Acta Pharmacologica Sinica (2012) 33: 27–33
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1671-4083/12 $32.00
www.nature.com/aps

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Original Article

Retraction: Lipid metabolism disturbances and AMPK activation in prolonged propofol-sedated rabbits under mechanical ventilation

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Aim: To explore the mechanisms underlying the propofol infusion syndrome (PRIS), a potentially fatal complication during prolonged propofol infusion.
Methods: Male rabbits under mechanical ventilation through endotracheal intubation were divided into 3 groups (n=6 for each) that were sedated with 1% propofol (Group P), isoflurane (Group I) or isoflurane while receiving 10% intralipid (Group II), respectively. Blood biochemical parameters were collected at 0, 6, 12, 18, 24, and 30–36 h after the initiation of treatments. The hearts were removed out immediately after the experiments, and the level of tumor necrosis factor (TNF)-α in the hearts were studied using immunohistochemistry. AMP-activated protein kinase (AMPK) and phospho-AMPK in the hearts were assessed using Western blotting.
Results: The mortality rate was 50% in Group P, and 0% in Groups I and II. The serum lipids and liver function indices in Group P were significantly increased, but moderately increased in Group II. Significant decreases in these indices were found in Groups I. All the groups showed dramatically increased release of creatine kinase (CK). Intense positive staining of TNF-α was found in all the heart samples in Group P, but only weak and neglectful staining was found in the hearts from Group II and Group I, respectively. AMPK phosphorylation was significantly increased in the hearts of Group P.
Conclusion: Continuous infusion of large dose of propofol in rabbits undergoing prolonged mechanical ventilation causes hyperlipidemia, liver dysfunction, increased CK levels, AMPK activation and myocardial injury. The imbalance between energy demand and utilization may contribute to PRIS.

Keywords: propofol; propofol infusion syndrome (PRIS); sedation; isoflurane; intralipid; tumor necrosis factor alpha; AMPK

Acta Pharmacologica Sinica (2012) 33: 27–33; doi: 10.1038/aps.2011.155; published online 12 Dec 2011

Introduction

Propofol is the most commonly used intravenous anesthetic, and it has gained widespread popularity because of its rapid onset and short duration of recovery. However, a potentially fatal complication after prolonged propofol infusion has been described in an increasing number of reports[1–3]. This complication has been termed propofol infusion syndrome (PRIS)[4] and includes progressive myocardial failure, bradycardia, lactic acidosis, rhabdomyolysis, hyperkalemia, lipemia, acute renal failure, and death. Unfortunately, little data on the mechanisms of PRIS are available.

PRIS resembles mitochondrial cytopathies and acquired carnitine deficiency[5, 6]. Malonyl-carnitine, C5-acylcarnitine, creatine kinase, troponin T (TnT) and myoglobin levels are increased in pediatric PRIS, which is consistent with impaired fatty acid oxidation due to a reduction of the mitochondrial entry of long-chain acylcarnitine esters and the failure of the respiratory chain. High concentrations of propofol impair mitochondrial respiration in rat brain synaptosomes (Marion *et al* citation)[6]. An uncoupling of oxidative phosphorylation due to an increase in the proton permeability of the inner mitochondrial membrane has been observed in isolated rat liver mitochondria[7]. Propofol uncouples oxidative phosphorylation and inhibits the electron transport chain, which decreases mitochondrial energy production, in isolated perfused guinea pig hearts[8]. The mitochondrial damage that is associated with propofol infusion is characterized by an imbalance between energy demand and utilization, which is a key pathogenetic mechanism in PRIS. AMP-activated protein kinase (AMPK) is also a primary regulator of the cellular response to lowered ATP levels in eukaryotes[9, 10]. AMPK is

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Received 2011-07-14 Accepted 2011-09-23

a serine/threonine protein kinase in the Snf1/AMPK protein kinase family[11]. AMPK activity requires the phosphorylation of the alpha subunit on Thr^{172} in its activation loop by one or more upstream kinases (AMPKKs)[11]. AMPK phosphorylation downregulates ATP-consuming processes, such as the synthesis of fatty acids, cholesterol, and protein, but it also upregulates ATP-producing catabolic pathways, such as fatty acid oxidation and glucose uptake[11, 12]. However, the role of AMPK in PRIS is not known.

This study examined the effects of a prolonged infusion of large doses of 1% propofol on the cardiac morphology and blood biochemical profiles in rabbits undergoing prolonged mechanical ventilation, and determined the role of AMPK signaling in PRIS. Our results demonstrated that the infusion of a large dose of propofol induced an emergent life-ending syndrome in rabbits that was consistent with PRIS and an increase in the phosphorylation of AMPK at Thr^{172}.

Materials and methods

Materials

Injections of 1% propofol (*w/v*) and 10% intralipid were purchased from AstraZeneca Pharmaceuticals (AstraZeneca, Cheshire, UK). Isoflurane was obtained from Baxter International, Inc (Deerfield, IL, USA). Antibodies against phospho-AMPK at Thr^{172} and α-AMPK were purchased from Cell Signaling Technology, Inc (China), and anti-TNF-α antibody was purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). ECL reagent was purchased from Pierce Thermo Fisher Scientific, Inc (Rockford, IL, USA). Other chemical agents were purchased from Sigma (St Louis, MO, USA).

Animals

Healthy male New Zealand rabbits weighing 2.5–3.0 kg at 3 months of age were used in this study (n=18). The rabbits were exposed to a 12-h light/dark cycle and received food and water *ad libitum*.

The animals were premedicated with a mixture of xylazine (50 μg/kg, im) and atropine (50 μg/kg, im) after an overnight fast and anesthetized with ketamine hydrochloride (0.05 g/kg, im) for endotracheal intubation 20 min later. Artificial ventilation was initiated using a pressure ventilator (ALC-V8S, Alcott Biotech Co, Ltd, Shanghai, China). The initial settings (flow rate, 8 L/min; respiratory rate, 20–25 breath/min; peak inspiratory pressure, 12 cmH_2O; and inspiration triggering, -2 cmH_2O) provided a tidal volume of approximately 6–8 mL/kg, and these settings were adjusted to maintain an arterial P_{aCO2} between 30 and 40 mmHg if required. The initial 40% FIO_2 (fraction of inspired O_2) was adjusted to achieve a P_{aO2} over 90 mmHg or an SpO_2 >95%.

A 5-lead electrocardiogram invasive arterial blood pressure, heart rate, SpO_2, respiratory rate and body temperature were continuously monitored (Hewlett Packard M3046A/Viridia). The levels of electrolytes and glucose were assessed every 4 h (Hitachi 7600, Japan). An $NaHCO_3$ solution was administered iv if metabolic acidosis occurred (20 mL $NaHCO_3$ 5% was administered within 1 h if pH<7.25 and HCO_3^- <15 mEq/L). Electrolytes and glucose levels were corrected if necessary. A dose of 300 IU of heparin sodium per kilogram of bodyweight was used via subcutaneous injection every 12 h for thrombosis prophylaxis. A central ear artery and two marginal ear veins were catheterized (20-gauge polyethylene catheter) to provide intra-arterial and IV access, respectively. The animals were placed in a supine position on a heated operation table and covered with an isothermic blanket to maintain normothermia. The urinary bladder was catheterized using an 8 CH Foley catheter to facilitate urine collection. Animals received either Ringer's lactated saline solution iv for electrolyte and fluid support or dextrose 5% saline during the experiment when glucose levels decreased to <0.8 g/L (normal range 0.75–1.40 g/L). Animals (n=6/group) were randomly assigned to sedation with 1% propofol (Group P), isoflurane (Group I), or isoflurane while receiving 10% intralipid (Group II). The Shanghai Jiaotong University Animal Care and Use Committee approved all experimental procedures and protocols, and all studies were performed in accordance with the National Research Council (US) Institute Guidelines for Laboratory Animal Care.

Measurement of blood biochemical variables

Venous blood samples were collected in test tubes and centrifuged at 3000×*g* for 10 min to obtain serum. The following variables were measured (ILAB 600 Analyser, Lichtenfels, Germany): potassium, sodium, glucose, urea, creatinine, lactate dehydrogenase (LDH), total protein content, albumins, total bilirubin, direct bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), cholesterol, triglycerides, and creatine kinase (CK).

Histological examination

Animals were exsanguinated after the termination of experiments, and the hearts were quickly excised and divided into two parts. One half was fixed in 10% neutral-buffered formaldehyde, embedded in paraffin wax, sectioned serially at 4 μm, and stained with hematoxylin-eosin (H&E). Immunohistochemical investigation of heart samples was performed utilizing an anti-TNF-α monoclonal antibody. Cardiac tissues slices were subjected to histological examination under an Olympus BX microscope by two independent researchers in a blinded manner.

The other half of the heart was rapidly frozen on dry ice. Frozen tissue was homogenized in ice-cold lysis buffer containing 10 mmol/L Tris, 100 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L NaF, 20 mmol/L $Na_4P_2O_7$, 2 mmol/L Na_3VO_4, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X-100, 10% glycerol, 10 mg/L leupeptin, 60 mg/L aprotinin, and 1 mmol/L phenylmethanesulfonyl fluoride. Cell lysates were centrifuged at 16 000×*g* for 10 min, and the supernatants were collected.

Immunoblotting

Western blotting (WB) analysis was performed on total cell lysates. Protein concentrations were measured using the Brad-

ford protein assay[13]. All samples were mixed with Laemmli sample buffer [sodium dodecyl sulfate (SDS)] and placed in a boiling water bath for 5 min. Proteins (50 μg) were resolved in 10% SDS-polyacrylamide gels, transferred to nitrocellulose, and incubated overnight with primary antibodies against phospho-AMPK at Thr[172] and α-AMPK (1:2000). Immunoblots were developed using horseradish peroxidase-conjugated goat antimouse or goat antirabbit immunoglobulin G (1:3000). Protein bands were visualized using the ECL reagent (Pierce Thermo Fisher Scientific, Inc, Rockford, IL, USA), and density was quantified using the Scion Image software (Scion Corp, Frederick, MD, USA).

Experimental protocols

Protocol 1 (Group P): effect of prolonged 1% propofol sedation on rabbits under mechanical ventilation

Animals were sedated using a continuous propofol infusion (1% Diprivan, AstraZeneca, Cheshire, UK) for 36 h or until death. Propofol was administered at the first signs of awakening from general anesthesia (restoration of corneal reflex) and at an initial rate (IR) of 10 $mg \cdot kg^{-1} \cdot h^{-1}$ using an infusion pump (Baxter AS40A, 1800 Williamson Ct Louisville, KY, USA). The IR was adjusted in 5 $mg \cdot kg^{-1} \cdot h^{-1}$ steps to maintain the desired level of sedation when necessary. The level of sedation was assessed using a corneal reflex response every 30 min; earlier assessments were performed only when clinical signs of awakening were observed (25% increase in heart rate and/or arterial blood pressure from the previous measurement, any increase in respiratory rate from the ventilator setting). The criteria for the desired level of sedation are listed in Table 1. The IR was reduced if necessary in 5 $mg \cdot kg^{-1} \cdot h^{-1}$ steps every 15 min until the initial signs of awakening were observed to avoid propofol overdose. The IR was returned to the previously identified safe dose to maintain an adequate level of sedation.

Table 1. Criteria for reflex response evaluation indicating light level of sedation in mechanically ventilated rabbits.

Reflex	Response
Palpebral	–
Corneal	+
Swallowing	–

–, loss; +, preservation.

Protocol 2 (Group I): Effect of isoflurane sedation on rabbits undergoing prolonged mechanical ventilation

Animals were sedated using an alternative anesthetic, isoflurane (Baxter International, Inc, Deerfield, IL, USA), for 36 h with a conventional vaporizer at an initial rate of 1% to distinguish the effects of propofol sedation from the effects of prolonged mechanical ventilation. The doses were adjusted if necessary to maintain a light level of sedation (Table 1).

Protocol 3 (Group II): Effect of 10% intralipid on isoflurane-sedated rabbits undergoing prolonged mechanical ventilation

One group of animals was sedated using the inhaled anesthetic, isoflurane, during 10% intralipid administration for 36 h to distinguish the effect of a 1% propofol infusion from lipid vehicle (10% intralipid) in sedated rabbits under mechanical ventilation. The initial isoflurane concentration was 1%, and this concentration was adjusted if necessary to maintain a light level of sedation. Intralipid (10%) was infused continuously at a rate that was equal to the average hourly 1% propofol infusion rate in Group P.

Statistical analysis

Data from the blood biochemical analysis are presented as the mean±SD. These data were subjected to a repeated measures analysis of variance. The differences between the mean values within time points in the same group were tested using the Bonferroni test, and differences between groups for the same time point were compared using the Student's *t*-test. A probability of $P<0.05$ was considered statistically significant. The results shown in the blots are representative of ≥3 independent experiments and represent the mean±SD. Densitometry data were analyzed using a 1-way ANOVA with a significant level of $P<0.05$.

Results

Mortality rate, blood biochemistry, and blood pressure

The mortality rate in Group P was 50%; one animal survived for 30 h and the other animals survived for 34 h. No deaths were observed in Groups I and II. The deterioration of the clinical course in Group P was evident at 10±2.12 h following the onset of propofol infusion and included a significant increase in serum lipid, GOT, GPT, LDH, total protein, total bilirubin, direct bilirubin, and CK levels (Table 2). A rapid drop in arterial pressure without a corresponding increase in heart rate followed these increases (Table 3), which may mean that the heart rate is more sensitivity to the effects of these drugs.

A significant increase ($P<0.05$) in serum CK levels was observed, but total protein content, albumin and creatinine levels exhibited a significant decrease 12 h after the onset of isoflurane administration in Group I (Table 4). CK levels were significantly lower in Group I than in Group P ($P<0.05$). Arterial blood pressure demonstrated a significant decrease in Group I at the termination of the experiment, but these values remained much higher than the levels in Group P.

Total protein content, total bilirubin, direct bilirubin, triglycerides, cholesterol, and CK gradually increased beginning at 12 h of infusion in Group II, and GOT, GPT, and LDH levels increased 24 h after infusion. Serum albumin levels decreased 12 h after the onset of 10% intralipid administration in isoflurane-sedated rabbits (Table 5). Triglycerides, CK levels and total protein content were significantly lower in Group II compared with Group P ($P<0.05$). No deviations in vital signs were noted during the experiments, except for a decrease in systemic arterial pressure. However, systemic arterial pres-

Table 2. Blood biochemical measurements in propofol-sedated rabbits. Mean±SD. *n*=6. [b]*P*<0.05 when compared with pretreatment (0 h) values.

Variable	Time (h) 0	12	24	30–36	Reference range
Creatinine (μmol/L)	72.0±5.6	61.1±6.2[b]	77.8±16.7	86.8±22.2	70–120
GOT (U/L)	20.0±4.9	101.8±36.2[b]	166.8±65.6[b]	143.0±40.4[b]	<30
GPT (U/L)	45.3±12.2	70.0±14.5[b]	180.0±45.0[b]	190.1±59.7[b]	<5–47
LDH (U/L)	242.3±34.4	352.7±26.4[b]	455.5±20.5[b]	620.5±12.9[b]	243±50
Total protein (g/L)	56.0±8.0	75.0±14.5	180.0±45.0[b]	190.1±59.7[b]	54–75
Albumins (g/L)	36±5.0	30±8.3	25±4.2[b]	20±5.0[b]	27–50
Total bilirubin (μmol/L)	11.1±5.5	144.4±8.6[b]	250.0±8.3[b]	227.8±12.1[b]	<26
Direct bilirubin (μmol/L)	5.6±2.1	55.6±11.0[b]	77.8±11.1[b]	44.4±11.0[b]	<7
Cholesterol (mmol/L)	3.98±2.4	11.4±1.2[b]	19.3±2.8[b]	18.3±3.1[b]	<5.2
Triglycerides (mmol/L)	3.7±1.1	305.5±7.9[b]	451.2±114.1[b]	386.7±6.9[b]	<2.0
CK (IU/L)	1059.3±383.0	5842.0±3428.6[b]	8958.3±3645.1[b]	11389.7±7462.6[b]	50–1300

CK, creatine kinase; LDH, lactate dehydrogenase; GOT, glutamic oxaloacetic transaminase; GST, glutamic pyruvic transaminase.

Table 3. Hemodynamic data of mechanically ventilated rabbits sedated with propofol (Group P), isoflurane (Group I) and isoflurane while receiving intralipid (Group II). Mean±SD. *n*=6. [b]*P*<0.05 when compared with pretreatment (0 h) values; [e]*P*<0.05 when compared with the same time-point values in Group P.

Variable	Time (h) 0	6	12	18	24	30–36
Group P						
AP syst (mmHg)	90.5±13.5	98.3±15.6	81.6±12.3[b]	79.5±10.1[b]	71.4±10.6[b]	33.2±4.4[b]
AP diast (mmHg)	60±19.3	61.6±8.5	53.6±5.2	45.6±5.4	44.3±5.6	24.1±3.2[b]
AP mean (mmHg)	69.5±10.5	73.1±11.3	62.8±7.8	56.3±7.3[b]	53.4±7.1[b]	26.8±3.8[b]
HR	220±13.3	223.5±27.1	229.2±19.1	222±11.6	208.5±13.3	187±9[b]
Group I						
AP syst (mmHg)	94.2±15.2	94.3±13.4	99.5±16.3	95±12.5	99.2±13.9	87.6±10.5
AP diast (mmHg)	70.8±9.2	65.1±8.2	72.4±8.6	70.1±7.6	73.3±8.1	55.3±4.7[be]
AP mean (mmHg)	75±9.2	67.8±8.3	79.1±10.4	78.4±8.7	80.1±9.4	65.2±7.1
HR	230.6±20.3	232.8±16.1	227±19.9	228.6±18.9	219.4±18.4	216.2±14.5
Group II						
AP syst (mmHg)	105.1±15.4	100.3±18.9	87.9±10.1[b]	86.5±8.9[b]	80.6±7.6[b]	70.3±7.5[be]
AP diast (mmHg)	66.3±6.1	67.2±6.7	64.3±5.8	66.1±5.6	60.1±5.4	51.2±4.9[be]
AP mean (mmHg)	79.1±8.9	77.6±7.1	71.2±6.7	73.1±6.7	66.5±6.2[be]	57.5±5.6[be]
HR	224.5±18.8	223.5±14.9	201.0±16.0	208.8±17.8	231.3±11.5	219.8±13.2

0 h=before intubation; AP, arterial blood pressure; HR, heart rate.

sure was significantly better than that in Group P.

Histological findings

The common characteristics of PRIS include cardiovascular collapse, cardiac arrhythmias, and metabolic acidosis[4, 14]. Therefore, the morphological features of heart specimens were investigated to determine whether these features were consistent with the features of PRIS. The histological examination revealed little infiltration of acute inflammatory cells in the myocardial fibers in all 3 groups (data not shown). However, TNF-α signals were intensely positive in all heart samples of Group P, but TNF-α staining intensity was weak in Group II and negligent in Group I (Figure 1).

Alterations in AMPK and phospho-AMPK expression in myocardial cells

This study revealed an increased phosphorylation of AMPK at Thr[172] in the heart following large doses of propofol infusion; little AMPK phosphorylation was observed in cardiac tissues of the other two groups (Figure 2).

Discussion

This study demonstrated that prolonged propofol sedation at high doses contributes to the incidence of fatal complica-

Table 4. Blood biochemical measurements in isoflurane-sedated rabbits. Mean±SD. *n*=6. [b]*P*<0.05 when compared with pretreatment (0 h) values. [e]*P*<0.05 when compared with the same time-point values in Group P.

Variable	Time (h) 0	12	24	30–36	Reference range
Creatinine (μmol/L)	72.2±5.6	61.1±6.2[b]	50.0±7.8[be]	38.9±5.6[be]	70–120
GOT (U/L)	5.5±2.0	25.0±3.3	35.5±3.0	31.5±6.0	<30
GPT (U/L)	41.2±4.8	45.0±3.2	42.2±1.1	46.0±9.1	<5–47
LDH (U/L)	236.5±90.5	241.1±76.6	253.3±152.2	201.1±92.1	243±50
Total protein (g/L)	59.1±6.1	40.1±18.2	31.1±9.0[be]	41.0±4.1[e]	54–75
Albumins (g/L)	40.0±2.0	26.1±10.1[b]	23.1±11.2[b]	25.2±2.0[be]	27–50
Total bilirubin (μmol/L)	15.1±2.0	14.4±8.6	25.0±8.3	27.8±12.1	<26
Direct bilirubin (μmol/L)	5.1±0.5	4.2±0.8	5.9±1.1	6.2±1.2	<7
Cholesterol (mmol/L)	2.6±0.6	1.9±0.4	2.3±1.2	3.9±1.1	<5.2
Triglycerides (mmol/L)	2.6±0.5	3.1±0.6	2.8±1.1	1.8±1.0	<2.0
CK (IU/L)	1032.2±226	3468.1±7965[b]	3548.2±1263[be]	3108.3±3746[be]	50–1300

CK, creatine kinase; LDH, lactate dehydrogenase; GOT, glutamic oxaloacetic transaminase; GST, glutamic pyruvic transaminase.

Table 5. Blood biochemical measurements in isoflurane-sedated rabbits receiving intralipid 10%. Mean±SD. *n*=6. [b]*P*<0.05 *vs* pretreatment (0 h) values. [e]*P*<0.05 *vs* the same time-point values in Group P.

Variable	Time (h) 0	12	24	30–36	Reference range
Creatinine (μmol/L)	77.7±5.6	55.6±11.2[be]	44.5±5.6[be]	46.6±5.2[be]	70–120
GOT (U/L)	25.2±5.6	35.6±9.5	152.2±35.1[b]	175.6±61.3[b]	<30
GPT (U/L)	38.5±12.5	34.6±17.2	195.2±37.6b[b]	252±62.1[b]	<5–47
LDH (U/L)	250.5±12.5	215.4±32.6	385.1±89.6[b]	575.1±124.2[b]	243±50
Total protein (g/L)	58.1±4.1	75.1±1.2	82.2±3.2[be]	133±4.1[be]	54–75
Albumins (g/L)	37.2±2.0	29.2±2.1	22.1±4.2[b]	24.2±1.0[b]	27–50
Total bilirubin (μmol/L)	11.1±5.6	94.5±11.2[be]	248.9±105.2[b]	351.1±88.9[be]	<26
Direct bilirubin (μmol/L)	5.6±0.2	55.6±16.7[b]	100.1±22.3[b]	102.2±25.6[be]	<7
Cholesterol (mmol/L)	3.4±0.2	12.5±0.2[b]	19.2±6.3[b]	22.5±5.2[b]	<5.2
Triglycerides (mmol/L)	6.17±2.7	31.6±1.2[be]	33.4±1.6[be]	35.2±2.2[be]	<2.0
CK (IU/L)	1006.1±1226	3168.1±10965[b]	3328.2±1562[be]	3468.3±284.6[be]	50–1300

CK, creatine kinase; LDH, lactate dehydrogenase; GOT, glutamic oxaloacetic transaminase; GST, glutamic pyruvic transaminase.

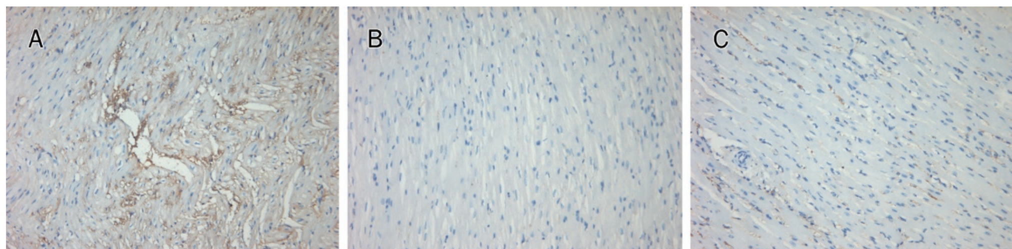

Figure 1. Immunohistochemical detection of tumor necrosis factor (TNF)-α in the heart. New Zealand rabbits under mechanically ventilation were subjected to sedation with 1% propofol (A), isoflurane (B) and isoflurane while receiving Intralipid 10% (C), respectively, for 30–36 h. Immunohistochemical detection of TNF-α with positive reaction in the propofol sedated heart (Brown reaction) (*n*=6). Original magnification ×100. Scale bar, 100 μm.

tions that resemble PRIS. This study also demonstrated that propofol produced an overexpression of TNF-α and AMPK activation, which has not been reported previously. The main features of PRIS include cardiovascular collapse, cardiac arrhythmias, and metabolic acidosis[4, 14]. Rhabdomyolysis, hyperlipemia, and hypertriglyceridemia are other commonly

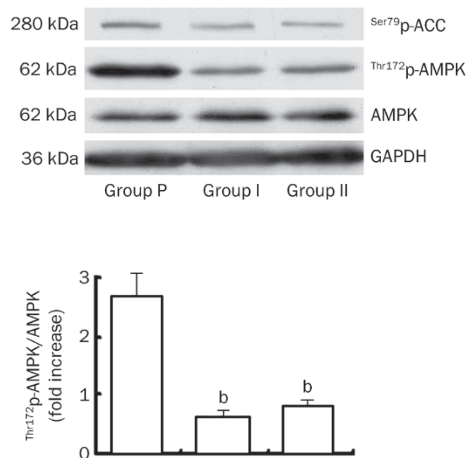

Figure 2. Immunoblot analysis of AMPK phosphorylation in the heart. New Zealand rabbits under mechanically ventilation were subjected to sedation with 1% propofol, isoflurane and isoflurane while receiving Intralipid 10%, respectively, for 30–36 h. AMPK phosphorylation at Thr 172 in myocardial tissues was observed by immunoblot analysis with phosphospecific antibody. The data shown are mean±SD. n=6. ^{b}P<0.05 *vs* propofol sedation.

described features, but these features are not consistently observed in all case reports and animal studies[15, 16]. Cardiac failure, which is characterized by progressive bradycardia that leads to asystole, is the major cause of death in patients with PRIS[17]. Cardiac function in propofol-sedated animals in this study did not reveal any conduction defects or bradycardia during most of the experiment, except for a slight decrease in heart rate during the last 3–4 h of the experiment (220±13 and 187±9 beat/min, at 0 and 30–36 h, respectively). Histological examination did not demonstrate myofibril degeneration or rhabdomyolysis. Therefore, the negative signs of cardiac injury explicate the absence of terminal bradycardia, which is characteristic of PRIS in patients[3, 17]. The immunohistochemical results demonstrated a strong positive reaction for TNF-α in propofol-infused cardiac myocytes, which indicated a rise in cardioinhibitory cytokines. The increase in TNF-α may be an adaptive response of a jeopardized myocardium[18]. Low- and high-dose propofol (1 and 10 mg·kg^{-1}·h^{-1}, respectively) exert beneficial effects on septic shock in rats and reduce serum TNF-α and IL-10 production after sepsis[19]. The difference between the results obtained here and these previous reports may be due to the administration of a long-term high-dose propofol infusion in this study; the use of high doses of propofol for prolonged periods is generally linked to a decrease in performance[3, 15, 17]. Cardiac arrhythmias, multiorgan damage, metabolic acidosis, rhabdomyolysis, hyperlipemia, and hypertriglyceridemia have been observed in critically ill patients receiving propofol for sedation[1–5]. However, the present study examined the effects of large doses of continuously infused propofol on lipid metabolism imbalance. Therefore, the primary outcome measurements in this study were blood biochemical deteriorations. The increases in LDH, GOT, GPT, cholesterol, triglycerides and creatine kinase (CK) were greater in Group P compared to the intralipid group. More importantly, the increased concentrations of these parameters presented 12 h after propofol administration (Table 2). Therefore, clinicians in the ICU should be extremely suspicious of PRIS development when hyperlipidemia and abnormal indices of liver function are observed. Propofol should be discontinued immediately in these cases. The marked increase in triglyceride concentrations may be directly caused by an impaired β-oxidation of FFAs, which is induced by propofol, or by an altered mobilization of FFAs from triglycerides. An increase in sympathetic nervous system outflow and elevated cortisol and growth hormone concentrations, which are commonly observed in critically ill patients, may promote lipolysis and lipid oxidation, which further increase FFA concentrations[1]. Liver dysfunction due to circulatory failure or direct hepatotoxic effects of propofol may also adversely affect lipid kinetics. Lipemia alone impairs mitochondrial oxygen uptake, which contributes to the accelerated development and refractory nature of the PRIS. AMPK is activated during metabolic stress. AMPK activates a number of energy-producing metabolic pathways, but it also inhibits energy-consuming pathways[11]. AMPK has been termed a 'fuel gauge' for cellular energy levels because of this dual effect[9–12]. The role of AMPK as a fuel gauge is particularly relevant in the heart, which has a remarkably high demand for energy. In addition to its 'fuel gauge' role, AMPK influences apoptotic processes in eukaryotic cells, and the prolonged stimulation of AMPK induces apoptosis through c-Jun-N-terminal kinase activation[20]. The present study demonstrated that the continuous infusion of high-dose propofol induced AMPK activation. Whether the activation of AMPK is beneficial or harmful during PRIS is not known. Further studies to explore this potential are required.

Intralipid infusion in isoflurane-sedated rabbits under prolonged mechanical ventilation was performed to investigate the possible side effects of the lipid vehicle. No serious deviations in vital signs were observed, and all animals survived the 36-h study period. Histological examination revealed no inflammation of the heart, and immunohistochemistry revealed little TNF-α staining. These results demonstrated that the lipid solvent caused few catastrophic manifestations of PRIS, which is consistent with previous studies[16, 21].

Additionally, rabbits were mechanically ventilated under isoflurane sedation for the maximum survival period of the propofol-sedated rabbits. Therefore, prolonged mechanical ventilation did not contribute to the detrimental effects. Positive results have been observed when prolonged isoflurane sedation was used with an anesthetic-conserving device in critically ill patients[13].

The present study included the following limitations. A more objective and sensitive method for the evaluation of sedation depth, such as the Bispectral Index or the electroencephalogram, would offer more precise adjustments of the sedative infusion rate or concentration. Additionally, the replication of human anesthetic dosing in rabbits was difficult. The initial propofol infusion rate was 10 mg·kg^{-1}·h^{-1}, and

this dose was increased up to 65 $mg \cdot kg^{-1} \cdot h^{-1}$, which is disproportionally higher than PRIS in humans (>4 $mg \cdot kg^{-1} \cdot h^{-1}$)[4, 22]. However, this result may explain the early onset of PRIS development in this species.

In summary, the continuous infusion of 1% propofol at large doses for sedation in rabbits undergoing prolonged mechanical ventilation induced detrimental changes in blood biochemistry, including hyperlipemia, liver dysfunction and a profound increase in CK levels, which indicated extended myocytolysis. These biochemical changes resembled PRIS. TNF-α expression increased in cardiac tissue specimens, which indicated myocardial injury. This observation that prolonged large doses of propofol infusion induce AMPK activation is novel. These results may provide a potential strategy for the elucidation of the mechanism of the imbalance between energy demand and utilization, which is a key pathogenetic mechanism in PRIS. The role of the lipid vehicle in the development of PRIS was minor, and this result was confined to late hyperlipidemia and liver dysfunction. Finally, isoflurane was a viable alternative sedation choice for prolonged mechanical ventilation in this rabbit model.

Acknowledgements
This study was supported by a grant from Shanghai Sixth Hospital.

Author contribution
Li WANG designed research and wrote the paper; Wei JIANG performed research; Zheng-bo YANG contributed equally to this work with Wei JIANG; Quan-hong ZHOU analyzed data; Xiang HUAN assisted to perform research.

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Acta Pharmacologica Sinica (2012) 33: i
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Retraction: Lipid metabolism disturbances and AMPK activation in prolonged propofol-sedated rabbits under mechanical ventilation

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The authors are retracting their article entitled "Lipid metabolism disturbances and AMPK activation in prolonged propofol-sedated rabbits under mechanical ventilation" [Acta Pharmacol Sin 2012; 33: 27–33]. Several expressions of this article were identical to those of previously published paper by Dr Petros YPSILANTIS *et al* [Anesth Analg 2007; 105: 155–66] and some original data were inappropriately manipulated. The authors would like to express their most sincere apology to Dr Petros YPSILANTIS and the Anesthesia & Analgesia and to the editors and readers of Acta Pharmacologica Sinica.

Acta Pharmacologica Sinica (2012) 33 (8): i; doi: 10.1038/aps.2012.113