Dexmedetomidine-ketamine Combination Mitigates Pulmonary Type-2 Cationic Amino Acid Transporter Isozymes Upregulation in Hemorrhagic Shock Rats

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Objective: Dexmedetomidine-ketamine combination has been reported to mitigate inducible nitric oxide synthase (iNOS) upregulation in rats with hemorrhagic shock. Type-2 cationic amino acid transporter isozymes, including CAT-2 and CAT-2B, are essential in regulating iNOS activity. We sought to elucidate the effects of dexmedetomidine-ketamine combination on regulating the expression of pulmonary CAT-2 isozymes in rats with hemorrhagic shock.

Methods: Forty adult male rats were randomized to one of four groups (10 rats in each group): sham-instrumentation (Sham); sham-instrumentation plus dexmedetomidine-ketamine combination (Sham-D+K); hemorrhagic shock (HS); or hemorrhagic shock plus dexmedetomidine-ketamine combination (HS-D+K). Rats in the HS and HS-D+K groups sustained controlled hemorrhagic shock (mean blood pressure was lowered to 40–45 mmHg by bloodletting for 60 minutes), followed by resuscitation with re-infusion of the shed blood mixed with saline. After close observation for 5 hours, the rats were sacrificed and the expression of CAT-2 isozymes was evaluated.

Results: Sham-instrumentation and dexmedetomidine-ketamine combination did not affect CAT-2 isozymes expression, as pulmonary CAT-2 and CAT-2B mRNA concentrations in the Sham and Sham-D+K groups were low. Hemorrhagic shock significantly upregulated CAT-2 isozymes expression as pulmonary CAT-2 and CAT-2B mRNA concentrations in the HS group were significantly higher than in the two Sham groups. Pulmonary CAT-2 and CAT-2B mRNA concentrations in the HS-D+K group were significantly lower than in the HS group, indicating that the effects of hemorrhagic shock on upregulating CAT-2 isozymes expression were attenuated by dexmedetomidine-ketamine combination.

Conclusion: Dexmedetomidine-ketamine combination mitigates pulmonary CAT-2 isozymes upregulation in rats with hemorrhagic shock.

1. Introduction

It is well established that hemorrhagic shock can readily cause acute lung injury.1 Upregulation of pulmonary inflammatory molecules, especially nitric oxide (NO) and its regulatory enzyme inducible NO synthase (iNOS), plays a crucial role in mediating the development of acute lung injury induced...
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by hemorrhagic shock. Previous data have demonstrated that therapies aimed at attenuating the up-regulation of pulmonary iNOS/NO may be beneficial against acute lung injury induced by hemorrhagic shock. Cellular uptake of L-arginine mediated by type-2 cationic amino acid transporter (CAT-2) isoforms, including CAT-2, the low affinity alternatively spliced CAT-2A, and the high affinity alternatively spliced CAT-2B, is one of the most crucial mechanisms involved in regulating NO production mediated by iNOS. Our previous data also confirmed that hemorrhagic shock can significantly upregulate the expression of CAT-2 isoforms.

Ketamine, a N-methyl D-aspartate receptor antagonist, is a potent anesthetic and analgesic. However, ketamine can cause undesired dose-dependent side effects, including emergence agitation and rise of intracranial pressure. Dexmedetomidine, a selective agonist of α2-adrenergic receptors, is a novel sedative and analgesic agent. It has been shown that a combination of dexmedetomidine and a subanesthetic dosage of ketamine provides effective sedation and analgesia in clinical practice. Moreover, the undesired effects of ketamine can be nullified with this combination.

Both dexmedetomidine and ketamine possess potent anti-inflammatory capacity. However, our previous data demonstrated that dexmedetomidine (at clinical dosage) alone or ketamine alone at a subanesthetic dosage did not exert significant effects on regulating the upregulation of pulmonary iNOS/NO in hemorrhagic shock rats. In contrast, a combination of a clinical dosage of dexmedetomidine and a subanesthetic dosage of ketamine could significantly attenuate pulmonary iNOS/NO upregulation induced by hemorrhagic shock. These data confirmed the potent anti-inflammatory capacity of a dexmedetomidine-ketamine combination. However, the effects of dexmedetomidine-ketamine combination on regulating upregulation of CAT-2 isoforms induced by hemorrhagic shock remain unstudied. Therefore, we conducted this animal study based on our hypothesis that a dexmedetomidine-ketamine combination can mitigate pulmonary CAT-2 isoforms upregulation in rats with hemorrhagic shock.

2. Methods

2.1. Animal preparation

This animal study was approved by the Animal Use and Care Committee of the Buddhist Tzu Chi General Hospital, Taipei Branch. The care and handling of the animals were in accordance with National Institutes of Health guidelines. Under halothane anesthesia, a polyethylene catheter (PE-50; Becton Dickinson, Sparks, MD, USA) was placed in the right femoral artery and left femoral vein of each rat. After tracheostomy, a 14-gauge intravenous (i.v.) catheter was inserted as a tracheostomy tube. Rats were mechanically ventilated (tidal volume, 4 mL of room air; rate, 35 breaths/min) with a small animal ventilator (Harvard Apparatus, South Natick, MA, USA). Mean arterial pressure (MAP) and heart rate were continuously monitored (BIOPAC Systems Inc., Santa Barbara, CA, USA).

2.2. Hemorrhagic shock and resuscitation protocols

Hemorrhagic shock was induced by bloodletting with a glass syringe over 10 minutes to lower the MAP from physiologic level (i.e. 100–120 mmHg) to hypotensive level (i.e. 40–45 mmHg), as we have previously reported. The shed blood was kept in the glass syringe already heparin-rinsed. Constant MAP was maintained by adjustment of withdrawal and re-infusion of blood as needed for 60 minutes. Then, resuscitation was performed by re-infusing the remaining shed blood plus normal saline (two times the maximum blood volume withdrawn). All rats were observed for 5 hours and then sacrificed by high-dose pentobarbital injection.

2.3. Experimental protocols

Forty adult male Sprague-Dawley rats (weight, 200–250 g; BioLASCO Taiwan Co. Ltd., Taipei, Taiwan) were randomly allocated to one of four groups (10 rats in each group): sham-instrumentation (Sham); sham-instrumentation plus dexmedetomidine-ketamine combination (Sham−D+K); hemorrhagic shock (HS); or hemorrhagic shock plus dexmedetomidine-ketamine combination (HS−D+K). All rats were anesthetized with halothane (0.75%) throughout the experiment. Rats in the Sham−D+K and HS−D+K groups received a loading dose of dexmedetomidine (1 μg/kg, i.v. infusion over 10 minutes; Hospira Inc., Lakeforest, IL, USA) followed by infusion of dexmedetomidine (0.5 μg/kg/hr, i.v.) and ketamine (1 mg/kg/hr, i.v.; Pfizer Pharmaceuticals Co., Taipei, Taiwan) until the end of the experiment. The administration of dexmedetomidine-ketamine combination was commenced immediately after resuscitation in the HS−D+K group or at the comparable time point in the Sham−D+K group.

2.4. Lung tissue collection

At the end of the experiment, sternotomy was performed and bilateral lung tissues were removed and divided. The freshly harvested lung tissues
were snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

2.5. Reverse transcription–polymerase chain reaction

Three independent reverse transcription–polymerase chain reaction (RT-PCR) assays were performed to determine the iNOS, CAT-2, CAT-2A and CAT-2B mRNA concentrations of each sample, as we have previously reported.14

In brief, total RNA was isolated from snap frozen lung samples with TRIzol Reagent (Life Technologies, Rockfield, MD, USA). RNA samples were then extracted by a phenol-chloroform technique. The RNA concentrations were quantified by measuring ultraviolet light absorbance at 260 nm. Maloney murine leukemia virus reverse transcriptase and random hexamer primers (Ready-To-Go RT-PCR Beads; Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) were used to reversely transcribe all mRNA species to complementary DNA (cDNA). The reaction progressed at 42°C for 30 minutes in a thermocycler. cDNA samples were then incubated at 95°C for 5 minutes to inactivate the reverse transcriptase. Separately carrying each sample through the PCR procedure without adding reverse transcriptase ensured the absence of genomic DNA contamination.

cDNA encoding iNOS, CAT-2, CAT-2A, CAT-2B and β-actin (as an internal standard) were then amplified using PCR. The primer sequences for each of the enzymes were designed in accordance with published rat DNA sequences and obtained from our previous report.14 The cycling conditions were: 35 cycles for iNOS/β-actin at 92°C for 40 seconds, 57°C for 40 seconds, 75°C for 75 seconds, and a final extension of products at 55°C for 5 minutes. Amplification of CAT-2/β-actin and CAT-2A/β-actin were performed using 35 cycles at 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minutes, and a final extension of products at 72°C for 7 minutes. Amplification of CAT-2B/β-actin was performed using 32 cycles at 94°C for 30 seconds, 58°C for 45 seconds, 72°C for 60 seconds, and a final extension of products at 56°C for 7 minutes.

PCR-amplified samples were electrophoretically separated on 1% ethidium bromide-stained agarose gels. The Gel Documentation System (Gel Doc 2000; Bio-Rad Laboratories, Hercules, CA, USA) was used to assay the PCR products. cDNA band densities were quantified using densitometric techniques with Scion Image (Scion Corp., Frederic, MD, USA) for Windows.

2.6. Statistical analysis

One-way analysis of variance with the Bonferroni test was used for multiple comparisons. Data are presented as mean±standard deviation. The significance level was set at 0.05. A commercial software package, SigmaStat (SPSS Inc., Chicago, IL, USA) for Windows, was used for data analysis.

3. Results

3.1. Pulmonary iNOS expression

Pulmonary iNOS mRNA concentrations (Figure 1) in the Sham and Sham–D+K groups were low, and that in the HS groups were significantly higher than in the Sham groups (p<0.001). In contrast, pulmonary iNOS mRNA concentration in the HS–D+K group was significantly lower than in the HS group (p=0.028). Densitometric analysis revealed that pulmonary iNOS mRNA concentration in the HS–D+K group was 70.46±5.18% of that in the HS group (Figure 1).

3.2. Pulmonary CAT-2, CAT-2A and CAT-2B expression

Pulmonary CAT-2, CAT-2A and CAT-2B concentrations are shown in Figures 2–4. Similar to iNOS, pulmonary CAT-2 and CAT-2B mRNA concentrations in the Sham and Sham–D+K groups were low, and that in the HS groups were significantly higher than in the Sham groups (p=0.006 and 0.001, respectively; Figures 2 and 4). Pulmonary CAT-2 and CAT-2B mRNA concentrations in the HS–D+K group, in contrast, were significantly lower than those in the HS groups (p=0.031 and 0.016, respectively; Figures 2 and 4). Densitometric analysis revealed that pulmonary CAT-2 mRNA concentration in the HS–D+K group was 65.76±4.12% of that in the HS group (Figure 2), while pulmonary CAT-2B mRNA concentration in the HS–D+K group was 73.07±3.10% of that in the HS group (Figure 4).

In contrast to iNOS, CAT-2 and CAT-2B, pulmonary CAT-2A mRNA concentrations in the four groups were similar (Figure 3). Pulmonary CAT-2A mRNA concentration in the HS–D+K group was 92.37±6.17% of that in the HS group (Figure 3).

4. Discussion

Findings from this study, in concert with our previous data,12 demonstrated that dexmedetomidine-ketamine combination could mitigate pulmonary iNOS upregulation in hemorrhagic shock rats. Our data also demonstrated that dexmedetomidine-ketamine combination could significantly attenuate hemorrhagic shock-induced upregulation of CAT-2 isozymes, including CAT-2 and CAT-2B, in rat lungs.
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**Figure 1** Representative gel photograph and densitometric data of inducible nitric oxide synthase (iNOS) mRNA concentrations in lung tissues harvested from rats in the sham-instrumentation (Sham), Sham plus dexmedetomidine-ketamine combination (Sham–D+K), hemorrhagic shock (HS), and HS plus dexmedetomidine-ketamine combination (HS–D+K) groups. The iNOS mRNA concentration in the HS–D+K group was compared to that of the HS group to determine the magnitude of change. Data are mean±standard deviation. *p<0.05 vs. Sham group; †p<0.05, HS–D+K group vs. HS group.

**Figure 2** Representative gel photograph and densitometric data of type-2 cationic amino acid transporter (CAT-2) mRNA concentrations in lung tissues harvested from rats in the sham-instrumentation (Sham), Sham plus dexmedetomidine-ketamine combination (Sham–D+K), hemorrhagic shock (HS), and HS plus dexmedetomidine-ketamine combination (HS–D+K) groups. The CAT-2 mRNA concentration in the HS–D+K group was compared to that of the HS group to determine the magnitude of change. Data are mean±standard deviation. *p<0.05 vs. Sham group; †p<0.05, HS–D+K group vs. HS group.

As CAT-2 and CAT-2B comprise a crucial part of the downstream pathways that regulate iNOS activity,4,5 these data provide clear evidence of the potent anti-inflammation capacity of dexmedetomidine-ketamine combination against hemorrhagic shock. Moreover, these data, together with those we previously obtained,12 seem to suggest that incorporating dexmedetomidine-ketamine combination as a therapeutic adjuvant in patients suffering from hemorrhagic shock will have beneficial effects.

It is well-established that iNOS expression is regulated by crucial upstream regulatory pathways, including nuclear factor (NF)-κB and mitogen-activated protein kinases (MAPKs).15,16 NF-κB and MAPKs are also crucial in mediating maximal transcription of a wide array of proinflammatory molecules, including...
intercellular adhesion molecule 1, cyclooxygenase-2, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α. Though the role of MAPKs in regulating CAT-2 isozymes expression remains unstudied, our previous data demonstrated that transcription induction of CAT-2 isozymes is mainly regulated by NF-κB. Hemorrhagic shock induces significant activation of NF-κB and MAPKs induced by hemorrhagic shock remains unresolved. However, previous data did confirm that ketamine attenuates NF-κB and MAPKs activation. Judging from these data, we speculate that dexmedetomidine-ketamine combination may act through inhibition of NF-κB and/or MAPKs activation to exert its effects on inhibiting iNOS, CAT-2 and CAT-2B up-regulation in the lungs of rats with hemorrhagic shock, as observed in this study.

![Figure 3](image1.png)

**Figure 3** Representative gel photograph and densitometric data of the low affinity alternatively spliced transcript of type-2 cationic amino acid transporter (CAT-2A) mRNA concentrations in lung tissues harvested from rats in the sham-instrumentation (Sham), Sham plus dexmedetomidine-ketamine combination (Sham−D+K), hemorrhagic shock (HS), and HS plus dexmedetomidine-ketamine combination (HS−D+K) groups. The CAT-2A mRNA concentration in the HS−D+K group was compared to that of the HS group to determine the magnitude of change. Data are mean±standard deviation.

![Figure 4](image2.png)

**Figure 4** Representative gel photograph and densitometric data of the high affinity alternatively spliced transcript of type-2 cationic amino acid transporter (CAT-2B) mRNA concentrations in lung tissues harvested from rats in the sham-instrumentation (Sham), Sham plus dexmedetomidine-ketamine combination (Sham−D+K), hemorrhagic shock (HS), and HS plus dexmedetomidine-ketamine combination (HS−D+K) groups. The CAT-2B mRNA concentration in the HS−D+K group was compared to that of the HS group to determine the magnitude of change. Data are mean±standard deviation. *p<0.05 vs. Sham group; †p<0.05, HS−D+K group vs. HS group.
Using an endotoxin-activated murine macrophage model, we previously showed that CAT-2A may account for the catecholamine-induced enhancement of L-arginine transport. However, in contrast to CAT-2 and CAT-2B, data from this study demonstrated that pulmonary CAT-2A expression was not affected by hemorrhagic shock and/or dexmedetomidine-ketamine combination. These data are consistent with those we previously obtained. Thus, we speculate that the role of CAT-2A in regulating hemorrhagic shock-induced iNOS upregulation in rat lungs is negligible.

As already noted, dexmedetomidine and ketamine possess potent anti-inflammatory capacity. However, our previous data demonstrated that dexmedetomidine-ketamine combination, but not dexmedetomidine (at clinical dosage) alone or ketamine (at subanesthetic dosage) alone, significantly attenuated pulmonary iNOS/NO upregulation as well as pulmonary inflammatory response and acute lung injury induced by hemorrhagic shock. In line with this notion, this study was conducted to investigate the effects of dexmedetomidine-ketamine combination but not the individual effects of dexmedetomidine and ketamine. This decision is justified by our previous data that showed that the anti-inflammatory effects of dexmedetomidine are evident only with higher dosages (approximately 10 times the clinical dosage) but not with clinical dosages. Previous data that the anti-inflammatory effects of ketamine in septic rats were found only with higher dosages (i.e. 5 and 10 mg/kg/hr) but not with lower dosages (i.e. 0.5 mg/kg/hr) also justified our decision.

There were certain study limitations. First, this study employed halothane to facilitate anesthesia. This could be a confounding variable as halothane has been reported to possess certain anti-inflammatory capacity. The effects of halothane per se on regulating CAT-2 isozymes expression in hemorrhagic shock rats were not evaluated in this study. Therefore, the question of whether dexmedetomidine-ketamine combination can exert similar effects without the presence of halothane remains to be elucidated. Second, the question of whether the effects of this combination are additive or synergistic remains unstudied.

In summary, dexmedetomidine-ketamine combination mitigates the upregulation of pulmonary CAT-2 isozymes in rats with hemorrhagic shock.

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References


