphine preconditioning (MPC) in rat cardiomyocytes using miRNA microarray analysis. Among these miRNAs, miR-133b-5p is the most upregulated miRNA, which directly inhibits the target gene Fas. However, the underlying mechanisms of how miR-133b-5p contributes to MPC-mediated cardioprotection need to be studied further. The purpose of this study was to investigate
Morphine preconditioning was performed by pretreatment with and the cells were then subjected to MPC followed by H/R injury. Morphine preconditioning was performed by pretreatment with 1 µmol litre⁻¹ morphine for 10 min before 5 h hypoxia–1 h reoxygenation injury. Cell viability was measured by the cell counting kit-8 assay, and lactate dehydrogenase activity in the culture medium was detected to evaluate cell injury. Cell apoptosis was assessed by Annexin V-FITC flow cytometry. Finally, total RNA and protein were extracted from H9C2 cells to detect miR-133b-5p and Fas mRNA by real-time quantitative RT-PCR and detect Fas protein by western blot.

The present study showed that transfection with miR-133b-5p inhibitor significantly decreased the expression of miR-133b-5p in cultured H9C2 myocardial cells, while negative control RNA transfection did not affect miR-133b-5p expression. Morphine preconditioning potently protected H9C2 cells from H/R injury by increasing cell viability and reducing lactate dehydrogenase concentration and cell apoptosis (Fig. 2). However, this protective effect was markedly blocked by the transfection of miR-133b-5p inhibitor, indicating the important role of miR-133b-5p in MPC-induced cardioprotection. The expression of miR-133b-5p was markedly decreased whereas the expressions of Fas mRNA and Fas protein were elevated after H/R injury. Pretreatment with morphine obviously elevated the expression of miR-133b-5p whereas it decreased both Fas mRNA and Fas protein expression. The effects of MPC on the expression of miR-133b-5p and Fas mRNA/Fas protein were abolished by miR-133b-5p inhibitor.

These results suggest that miR-133b-5p and its target gene Fas may play an important role in morphine-induced cardioprotection, indicating a potential novel therapeutic target for treatment of ischaemic heart disease.

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Reference

Integrin β3 positively regulates Toll-like receptor (TLR)-triggered inflammatory responses by targeting CD14 expression via a TLR4/MyD88-dependent and TRIF-independent pathway in murine sepsis

Z. Chen, X. Ding, S. Mei, S. Jin, Y. Tong and Q. Li
Department of Anesthesiology, East Hospital, Tongji University School of Medicine, Shanghai, China

Sepsis is one of the life-threatening diseases worldwide. It is characterized by inappropriate amplification of the systemic inflammatory response that may promote multiple organ dysfunction and mortality. Activation of Toll-like receptor (TLR) plays a key role in sepsis. 1 CD14 is necessary for the TLR-dependent inflammatory responses by targeting CD14 expression

**Integrin β3 positively regulates Toll-like receptor (TLR)-triggered inflammatory responses by targeting CD14 expression via a TLR4/MyD88-dependent and TRIF-independent pathway in murine sepsis**

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Department of Anesthesiology, East Hospital, Tongji University School of Medicine, Shanghai, China

Sepsis is one of the life-threatening diseases worldwide. It is characterized by inappropriate amplification of the systemic inflammatory response that may promote multiple organ dysfunction and mortality. Activation of Toll-like receptor (TLR) plays a key role in sepsis. 1 CD14 is necessary for the TLR-dependent induction of pro-inflammatory cytokines. 2 Recent studies have indicated that integrin β3 is involved in TLR-triggered innate immunity. 3 Our previous study found that the integrin inhibitor Arg-Gly-Asp-Ser peptides (RGDs) alleviated sepsis-induced lung injury and improved survival rate. However, the interaction
between integrin β3 and TLR activation in sepsis remains largely unknown. This study aimed to uncover the mechanism of integrin β3 and TLR activation in an in vivo and in vitro study.

Mice undergoing caecal ligation and puncture (CLP)-induced sepsis were killed at specified times. Serum was obtained for analysis of the inflammatory cytokine interleukin-6 (IL-6) by enzyme-linked immunosorbent assay. Tissues were fixed with 4% paraformaldehyde for Haematoxylin and Eosin staining and immunohistochemistry. Tissue lysate was obtained for measurement of CD14 expression by western blot, and the interaction between TLR4 and integrin β3 was assayed by co-immunoprecipitation. Peritoneal macrophages isolated from C57bl/6 mice were pretreated with RGDs for 1 h and stimulated with lipopolysaccharide at specified times or treated with vitronectin. Protein was collected for measurement of CD14 expression through western blot and immunohistochemistry. Medium was collected for measurement of TNF-α and IL-6 release via enzyme-linked immunosorbent assay.

The serum IL-6 concentration increased significantly in wild-type (WT) but not in integrin β3−/− mice after CLP. Histological analysis of the lungs, liver, and kidney, showed that CLP caused destruction of the micro-architecture characterized in WT mice (Fig. 3). The CLP-provoked tissue damage was suppressed in integrin β3−/− mice. CD14 expression was significantly increased compared with the control group after CLP in the lung, liver, and kidney. These increases were attenuated in integrin β3−/− mice. Co-immunoprecipitation results showed that the interaction between TLR4 and integrin β3 was enhanced after CLP. The elevation in IL-6 concentration was significantly inhibited in TLR4−/− mice of the CLP group at 24 h. Interleukin-6 release was also markedly attenuated in the CD14−/− and Myd88−/− mice of the CLP group from 4 to 24 h, but not in TRIF−/− mice of the CLP group, which was consistent with the histological results. Increased expression of CD14 in the lung and liver were attenuated in TLR4−/−, CD14−/−, and Myd88−/− mice but not in TRIF−/− mice.

Pretreatment with RGDs significantly inhibited the upregulation of CD14 and the release of inflammatory cytokines induced by lipopolysaccharide in peritoneal macrophages. Vitronectin-integrin β3 ligation did not affect either CD14 expression or cytokine release in macrophages.

Integrin β3 positively regulates TLR-triggered inflammatory responses by targeting CD14 expression by a TLR4/MyD88-dependent and TRIF-independent pathway in murine sepsis. Inhibition of the ‘integrin β3–CD14’ axis may be a potential treatment strategy in sepsis.

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**References**


**Mechanical ventilation augments Poly(I:C)-induced lung injury via WISP1-integrin β3 signalling in mice**

S. Q. Jin, Z. X. Chen, X. B. Ding, Y. Tong, X. Jiang and Q. Li

Department of Anesthesiology, Shanghai East Hospital, Tongji University, Shanghai, China

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Fig 3 (a and s) Integrin β3−/− mice are resistant to septic inflammation induced by caecal ligation and puncture. (c) Mice with inhibition of integrin β3 are resistant to sepsis-induced upregulation of CD14 in the lung, liver, and kidney. (o) The upregulation of CD14 in the tissues of septic mice is decreased in TLR4−/−, CD14−/−, and Myd88−/− mice but not in TRIF−/− mice.
Mechanical ventilation (MV) is an effective therapy for patients with serious viral infection in the lung. Polyinosinic–polycytidylic acid [Poly(I:C)] is an analogue of a natural double-stranded RNA virus, which can stimulate an immune response. Previously study indicated that MV augmented Poly(I:C)-induced lung inflammation, but the pathogenesis remained unknown. The deleterious effects of MV may be mediated by endogenous substances. Our previous study found that WNT1 inducible signalling pathway protein 1 (WISP1) contributed to ventilation-induced lung injury. The effect and mechanism of MV on virus-induced lung injury need further study, and WISP1 could be an important candidate for novel therapy.

C57 BL/6J wild-type mice were randomly assigned into four groups: control, moderate tidal volume ventilation (MTV), Poly (I:C), and Poly(I:C)+MTV. First, lung inflammation and permeability were assessed by Haematoxylin and Eosin staining, Evans blue albumin (EBA) wet-to-dry ratios, and total protein and inflammatory cytokine concentrations in bronchoalveolar lavage fluid. Second, WISP1 expression in lung tissue was assessed by western blot and RT-PCR, and anti-WISP1 antibody was used to explore the effect of WISP1 on lung injury. Third, integrin β3 expression was detected to observe whether integrin β3 was related to the injury, and co-immunoprecipitation was performed to observe the interaction between WISP1 and integrin β3. Finally, an in vitro study of peritoneal macrophages from integrin β3 knock-out mice was carried out to explore the exact mechanism of WISP1–integrin β3 signalling during Poly(I:C) exposure.

Moderate tidal volume ventilation amplified Poly(I:C)-induced lung injury, including an increase in the EBA permeability, wet-to-dry ratio of the lung, and total protein and inflammatory cytokine concentrations in bronchoalveolar lavage fluid. The expression of WISP1 was consistent with lung injury, and we found that the amplification of lung injury by MTV can be alleviated by anti-WISP1 antibody (Fig. 4). Poly(I:C)+MTV increased integrin β3 expression compared with Poly(I:C) alone. The co-immunoprecipitation results showed there was an interaction between WISP1 and integrin β3. In the in vitro study, WISP1 and Poly(I:C) also promoted phosphorylation of extracellular signal-related kinase (ERK), and the synergistic effect of WISP1 and Poly(I:C) on TNF-α release was significantly inhibited by ERK inhibitor in a dose-dependent manner.

Fig 4 (A) Increases in EBA permeability were seen in Poly(I:C)-treated mice compared with control animals. The increases were amplified by MTV. (B) Western blot showed that WISP1 expression in the Poly(I:C)+MTV group increased significantly compared with the Poly(I:C) group. (C) Anti-WISP1 reduces EBA permeability of lung tissue compared with control IgG antibody. (D) Enzyme-linked immunosorbent assay showed that the synergy of Poly(I:C) and WISP1 to increase the TNF-α concentration was significantly inhibited in integrin β3 knockout mice.
Moderate tidal volume ventilation did not cause extensive lung injury in normal lungs, but augmented Poly(I:C)-induced lung injury. The mechanism may be that MTV enhanced the expression of WISP1 and integrin β3 in Poly(I:C)-injected lung. WISP1 and Poly(I:C) showed a synergistic effect on the inflammatory response through the ERK pathway, resulting in aggravation of lung injury.

**References**


**Effect of ifenprodil on intrathecal morphine-induced pruritus and its mechanism**

W. J. Wang, L. Shen and Y. G. Huang

Department of Anesthesiology, PUMC Hospital, CAMS, Beijing, China

Pruritus is the most common complication of intrathecal (i.t.) morphine, mediated through the μ-opioid receptor (MOR) isoform (MOR1D). Existing research suggested that an NR2B-selective antagonist could be a potent drug for the treatment. The purpose of the present study was to explore whether ifenprodil could relieve intrathecal morphine-induced pruritus in mice, and its effects on analgesia, with expression of pERK1/2 in the dorsal horn of lumbar spinal cord as a marker for neuronal activation.

With animal ethics committee approval, male C57BL/6 mice were randomly divided into the NS group (i.t. ns), the Mo group (i.t. morphine), and the Mo+Ifen group (i.t. morphine+ifenprodil). The extent of pruritus was evaluated by counting the number of scratches 30 min after injection. To study analgesia, we performed the tail-flicking test before and 30, 60, 90, and 120 min after the injection. The results were expressed as a percentage of the maximal possible effect (%MPE) and the area under the curve (AUC). For the molecular experiment, mice were killed 5 min after injection. The dorsal horn of the lumbar spinal cord was assayed by western blot to detect the amount of pERK1/2 and ERK1/2 protein.

Compared with the NS group, morphine 0.5 μg i.t. induced significant pruritus in mice (mean (sd) 38(22), n=8, P<0.001). In the Mo+Ifen group, ifenprodil 0.1 and 0.5 μg almost abolished the scratching behaviour induced by morphine 0.5 μg i.t. injection (mean (sd) 13.20(6.50) and 1.00(1.27), n=8, respectively, P<0.001). As for analgesia, in the Mo+Ifen Group, morphine 0.5 μg i.t. co-injected with ifenprodil 0.1 and 0.5 μg significantly increased the AUC by 32.40 and 35.64%, respectively (n=8, P<0.05), compared with morphine 0.5 μg i.t. Compared with morphine 0.5 μg i.t., the protein expression of pERK1/2 in the dorsal horn of the lumbar spinal cord in the Mo+Ifen Group was significantly attenuated (P<0.05).

Ifenprodil could alleviate the pruritus induced by intrathecal morphine by inhibiting sensory neurone activation. Co-administration of intrathecal morphine and ifenprodil could enhance the analgesic effect of morphine, which may provide a way of optimizing intrathecal analgesia.

**References**


**Sevoflurane postconditioning protects neurones against oxygen–glucose deprivation and resuscitation via downregulation of Bid, Bim, and Puma mediated by inhibition of the mitochondrial permeability transition pore**

L. M. Zhang, X. C. Zhao, R. Li, W. B. Sun and Q. Wang

Department of Anesthesiology, Cangzhou Central Hospital, Cangzhou, China

Temporal postconditioning to induce neuroprotection against brain ischaemia–reperfusion injury insult is considered to be an effective intervention, but the exact mechanisms of sevoflurane postconditioning are poorly understood. The essential axis of activator Bid, Bim, and Puma (BH3s), BAX, and BAK in activating the mitochondrial death programme might offer common grounds for a cell death signal. In addition, inhibition of the opening of the mitochondrial permeability transition pore (mPTP) contributed to the protection by inhalation anaesthetic against cell death induced by hypoxia–ischaemia. We hypothesized that sevoflurane postconditioning might decrease the expression of Bid, Bim, and Puma and inhibit the opening of the mPTP to reduce neuronal death.

To test this hypothesis, we exposed primary cultures of cortical neurones to oxygen and glucose deprivation (OGD) for 1 h and resuscitation for 24 h (OGD/R). The assays of MTT, Annexin V, fluorescein isothiocyanate, and propidium iodiude uptake, JC-1 fluorescence, optical density (ΔOD_{560}) and western blot demonstrated reduced cell viability (P<0.05), increased cell death (P<0.05), decreased mitochondrial membrane potential and opening of the mPTP (P<0.05), and the expressions of Bid, Bim, and Puma with OGD/R exposure. The mPTP-opener atractyloside could attenuate the increase in neuronal viability and mitochondrial membrane potential mediated by sevoflurane postconditioning, and also led to a decrease in cell death, opening of the mPTP, and expression of Bid, Bim, and Puma after OGD/R treatment.

The results demonstrated that sevoflurane postconditioning markedly reduced death of cortical neurones exposed to OGD/R via downregulation of Bid, Bim, and Puma expression mediated by inhibition of the opening of the mPTP.

**References**

Role of serotonergic neurones in dorsal raphe nucleus in the facilitative effect of orexinergic signal on emergence from isoflurane anaesthesia

C. Yang and H. L. Dong

Department of Anesthesiology, Xijing hospital, Fourth Military Medical University, Xi’an, China

General anaesthesia has been used clinically for ~170 yr, but its underlying mechanism is not fully understood. At present, it is believed that a variety of neurotransmitters and neural pathways in the brain are involved in the generation and modulation of general anaesthesia.1 Recently, our group has proved that neuropeptide hypocretins (orexins) play an important role in the emergence from general anaesthesia.2,3 Studies have shown that orexin can activate the serotonergic neurons in the dorsal raphe nucleus (DRN). There is also evidence proving that 5-hydroxytryptamine has a role in anaesthesia.4,5 The present studies aimed to demonstrate that the orexinergic signal can regulate the function of serotonergic neurones to promote wakefulness during isoflurane anaesthesia.

The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation and was conducted according to the Guidelines for Animal Experimentation of our institutes. We chose adult male Sprague-Dawley rats, body weight 230–280 g.1 The animals were anaesthetized with chloral hydrate (10%, 1 ml kg−1, i.p.) and the guide cannulas for microinjection into the DRN were embedded 5–7 days before the experiment. We put the rats in the anaesthesia box filled with oxygen at a flow rate 1.5 litres min−1 and 1 MAC (1.4%) isoflurane. Fifteen minutes later, we microinjected orexin-A [30 and 100 pmol (0.3 µl)−1], orexin-B [30 and 100 pmol (0.3 µl)−1] or saline (0.3 µl). The other two groups were microinjected with the orexin type I receptor antagonist SB334867 [5 and 20 µg (0.3 µl)−1], the type II receptor antagonist TCS-OX2-29 (20 µg) −1 and 1 MAC (0.3 µl)−1], or its solvent, DMSO (0.3 µl). After 15 min, we stopped anaesthetic inhalation and observed the emergence time.2 To observe the chronic induction, the protocols were the same as above except that the reagents were given 15 min before anaesthesia. The induction time was recorded.3 Guide cannulas for microinjection to the DRN nuclei and four stainless-steel screws for monitoring EEG were embedded. Five to seven days later, the rats were anaesthetized in the anaesthesia box filled with oxygen in a flow rate of 1.5 litres min−1 and 1 MAC isoflurane (1.4%). Microinjections of orexin-A [100 pmol (0.3 µl)−1], orexin-B [100 pmol (0.3 µl)−1] or saline (0.3 µl) were executed 30 min after anaesthesia. EEGs were recorded, and the burst suppression ratios were calculated and compared with the control group.

Data are presented as mean (±SD). Compared with the saline group 13.91 (0.9) min microinjections of orexin at 100 pmol (10.05 (0.36) min) and 30 pmol (11.6 (0.36) min) into the DRN could significantly shorten the emergence time (P<0.05). Microinjection of orexin-B (100 pmol) into the DRN also had an effect to promote awaking of the rat from anaesthesia (10.59 (0.40) min, in comparison to the control; P<0.05), whereas orexin-B 30 pmol injection had no impact on the emergence time (P>0.05). After microinjection of orexin type I receptor antagonist SB334867 (20 µg), the emergence time [13.81 (0.18) min] was prolonged (P<0.05), in comparison to the DMSO control (11.33 ± 0.35 min), while the 5 µg injection [11.84 (0.46) min] had no such effect (P>0.05 vs the control). Microinjection of orexin type II receptor antagonist TCS-OX2-29 (20 µg) had no effect on the emergence time (P>0.05). During the experiment of chronic induction, microinjections of all reagents had no effect on the induction time of anaesthesia (P>0.05). Compared with the control [18.72 (2.69) min], microinjection of orexin-A into the DRN reduced the burst suppression ratio [9.55 (1.54) %] of the EEG (P<0.05), and the electroencephalogram was changed to the waveform of wakefulness.

This experiment proves that orexin can promote the emergence from anaesthesia, change the EEG pattern to an arousal waveform, and reduce the 5 wave in isoflurane anaesthesia by regulating the activities of serotonin neurones in the DRN.

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References


NR2B-containing NMDA receptor contributes to remifentanil-induced hyperalgesia via activation of DMT1(−)IRE

R. Shu1,2, L. Zhang1,2, C. Y. Wang1,2, N. Li1,2, H. Wang1,2 and G. Wang1,2

1Department of Anesthesiology, Tianjin Medical University General Hospital, Tianjin, China, and 2Tianjin Research Institute of Anaesthesiology, Tianjin, China

Intraoperative analgesia with remifentanil is limited by the high incidence of hyperalgesia. We recently found that NR2B-containing N-methyl-D-aspartate (NMDA) receptor trafficking is responsible for remifentanil-induced hyperalgesia,1 while its downstream molecules are still unclear. Divalent metal transporter 1 (DMT1)-mediated iron overload is suggested to participate in NMDA neurotoxicity, which is prevented by iron chelation.2,3 This study aimed to determine whether the NR2B-containing NMDA receptor contributes to remifentanil-induced hyperalgesia via activation of DMT1.

Behavioural testing was used to assess thermal and mechanical hyperalgesia in rats. The expression of spinal DMT1(−)IRE and DMT1(+)-IRE was detected by western blot analysis. Spinal iron concentration was measured using Perl’s stain. Rat spinal dorsal horn neurones were cultured, and DMT1(−)-IRE small interfering RNA transfection with packaging lentivirus was applied in

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the experiment in vitro. Neurones were incubated in remifentanil (40 nM) for 60 min, and intracellular Fe\(^{2+}\) and Ca\(^{2+}\) were detected with the molecular probes FeRhoNox-1 and fluo-3 AM, respectively (Fig. 5). A selective NR2B antagonist (Ro 25 6981) and an iron chelator (SH) were applied in the mechanistic study on the role of DMT1, and for prevention of hyperalgesia.

Through a combination of in vivo and in vitro studies, we showed that DMT1\(^{−}\)IRE, but not DMT1\(^{+}\)IRE, was associated with remifentanil-induced hyperalgesia. Ro 25 6981 attenuated DMT1\(^{−}\)IRE overexpression and prevented nociceptive hypersensitivity. DMT1\(^{−}\)IRE small interfering RNA silencing inhibited remifentanil-induced Fe\(^{2+}\) accumulation and Ca\(^{2+}\) overload in spinal dorsal horn neurones. Iron chelation protected against hyperalgesia in a dose-dependent manner.

Our study identifies that spinal DMT1\(^{−}\)IRE regulated by the NR2B-containing NMDA receptor contributes to remifentanil-induced hyperalgesia, while providing the rationale for development of ‘iron-targeted’ therapies.

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**References**


**Oestrogen replacement-induced neuroprotection against brain ischaemia-reperfusion injury involves activation of astrocytes via oestrogen receptor β**

Y-L. Ma\(^1\), H. Guo\(^1,2\), Y. Li\(^1\), L. Tao\(^1\), A-Q. Yin\(^1\), H-L. Dong\(^1\), W-G. Hou\(^1\) and L-Z. Xiong\(^1\)

\(^1\)Department of Anesthesiology, Xijing hospital, The Fourth Military Medical University, Xi’an, China, and \(^2\)Department of Anesthesia, General Hospital of Chinese PLA Beijing Command, Beijing, China

The incidence of ischaemic stroke is significantly increased in postmenopausal women. However, the neuroprotective effects of oestrogen replacement treatment against stroke remain controversial, and the role of astrocytes in oestrogen replacement treatment has rarely been explored. To address these questions,
we investigated the effects of oestrogen and the selective oestrogen receptor (ER) agonists propyl pyrazole triol (PPT) for ERα and diarylpropionitrile (DPN) for ERβ on astrocytes and neuronal apoptosis in conditions of oxygen and glucose deprivation and global cerebral ischaemia. Using immunofluorescence and western blot analysis, we demonstrate that hippocampal astrocytes primarily express ERβ both in vivo and in vitro. In primary cultured astrocytes, treatment with 17β-oestradiol (2.5–20 nM) or DPN (10 nM), but not PPT (10 nM), significantly increased glial fibrillary acidic protein expression. Further analyses indicated that pre-treatment with either 17β-oestradiol (10 nM) or DPN (10 nM) significantly reduced neuronal apoptosis and cleaved caspase-3 expression after the subjection of astrocytic and neuronal co-cultures to oxygen and glucose deprivation and reperfusion. Using in vivo experiments, we found that either 17β-oestradiol (50 μg kg⁻¹) or DPN (8 mg kg⁻¹) replacement (3 weeks) significantly increased glial fibrillary acidic protein expression and reduced global cerebral ischaemia-induced neuronal apoptosis in the hippocampal CA1 region of ovariectomized mice. Collectively, these results indicate that oestrogen replacement-induced neuroprotection against brain ischaemia–reperfusion injury involves the activation of astrocytes via ERβ. Thus, the discovery and design of astrocyte-selective ERβ modulators may offer a new strategy for oestrogen replacement treatment of ischaemic stroke.

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Morphine-induced conditioned place preference and alteration of NR2B subunit expression in offspring of rats that underwent late-gestational morphine exposure

Y. Liu, Y. Weidong, Q. Ming and J. Xiaoju

Department of Anesthesiology, Yijishan Hospital, Wannan Medical College, Wuhu, China

Opioid abuse by pregnant women results in neonatal abstinence syndrome of 95% newborns.1 Studies have reported that offspring exposed to morphine show alterations in neurobehavioural development, including tolerance to morphine.2 However, the mechanism of susceptibility to morphine addiction remains unclear in rat offspring. As we know, there are individual differences in susceptibility to morphine addiction.3 Exploration of the neurobiological mechanism, therefore, has important theoretical and real-life significance. The N-methyl-D-aspartate (NMDA) receptor is a subtype of the ionic glutamate receptor and is closely associated with opioid drug dependence.4 The formation of morphine addiction is closely related to glutamate sites and is located in the NR2B-containing NMDA receptor.5 We examined the relationship of NR2B subunit expression and susceptibility of morphine-induced conditioned place preference (CPP) in offspring of rats that had undergone late-gestational morphine exposure.

Forty-eight late pregnant Sprague–Dawley rats were randomly divided into a morphine group (Group M) and a saline group (Group C). Pregnant rats in Group M were administered morphine s.c. at the back of neck with an initial dose of 3 mg kg⁻¹, with a daily increment of 1 mg kg⁻¹ to a final dosage of 6 mg kg⁻¹ on days 12–18 of pregnancy. Pregnant rats in Group C were treated with the same volume of saline in the same protocol.

Seventy offspring rats of similar body weight were selected from the two groups and conventionally fed for 8 weeks. A constant dosage of morphine (3 mg kg⁻¹ s.c.) was administered for 7 days to start the CPP protocol, and the CPP effects were examined after 24 h of condition. Then the rats of Group M were divided into high-CPP (n=10), moderate-CPP (n=30), and low-CPP groups (n=10) according to the CPP scores measured. To study whether susceptibility differences exist in the offspring rats, we used only the high-CPP and low-CPP groups for analysis. Western blot was performed to determine the NR2B expression in offspring rats with high-CPP, low-CPP, and Group C.

Results are presented as mean (SD). The CPP cardinality value before the test showed no statistical difference for the three groups (P=0.4625), yet the score was significantly higher in the high-CPP group 552.6 (132.6) s than in the low-CPP group 223.4 (55.14) s and Group C 42.93 (31.78) s. The difference was significant (P<0.01). Western blotting revealed that the high-CPP group had higher NR2B expression 0.81 (0.01) than the low-CPP group 0.64 (0.01) and Group C 0.55 (0.01); P<0.01; Fig. 6.

Higher susceptibility of morphine-induced CPP was found in offspring of rats that had undergone late-gestational morphine exposure, suggesting that this vulnerability may be associated with upregulated NR2B expression. Narita and colleagues6 have reported that NR2B subunit protein was specifically upregulated in the limbic forebrain of morphine-conditioned mice and that intracerebroventricular treatment with an antibody against NR2B subunits abolished the morphine-induced place preference. To sum up, alteration of NR2B subunit expression plays an important role in the offspring rats of with late-gestational morphine exposure, and they show susceptibility to morphine-induced addiction.

Fig 6 NR2B subunit expression in NAc of high- (H) and low-CPP (L) groups and control group (C). Top panel shows a protein imprinting image of the NR2A subunit of three groups of rats in NAc. Bottom panel shows the protein imprinting image greyscale comparison of NR2A subunits of the three groups of rats in NAc. The y-axis indicates the grey ratio of the NR2A and β-actin belt in each group.
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References

Goal-directed intraoperative fluid therapy guided by mini-fluid challenge in elective colorectal resection: a prospective randomized study

L. Zhang, S. Y. Wang and B. Liu

Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, Sichuan, China

Goal-directed fluid therapy during surgery may improve postoperative recovery.1 Mini-fluid challenge has been shown to be able to predict fluid responsiveness.2 In the present study, we aimed to explore whether protocolized fluid administration guided by mini-fluid challenge with transoesophageal echocardiography can improve the postoperative outcome in patients undergoing elective colorectal resection.

With ethical committee approval and informed consent, 42 patients undergoing elective colorectal resection were recruited into a prospective randomized controlled trial. A transoesophageal echocardiography probe was placed in each patient to monitor the subaortic velocity-time index. The intervention group received additional colloid based on the assessment of fluid responsiveness by mini-fluid challenge, whereas the control group received perioperative fluid at the discretion of the anaesthetist. Main outcomes were postoperative complications, return of gastrointestinal function, postoperative stay, and cytokine markers of the systemic inflammatory response.

During surgery, patients in the intervention group received more colloid mean (SD) [616 (273) vs 432 (228) ml, P < 0.05] and experienced fewer episodes of hypotension mean (SD) [1.8 (1.2) vs 3.2 (1.5), P < 0.05]. The velocity-time index at the end of surgery was higher in the intervention group [23.16 (3.58) vs 20.88 (2.70) cm/s, P < 0.05]. Patients in the intervention group had a significantly reduced time to the first bowel movement [2.5 (0.6) vs 3.6 (1.4) days, P < 0.05]. Although not statistically significant, the postoperative length of stay was shorter in the intervention group. However, there was no difference in postoperative complications in these two groups. The interleukin-6 concentration on the first day after surgery was higher in the control group [87.02 (31.98) vs 132.88 (67.50) pg ml⁻¹, P < 0.05].

A protocol-based fluid-optimization programme guided by mini-fluid challenge with transoesophageal echocardiography leads to speedier return of bowel function and attenuates the inflammatory response to surgical trauma. In addition, this fluid strategy may shorten hospital stay.

References

Novel combined left and right atrial pressure-monitoring catheter: a simple and reliable left atrial pressure-monitoring method in paediatric cardiac surgery

J. Ding, Q. Y. Zhang, Q. P. Luo and F. X. Yan

Department of Anesthesiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Left atrial pressure (LAP) monitoring is important to assess left atrial function and manage haemodynamic stabilization of paediatric congenital heart disease patients during the perioperative period.1 Existing LAP monitoring methods are associated with a high risk of haemorrhage and need double catheterization.2 3 This study reports a novel LAP-monitoring method that can monitor LAP and central venous pressure (CVP) and administer a drug or infusion via only one catheter, by inserting a micro-tube into a reformed triple-lumen catheter. We also compare it with the traditional double catheterization method in paediatric cardiac surgery (Fig. 7).

With ethical committee approval and informed consent, 240 paediatric patients, undergoing surgery for congenital heart disease and requiring LAP monitoring, were recruited. The novel catheter was used for the test group; a central venous catheter for CVP monitoring and a single-lumen catheter for LAP monitoring were used for the control group by the traditional method. We compared the pressure-monitoring accuracy of LAP and CVP, arrival and prolappe of the LAP-monitoring catheter, feasibility of infusion or drug administration, and catheter-related complications (arrhythmia, infection, and thrombosis) between the two groups.

Both the novel catheter and the traditional method could accurately measure LAP and CVP. The pressure-monitoring concordance rate of LAP and CVP were both higher than 95.00% and had no difference between them. The arrival rates of the LAP-monitoring catheter in the two groups were both 100%, but prolappe in the test group (five patients) was significantly less than in control group (12 patients). Infusion or drug administration was feasible in each group. There were no catheter-related complications or serious adverse events in either group.

The use of this novel combined left and right atrial pressure-monitoring catheter, for the first time in this study, has proved to be safe and reliable to monitor LAP and to provide CVP monitoring and infusion via only one catheter without the disadvantages of existing monitoring methods. This new method has clinical advantages, especially in paediatric open-heart surgery.

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The novel combined catheters used in this study were provided by Yixinda.

References
Comparison of qCON and BIS monitoring hypnotic effect during general anaesthesia

Y. Pan, S. Ge, Q. W. Liao, P. Fort and E. W. Jensen

Department of Anaesthesia, Zhongshan Hospital, Shanghai, China

The qCON (qCON2000; Quantium Medical, Spain)\(^1\)\(^2\) is an index to assess the level of hypnosis during sedation and analgesia. It is calculated from the raw EEG using technology based on Adaptive Neuro Fuzzy Inference Systems (ANFIS). ANFIS is a data-driven approach, rather than assuming an underlying function governing the relationship between an input and an output. The qCON monitor uses a three-electrode montage: middle forehead (+), malar bone (−), and left forehead electrode used as reference. The objective of the present study was to compare two depth-of-anaesthesia indices, the qCON2000 and the BIS (BIS VISTA; Covidien, USA).\(^3\) Both indices are derived from the frontal EEG.

The study was approved by the ethics committee at the Zhongshan Hospital in Shanghai (China). The database contains data recorded from 19 patients [11 men, mean (±) age 56 (14) yr] undergoing major surgery under sedation and analgesia with propofol, remifentanil, and sevoflurane. The qCON was assessed simultaneously with the BIS as a reference for the qCON.

The mean for each minute was calculated for the qCON and BIS indices. Frames with low signal quality were excluded. The BIS was used as a reference divided into four levels. The prediction probability, Pk, between the qCON and BIS indices was calculated to assess the probability of the qCON to predict the level of consciousness. Also, the Pearson product–moment correlation coefficient, r, was calculated to assess the correlation between the two indices. The prediction probability, Pk, between qCON and BIS was mean (±) 0.82 (0.12). The correlation coefficient, r, was 0.67 (95% CI 0.47–0.87).

Figure 8 shows an example of the hypnotic indices recorded with the two monitors.

Acknowledgement

The novel combined catheters used in this study were provided by Yixinda.

References


Can sonographic measurements of mandibular condyle mobility predict difficult laryngoscopy?

W. D. Yao\(^1\)^\(^3\), B. Wang\(^1\), T. Yu\(^1\), Z. B. Shen\(^2\), H. Wu\(^1\), X. J. Jin\(^1\) and Y. H. Li\(^3\)

\(^1\)Department of Anesthesiology, \(^2\)Department of Ultrasonic Medicine, The First Affiliated Hospital of Wannan Medical College, Wuhu, China, and \(^3\)Department of Anesthesiology, The First Affiliated Hospital of Anhui Medical University, Hefei, China

Mandibular condylar mobility, as measured by sonography, is a new reliable method for assessment of temporomandibular joint mobility.\(^1\) Compared with other related assessment methods,\(^2\) however, it is still uncertain whether sonographic measurements indicating limited mandibular condylar mobility can...
predict difficult laryngoscopy. The purpose of this study was to observe its capacity for predicting difficult laryngoscopy.

Patients who were administered tracheal intubation for elective surgery under general anaesthesia were enrolled in the study. Mandibular condylar mobility was assessed by sonography through condylar translation measurements (Fig. 9A–D). Other variables that were correlated with temporomandibular joint mobility, such as inter-incisor distance, mandibular protrusion distance, upper lip bite test, and whether the condyle–tragus distance was less than one finger breadth, were also evaluated before administration of anaesthesia. The Cormack–Lehane level was also documented before intubation. The correlation analysis, internal agreement tests, and receiver operating characteristic curve analysis were used.

A total of 536 patients were prospectively included, and difficult laryngoscopy was reported in 47 patients. The condylar translation prediction criteria for difficult laryngoscopy determined by the Youden index was condylar translation ≤10 mm (defined as limited condylar translation). The condylar translation showed the highest correlation with the Cormack–Lehane levels (r=–0.476, P<0.001). Among observed predictors, the limited condylar translation had the highest k value for difficult laryngoscopy (k=0.687, 95% confidence interval [95% CI]: 0.586–0.788). Additionally, the limited condylar translation had the highest area under the receiver operating characteristic curve (0.958, 95% CI: 0.937–0.973; Fig. 9E), odds ratio (122, 95% CI: 47–313), sensitivity (0.872, 95% CI: 0.743–0.952), and specificity (0.947, 95% CI: 0.923–0.965) for predicting difficult laryngoscopy.

Mandibular condyle mobility, as assessed by sonography, was highly correlated with difficult laryngoscopy and had powerful predictive capacity. This method may become a new useful way to predict a difficult airway.

![Image](https://academic.oup.com/bja/article-abstract/116/6/e912/2566337)
Laryngoscope and a new tracheal tube assist lightwand intubation in difficult airways resulting from an unstable cervical spine

C. N. Wu¹, W. H. Ma¹, J. Q. Wei¹, H. F. Wei², Q. Y. Cen¹ and Q. X. Cai¹

¹Department of Anesthesiology, the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, China, and ²Department of Anesthesiology and Critical Care, University of Pennsylvania, Philadelphia, PA, USA

Patients with cervical spine instability usually present with a limited range of neck motion and a challenging airway for tracheal intubation.¹ A special tracheal tube designed by H.F.W. called the WEI Jet Endotracheal Tube (WEI JET; Wei Medical LLC, Cherry Hill, NJ, USA) applies supraglottic jet oxygenation and ventilation during tracheal intubation and assists blind tracheal intubation in patients with a difficult airway.² We evaluated the effectiveness and usefulness of the WEI JET in combination with a lightwand under direct laryngoscopy in difficult tracheal intubation resulting from an unstable cervical spine.

This study was approved by the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine. Written informed consent was obtained from all patients. Ninety patients with unstable cervical spine disorders (ASA I–III) undergoing general anaesthesia were included and randomly assigned to three groups, based on the device used for intubation: lightwand only (LW); lightwand under direct laryngoscopy (DL); or lightwand with WEI JET under direct laryngoscopy (WEI; Fig. 10).

No statistically significant differences were detected among the three groups with respect to patient characteristics and Cormack–Lehane grade. There were statistically significant differences between the three groups for overall intubation success rate (P=0.015) and first-attempt success rate (P=0.000). The intubation time was significantly longer in the WEI group mean (SD) [110.8 (18.3) s] than in the LW group [63.3 (27.5) s, P=0.000] and DL group [66.7 (29.4) s, P=0.000], but the lowest SpO₂ in the WEI group was significantly higher than in other two groups (P<0.01). The WEI/JET significantly reduced successful tracheal intubation attempts compared with the LW group (P=0.043). The severity of sore throat was similar in the three groups (P=0.185).

The combined use of the WEI/JET under direct laryngoscopy helps to assist tracheal intubation and improves oxygenation during intubation in patients with a difficult airway secondary to unstable spine disorders.

References

Simvastatin attenuates neuropathic pain by inhibiting the RhoA/LIMK/cofilin pathway

Y. Qiu and Y. G. Huang

Department of Anesthesiology, PUMC Hospital, CAMS and PUMC, Beijing, China
Statins, the inhibitors of HMG-CoA reductase, are well known as a type of cholesterol-lowering agent. In addition, statins also exert a number of cholesterol-independent, pleiotropic effects. There is accumulating evidence demonstrating that statins may protect against neuropathic pain, through the ability to prevent the isoprenylation of small molecular G proteins, such as Rho and Ras. In the present study, we investigated the anti-nociceptive effects of intrathecal simvastatin treatment on chronic constriction injury (CCI) nociceptive behaviour and its inhibiting effect on the RhoA/LIMK/cofilin pathway in rats.

Forty-two male Sprague–Dawley rats weighing 180–200 g were randomly divided into a CCI group (n=30), a sham group (n=15), and a naïve group (n=15). Rats in each group received chronic sciatic nerve constriction injury (CCI). The paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) were used to evaluate and measure the behavioural changes before operation and on days 1, 3, 7, 14, and 21 after surgery. The L4–L6 dorsal root ganglia were removed on days 1, 3, 7, 14, and 21 after surgery. RT-qPCR was used to test the mRNA expression of RhoA and its downstream effector, Rho kinase. Western blot was performed to explore the protein expression of RhoA, phosphorylated LIMK, phosphorylated cofilin and the membrane-to-cytosol ratio of RhoA. To observe whether activation of the RhoA/LIMK/cofilin pathway was associated with the behavioural change of CCI, Y-27632, the Rho kinase inhibitor, was administrated intrathecally after CCI surgery, and PWMT and PWTL were recorded on postoperative day 1, 3, 7, and 14. Western blot was performed to explore the protein expression of phosphorylated LIMK, phosphorylated cofilin and the membrane-to-cytosol ratio of RhoA. To evaluate the influence of simvastatin on neuropathic pain and its molecular mechanism, simvastatin (10 µg µl⁻¹) or the same volume of vehicle control was applied through the intrathecal tube once daily after CCI. Behavioural tests were performed to observe the therapeutic effect on mechanical and thermal hyperalgesia. The L4–L6 dorsal root ganglia were removed on postoperative day 14, followed by RT-qPCR and western blot evaluation of the RhoA/LIMK/cofilin pathway activation.

Compared with the sham group and the naïve group, an increase of PWTL was observed from day 3 after surgery, whereas PWMT increased from day 7 after surgery in the CCI group (P<0.05). Furthermore, the expression of RhoA was greatly increased from day 7 after surgery in the CCI group in terms of western blot and RT-qPCR (P<0.05). Administration of Y-27632 attenuated behavioural changes in CCI rats (P<0.05) and decreased the expression of phosphorylated LIMK and phosphorylated cofilin on postoperative day 14 (P<0.05). When simvastatin was administrated intrathecally, the pain behaviours of the CCI rats were significantly improved (P<0.05). Simvastatin also inhibited the protein expression of RhoA, phosphorylated LIMK, and phosphorylated cofilin on postoperative day 7 (P<0.05). Meanwhile, both Y-27632 and simvastatin decreased the membrane-to-cytosol ratio of RhoA significantly, which increased in the CCI group on postoperative day 14 and was highly dependent on the cytoskeleton.

The overactivated cytoskeleton caused by the activation of RhoA/LIMK/cofilin pathway may act as a scaffold for nociceptive signalling to trafficking, leading to the persistence of chronic neuropathic pain. Simvastatin can attenuate neuropathic pain of CCI rats and may inhibit the actin-mediated intracellular trafficking by inhibiting the RhoA/LIMK/cofilin pathway.