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Dexmedetomidine Protects Against Traumatic Brain Injury-Induced Acute Lung Injury in Mice

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Background: Traumatic brain injury (TBI) leads to acute lung injury (ALI), in which the inflammatory response plays an important role in its pathophysiology. Recent studies suggest that dexmedetomidine (Dex) plays a protective role in acute inflammatory diseases. However, whether Dex has a protective effect on TBI-induced ALI is not clear. The aim of this study was to investigate the effect of Dex on TBI-induced ALI in mice.

Material/Methods: Mice were randomly divided into 5 groups: 1) sham group; 2) TBI group; 3) TBI+Dex group; 4) TBI+atipamezole (Atip) group; and 5) TBI+Dex+Atip group. Dex (50 µg/kg) was intraperitoneal injected immediately after TBI. The α_2 adrenergic antagonist Atip (250 µg/kg) was intraperitoneal injected 15 minutes prior to Dex treatment. Then 24 hours later, the protein concentration in the bronchoalveolar lavage fluid (BALF), lung wet to dry weight ratio, hematoxylin and eosin (H&E) staining of lungs, the level of high-mobility group box protein 1(HMGB1) in serum, and the receptor for advanced glycation end products (RAGE) expression in lung were detected.

Results: Dex ameliorated the score of lung histological examination, as well as the severity of pulmonary edema and permeability. Moreover, Dex was observed to significantly suppress the expression of HMGB1 and RAGE. However, the protective effects of Dex were partially reversed by the administration of Atip.

Conclusions: Dex may protect against TBI-induced ALI via the HMGB1-RAGE signal pathway, and this protective effect is partly dependent on its α_2 adrenoceptor agonist action.

MeSH Keywords: **Acute Lung Injury • Brain Injuries • Dexmedetomidine**

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Background

Traumatic brain injury (TBI) is caused by mechanical forces applied to the brain, including subdural hematoma, subarachnoid hemorrhage, and brain contusion [1]. Every year, the increased incidence of craniocerebral injury leads to high mortality of unfavorable outcomes and shares the largest source of donor lungs [2]. Besides, patients with TBI always suffer peripheral organs dysfunction such as lung, liver, kidney, or intestine. Evidence has shown that these complications are associated with an increased incidence of mortality [3,4]. Acute lung injury (ALI) [5], as well as other pulmonary complications including ventilator-associated pneumonia (VAP), acute respiratory distress syndrome (ARDS), and neurogenic pulmonary edema (NPE) are the most prevalent non-neurologic organ dysfunctions in the TBI population [6,7]. However, the pathophysiological mechanism remains unclear.

Dexmedetomidine (Dex), a kind of α_2 adrenergic receptor agonist, was often used in craniotomy and associated with fewer respiratory adverse events in previous research [8]. Dex is considered to confer protective effects on ALI induced by intestinal ischemia reperfusion, sepsis, orthotopic autologous liver transplantation, or other diseases [9,10]. However, as there are limited reports investigating the effect of Dex on TBI-induced ALI, we would like to determine whether Dex could improve TBI-induced ALI in mice and explore its potential mechanisms.

Previous studies demonstrated that many inflammatory cytokines were involved in the inflammatory responses after TBI, including IL-1 β , IL-6, TNF- α , and damage related molecular patterns (DAMPs) [11–13]. Though the specific mechanism remains unknown, the release of DAMPs by the nervous system was involved in immune responses [13]. High-mobility group box protein 1 (HMGB1) is a type of DAMPs whose receptors are receptor for advanced glycation end products (RAGE) and Toll-like receptor 4 (TLR4). During endotoxin shock and severe sepsis, RAGE signal could activate downstream signaling pathways such as promoting the activation of MAPK and NF- κ B, mediating pro-inflammatory responses [14,15]. In addition, RAGE is a direct index of lung inflammation in experimental lung inflammation models [16]. It has reported that Dex could alleviate CLP-stimulated ALI via downregulating the expression of HMGB1-RAGE and reducing NF- κ B and MAPK phosphorylation [17]. Therefore, in our research, we hypothesized that whether Dex attenuates the TBI-induced lung injury in mice and whether Dex interacts with the HMGB1-RAGE signaling pathway.

Material and Methods

Animals

Healthy 6–8-week-old C57BL/6 male mice, weighing 18–22 g, were used in our study. All mice were purchased from the Experimental Animals Center of Naval Medical University (Shanghai, China). They were housed in cages under standard conditions at a temperature of 18–22°C with a relative humidity of 50–60%, a 12 h light-dark cycle, and allowed free access to water and food. All the animals used were approved by the Animal Care and Use Committee of Naval Medical University.

Experiment procedure

C57BL/6 mice were randomly assigned to the following groups (n=8, each): 1) sham group; 2) TBI group; 3) TBI+Dex group; 4) TBI+atipamezole (Atip) group; and 5) TBI+Dex+Atip group. To establish the severe traumatic brain injury, a validated controlled cortical impact was performed by Feeney's weight-drop model [18]. After being anesthetized with sevoflurane, mice were placed in a stereotaxic frame and underwent a craniotomy with a diameter of 3 mm over the left parietal cortex with the center between the bregma and lambdoid suture. After TBI, the bone flap was repositioned and the skin was closed with continuous sutures. Each mouse was intraperitoneally injected the same volume (200 μ L) of vehicles phosphate-buffered saline (PBS) or Dex (50 μ g/kg) immediately [19]. In the TBI+Atip group and the TBI+Dex+Atip group, Atip (250 μ g/kg) was intraperitoneally injected 15 minutes before TBI. This model is generally associated with 40% mortality within the first 5 minutes after injury and neurological deficit scores of III within 24 hours after injury [20].

Histology and lung injury scoring

At 24 hours after TBI challenge, mice were anesthetized with sevoflurane and underwent cardiac perfusion with 50 mL 4% paraformaldehyde to remove the blood in lungs and to fix lung tissues. Then, the left lung was fixed in 4% paraformaldehyde for 48 hours and embedded in paraffin. Sections were cut at a thickness of 4–5 μ m for hematoxylin and eosin (H&E) staining. Lung sections were scanned and evaluated blindly by 2 experienced pathologists under light microscopy. Alveolar congestion, hemorrhage, aggregation of inflammatory cells, and the thickness of the alveolar walls were assessed, according to the criteria described previously [21].

Detection of proteins in the bronchoalveolar lavage fluid (BALF)

To obtain the bronchoalveolar lavage fluid (BALF), lungs were lavaged twice with 1 mL of ice-cold PBS and the recovery ratio

of the total fluid maintained at about 90%. Both aliquots were pooled and centrifuged at 12 000 rpm for 10 minutes at 4°C. The cell-free supernatant was used for albumin measurement using the BCA Protein Assay kit (Thermo Scientific, IL, USA).

Lung wet-to-dry (W/D) weight ratio measurement

At 24 hours after the TBI challenge, mice were sacrificed, and lung tissues were excised and weighed. Then the lung tissues were placed in an oven at 80°C wrapped with a tinfoil for 72 hours to achieve dry weight [22]. And the wet-to-dry (W/D) weight ratio was calculated to assess edema.

Enzyme-linked immunosorbent assay (ELISA) analysis for HMGB1 in serum

Mice were anesthetized by isoflurane 24 hours after the TBI challenge and blood was collected by heart puncture. Serum was separated by centrifugation at 12 000 rpm for 10 minutes at 4°C. The concentration of HMGB1 in serum was detected by enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, MN, USA) according to the manufacturer's instructions.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from lung tissues using TRIzol reagent (Takara Biotechnology, Dalian, China). The concentration and purity of the total RNA were determined at 260/280 nm. Complementary DNA (cDNA) was reverse-transcribed using a Prime Script RT Reagent Kit (Takara Biotechnology, Dalian, China). The quantification of relative messenger RNA (mRNA) concentrations were conducted using a StepOnePlus™ Real Time PCR System (Applied Biosystems, CA, USA). Specific primers were as follows: RAGE: forward, ACCACTCCAACAGACCTG; reverse, GGTACTCCAGAAGACCAGAGG; GAPDH: forward, ACCACAGTCCATGCCATCAC; reverse, TCCACCACCCTGTGCTGTA. Normalized by the amount of GAPDH, the levels of RAGE mRNA were expressed as fold-changes.

Western blot analysis

At 24 hours after the TBI challenge, the lung samples were homogenized in protein extract solution and protease inhibitors (Beyotime Biotechnology, Shanghai, China) and centrifuged at 12 000 rpm for 10 minutes at 4°C. The total protein concentration was evaluated by the BCA protein assay kit (Thermo Scientific, IL, USA). Equal amounts of total protein were loaded per well on a 12% sodium dodecyl sulfate-polyacrylamide gel and transferred into a polyvinylidene difluoride membrane. The membranes were blocked with 5% BSA for 2 hours at room temperature. Then the membranes were incubated with appropriate dilution of specific primary antibodies (anti-RAGE 1: 1000 and anti-GAPDH 1: 1000) for 12 hours at 4°C. Then the

membranes were incubated with the horseradish peroxidase-linked secondary antibody (1: 1000; Beyotime Biotechnology, Shanghai, China). Protein bands were demonstrated by enhanced chemiluminescence Western blot kit (Thermo Scientific, IL, USA). Signals were densitometrically assessed and normalized to GAPDH signals.

Statistical analysis

All data were normally distributed and analyzed statistically with GraphPad Prism 5 (GraphPad Software Inc., CA, USA). Results are expressed as means ± standard error of the mean (SEM). Differences between groups were analyzed by unpaired *t*-tests and analysis of variance and Tukey post hoc test. *P*<0.05 was considered statistically significant.

Results

Dexmedetomidine (Dex) ameliorated the severity of TBI-induced ALI

At 24 hours after the TBI challenge, mice in the TBI group showed apparent pathological changes in lungs as evidenced by thickened alveolar wall, disordered lung tissues, and obvious inflammatory cells infiltration. And the histopathologic changes were significantly ameliorated with the treatment of Dex (Figure 1A). Compared with TBI+Dex group, the TBI group had higher ALI pathological scores (Figure 1B). The protein concentration in the BALF was decreased after Dex administration (Figure 1C). Compared with the TBI group, Dex also alleviated the severity of pulmonary edema as evidenced by the lower W/D ratio (Figure 1D). Collectively, our results showed that Dex was able to ameliorate the severity of TBI-induced ALI in mice.

It is well known that Dex is a highly selective α_2 adrenergic receptor agonist. Therefore, we used Atip, an α_2 adrenergic receptor antagonist, to determine whether the protective effect of Dex on TBI-induced ALI in mice was related to its α_2 adrenergic receptor activating effect. Results showed that, compared with the TBI+Dex group, the pathological changes (Figure 1A, 1B), total protein concentrations in the BALF (Figure 1C), and lung W/D ratio (Figure 1D) were significantly increased after the administration of Atip. In addition, there was no difference between the TBI group and the TBI+Atip group. These results suggested that Atip partly reversed the protective effect of Dex on TBI-induced ALI in mice.

Dexmedetomidine (Dex) negatively regulated HMGB1-RAGE pathway

Since HMGB1-RAGE pathway plays a vital role in TBI-induced lung injury in mice [16], we then examined whether Dex

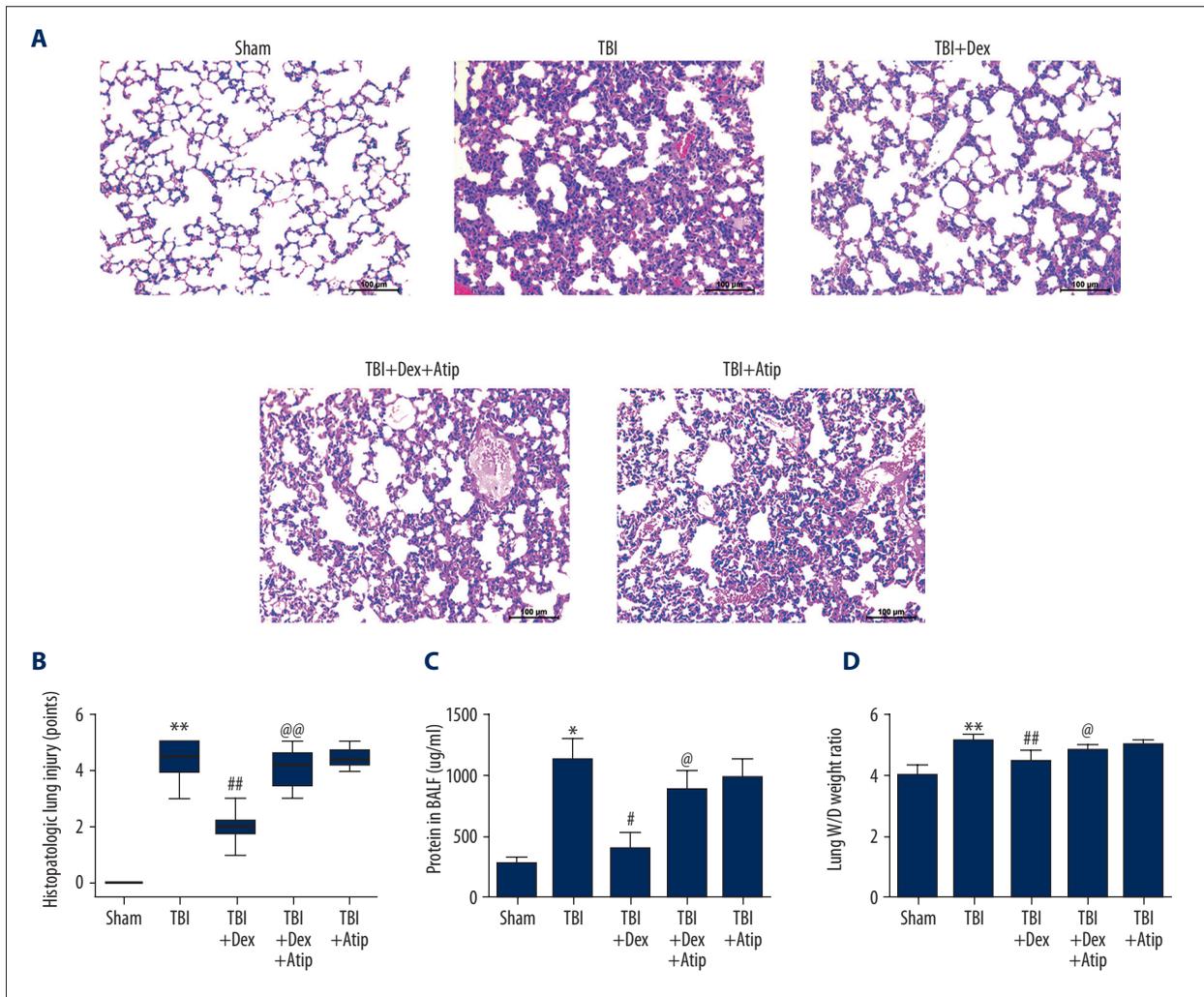


Figure 1. Dexmedetomidine (Dex) ameliorated TBI-induced ALI in mice. After 24 hours of TBI, lung tissue and the BALF in each group were collected. (A) The example of micrographic images (200 \times) from the sham group, the TBI group, the TBI+Dex group, the TBI+Atip group, and the TBI+Dex+Atip group. (B) Histopathological lung injury scores in each group. (C) Total protein concentration in the BALF of mice in each group. (D) The lung wet-to-dry ratio. The values are presented as the means \pm SEM. * $P < 0.05$, ** $P < 0.01$ versus the sham group, # $P < 0.05$, ## $P < 0.01$ versus the TBI group, @ $P < 0.05$, @@ $P < 0.01$ versus the TBI+Dex group.

ameliorated TBI-induced lung injury in mice through regulating the HMGB1-RAGE pathway. Compared with the sham group, the level of HMGB1 in serum of mice in the TBI group was significantly elevated, which was largely decreased by the administration of Dex (Figure 2A). Results from real-time PCR and western blot analysis suggested that the expression of RAGE was increased significantly in the TBI group. However, all these changes were reversed by the administration of Dex (Figure 2B–2D).

Compared with the TBI+Dex group, the serum HMGB1 level (Figure 2A) and the expression of RAGE in lung tissues (Figure 2B–2D) were increased in the Atip administrated group. Collectively, these results indicated that Dex negatively

regulated the HMGB1-RAGE pathway in the TBI-induced ALI in mice and that Atip partly reversed the effect of Dex through the HMGB1-RAGE pathway.

Discussion

Depending on the severity of the trauma, TBI patients may suffer from complications of non-neurologic organ dysfunction (NNOD). Pulmonary complications are among the most prevalent NNOD encountered in the TBI population [23]. In this study, Feeney's weight-drop model was used to imitate the pathogenesis of acute lung injury after TBI. The total protein concentration in the BALF and lung W/D ratio are sensitive

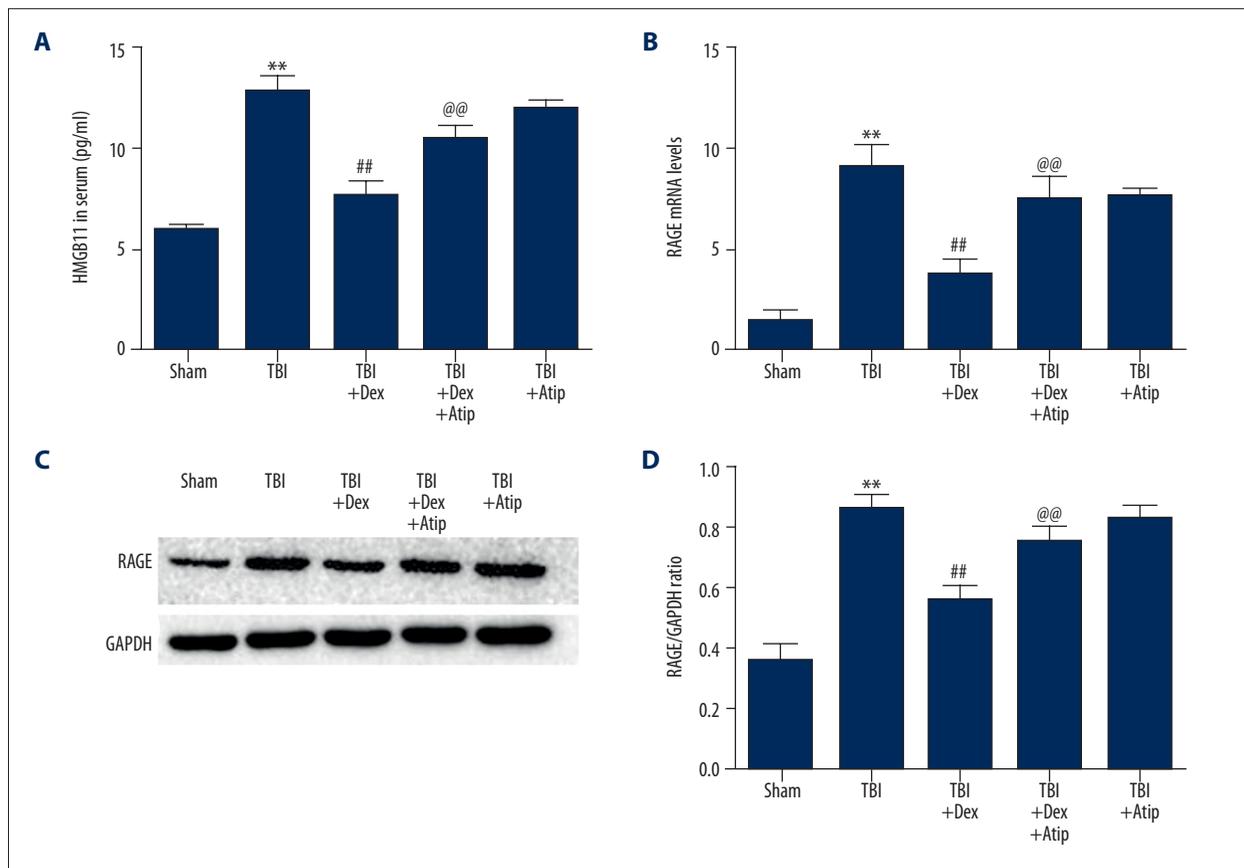


Figure 2. Dexmedetomidine (Dex) negatively regulated HMGB1-RAGE pathway. At 24 hours of TBI, lung tissues and blood in each group were collected. **(A)** HMGB1 level in serum of mice in each group. **(B)** The mRNA expression of RAGE in lung tissues. **(C)** Western blot analysis for RAGE in lung tissues. **(D)** Density quantification of RAGE. The values are presented as the means \pm SEM. ** $P < 0.01$ versus the sham group, ## $P < 0.01$ versus the TBI group, @@ $P < 0.01$ versus the TBI+Dex group.

indicators of ALI, and lung pathological change is one of the main classical characteristics of ALI [24]. In this study, mice were sacrificed at 24 hours after TBI and the total protein concentration in the BALF, the lung W/D ratio, and the pathological changes were measured to assess the severity of ALI induced by TBI. Previous studies have suggested that Dex has an organ protective effect in addition to its sedation and anxiolytic activity [25,26]. In this study, our results showed that Dex decreased lung W/D ratio and the total protein concentration in the BALF, and ameliorated lung pathological changes in TBI mice, which indicated that Dex could alleviate TBI-induced ALI in mice.

Although the pathophysiology of TBI-induced ALI is unclear, it may relate specifically to the release of the DAMPs in nervous system. DAMPs are a family of molecules that are released in response to sterile inflammation, such as TBI [27]. A previous study illustrated that TBI causes injury of the cerebral vasculature resulting in the disruption of the blood-brain barrier. The secreted inflammatory mediators, such as DAMPs released by injured cells, trigger further brain inflammation and

affect distal organs including the lungs [2]. HMGB1, a type of DAMPs, belongs to a family of non-histone chromosomal proteins. When it is not acetylated, HMGB1 remains localized in the nucleus, and is not secreted or released regardless of injury or insult [28]. However, hyper-acetylation of the molecule results in cytosolic relocation, allowing for further secretion into the cellular milieu under proper signaling stimulation [29]. HMGB1 leads to the activation of the nuclear factor (NF)- κ B pathway with the generation of inflammatory cytokines through binding to the RAGE receptor [23]. HMGB1-RAGE signal pathway plays a pivotal role in the development of TBI-induced ALI [16]. In this study, compared with the administration of Dex, serum HMGB1 level and the expression of RAGE in lung tissues were significantly increased after TBI. Previous studies showed that the neutralization of HMGB1 or genetic knockout of the RAGE receptor has been shown to attenuate post-TBI lung injury and hypoxia [16,30]. Since our results proved that Dex negatively regulated HMGB1-RAGE pathway, it can be inferred that the mechanism of Dex in TBI-induced ALI may be partly through the regulation of HMGB1-RAGE pathway.

It is well known that Dex is a highly selective α_2 adrenergic receptor agonist. Therefore, we used Atip, an α_2 adrenergic receptor antagonist, to determine whether the protective effect of Dex on TBI-induced ALI in mice was related to its α_2 adrenergic receptor activating effect [31]. Results showed that Atip partly reversed serum HMGB1 levels and the expression of RAGE in lung tissues. Accordingly, TBI-induced ALI was also partly reversed by Atip. These results suggested that Atip partly reversed the protective effect of Dex. Based on these findings, we concluded that Dex may protect against TBI-induced ALI in mice via the HMGB1-RAGE signal pathway, and this protective effect is partly dependent on its α_2 adrenoceptor agonist action.

Our study had several limitations. First, although we found the protective effects of Dex on TBI-induced acute lung injury, we did not further study how Dex interacted with the HMGB1-RAGE pathway. Second, we adopted only 1 time point (24 hours after TBI) and 1 dosage of Dex (50 $\mu\text{g}/\text{kg}$). We did not further discuss whether changing the dose of Dex or the time points

will lead to different results. At last, our study only put focus on the activation of the HMGB1-RAGE pathway. However, whether other pathways, such as NF- κB and MAPK signals, was involved needs to be clarified in future studies.

Conclusions

Our study suggested that Dex administration could decrease TBI-induced ALI in mice models. The protective effects may act through the regulation of the HMGB1-RAGE pathway, which is partly dependent on the α_2 adrenoceptor agonist action of Dex. Further studies are warranted to investigate the detailed molecular pathways of Dex in protecting against TBI-induced ALI.

Conflict of interests

None.

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