Enhanced pulmonary inflammation following experimental intracerebral hemorrhage

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Abstract

The association between brain damage and respiratory dysfunction has been recognized although mechanistic link between the two is still poorly defined. Intracerebral hemorrhage is accompanied by brain injury, stroke, and parenchymal hematoma formation with surrounding inflammation. Increase intracranial pressure as a result of intracerebral hemorrhage may promote localized activation of cytokines and coagulation system including tissue factor release. However, whether intracerebral hemorrhage triggers inflammation in noncerebral organs has not been elucidated. The aim of the present study was to examine the impact of intracerebral hemorrhage on lung inflammatory response. Intracerebral hemorrhage was induced by stereotaxic intrastriatal administration of bacterial collagenase. Expression of intracellular adhesion molecule-1 (ICAM-1), IκB-α, tissue factor, tumor necrosis factor-α (TNF-α), and tumor necrosis factor-β (IL-1β) was evaluated by Western blot analysis. Our results revealed that intracerebral hemorrhage upregulated expression of ICAM-1 and tissue factor in both brain and lung, whereas it enhanced TNF-α and IL-1β mainly in brain within 6 and 24 h of the brain injury. Levels of IκB-α remained unchanged in brain and lung tissues. Appearance of inflammatory markers in the lung was accompanied by morphological pulmonary damage. These data suggest that intracerebral hemorrhage may trigger acute inflammatory response in both brain and lung.

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Introduction

Intracerebral hemorrhage is a serious medical problem for which there is no effective therapy (Aronowski and Hall, 2005; Gong et al., 2000). Clinical and experimental evidence suggested that brain injury resulted from intracerebral hemorrhage elicits a robust acute inflammatory response in the injured brain (Gong et al., 2000). Several pro-inflammatory cytokines including transcription factor nuclear factor-kB (NFκB) and intracellular adhesion molecule-1 (ICAM-1) are rapidly upregulated with the onset of intracerebral hemorrhage followed by microglia activation, neutrophil accumulation, astrogliosis, and complement activation (Aronowski and Hall, 2005; Jean et al., 1998; Whalen et al., 1999; Gong et al., 2000). Nonetheless, it is not clear if the acute inflammatory response resulting from cytokines, infiltrating leukocytes, and activated microglia is limited to cerebral region only, or it may trigger a secondary cascade of noncerebral cytotoxic injury. Acute intracerebral hemorrhage is often accompanied with multiple organ damage or systemic inflammatory response syndrome (SIRS) including pulmonary and ventricular dysfunction (Fisher et al., 1999; Goel et al., 2005; Schranz et al., 1983; Ren and Wu, 2006). One of the most common complications resulting from intracerebral hemorrhage and other brain injuries including seizures, head trauma, tumors, and neurosurgical procedures is neurogenic pulmonary edema. The pathogenesis behind neurogenic pulmonary edema has not been completely elucidated although two theories are well acknowledged, namely the blast theory and the permeability defect theory (Pyeron, 2001). It has been speculated that the hyperactive sympathetic–adrenergic reactions and catecholamine spillover contributes to direct organ “blast” insult injury or increased vascular permeability, which lead to ultimate
cardiopulmonary dysfunction following intracerebral hemorrhage (Schranz et al., 1983; Shivalkar et al., 1993). In addition, several observations have also provided potential alternative explanation for intracerebral hemorrhage-induced pulmonary injuries such as disruption of blood–brain barrier (Lampl et al., 2005), alteration in cerebrospinal fluid ubiquitin (Earle et al., 2005), and pulmonary contusion as a result of hypoxemia (Orliaguet et al., 2000). Nevertheless, none of these rationales has been fully validated clinically. We hereby hypothesized that the pulmonary injury secondary to intracerebral hemorrhage may be due to enhanced inflammatory response of lung as a result of coagulation activation and tissue factor release from the brain. The aim of the present study was to examine the impact of intracerebral hemorrhage on pulmonary coagulating and inflammatory markers including tissue factor, ICAM-1, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IκB, the negative regulator of transcription factor NFκB.

Materials and methods

Induction of intracerebral hemorrhage

All animal experimental procedures were in accordance with our institutional guidelines. Intracerebral hemorrhage was induced by stereotaxic intrastriatal administration of bacterial collagenase type IV (Sigma Chemicals, St. Louis, MO) in adult female Sprague–Dawley rats (250–350 g) (Xue and Del Bigio, 2000; Paxinos et al., 1985). In brief, following anesthesia with pentobarbital (50 mg/kg, i.p.), rats were placed onto a stereotaxic frame instrument (David Kopf Instruments, Tujunga, CA). Rectal temperature was maintained at 37.0 ± 0.5°C using a temperature-controlled heating blanket during surgery. A burr hole was made, and a 26-gauge Hamilton syringe needle was inserted into the striatum (location: 3.0 mm right lateral to the midline, 0.2 mm posterior to bregma, and 6 mm in depth below the skull). Hematoma-induced and sham groups received 5 μl collagenase solution (containing 0.2 U/μl Type IV collagenase) and 5 μl saline, respectively, during a 10-min injection duration. Following the injection, the needle was removed with a 5-min delay to prevent reflux, and the skin was sutured. During the recovery period, rats were assessed for forelimb flexion and contralateral circling to confirm intracerebral hemorrhage procedures. No seizure was observed during this procedure. After recovery from anesthesia, the rats were kept in air-ventilated cages at 24.0 ± 0.5°C under a circadian cycle of 12-h light–12-h dark with free access to food and water.

Western blot analysis of ICAM-1, IκB, TNF-α, tissue factor, and IL-1β

Experimental animals were euthanized under anesthesia at 6, 12, and 24 h following the intracerebral hemorrhage procedure. Sham-operated rats were sacrificed at 12 h after the sham surgery. Following intracardiac perfusion with TBS, the brain tissues adjacent to the hematoma and lung tissues were removed and stored at −80°C until experimentation. Brain and lung tissues were then homogenized in a lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS, and 1% protease inhibitor cocktail and were centrifuged at 12000 × g for 15 min at 4°C. Proteins (35 μg/lane) were separated on 10–15% SDS-polyacrylamide gels in a minigel apparatus (Mini-PROTEAN II, Bio-Rad) and transferred to polyvinylidene difluoride membranes. The membranes were blocked with TBS–0.05% Tween-20 (TBS-T) with 5% nonfat dry milk for 60 min and was then incubated with mouse anti-rat CD54 (1:1000, Biologicend, San Diego, CA), Armenian hamster anti-mouse/rat TNF-α (1:1000, Biologicend), rabbit anti-IκB-α (1:1000) (Cell Signaling, Beverly, MA), Armenian hamster anti-mouse/rat IL-1β (1:1000, Biologicend), rabbit anti-mouse tissue factor (5.95 mg/ml, kindly provided by Dr. James H. Morrissey, University of Illinois at Urbana–Champaign, Urbana, IL), and mouse anti-mouse β-actin (1:5000, Cell Signaling, used as loading control) antibodies in TBS-T with 5% BSA overnight at 4°C. After corresponding secondary

Fig. 1. Western blot analysis of ICAM-1 (panel A) and IκB-α (panel B in adult rat brain and lung tissues from control (cont), sham-operated (at 12 h) as well as 6, 12, and 24 h post-intracerebral hemorrhage groups. Inset displays representative immunoblots of ICAM-1 and IκB-α using specific anti-ICAM-1 and anti-IκB-α antibodies. Mean ± SEM, n = 4–6, *P < 0.05 vs. control group.
antibody treatment, the membranes were exposed to 2 ml of a mixture of luminol plus hydrogen peroxide under alkaline conditions (SuperSignal® West Dura Extended Duration Substrate, Pierce, Rockford) for 1 min, and the resulting chemiluminescent reaction was detected by Kodak X-OMAT AR Film (Eastman Kodak, Rochester, NY). Then the film was scanned, and the intensity of immunoblot bands was detected with a Bio-Rad Calibrated Densitometer (Model: GS-800).

**Histology processing**

Rats were euthanized under anesthesia at 6, 12, and 24 h following the intracerebral hemorrhage procedure. Following intra-cardiac perfusion with TBS, the lungs were excised and fixed in 10% formalin. Instillation of formalin into the airways was omitted to prevent damage to alveolar structures. The specimens were embedded in paraffin and cut by a microtome into 10-μm sections. Staining was performed with hematoxylin and eosin [H&E] (Knoferl et al., 2003).

**Statistical analyses**

Data were presented as Mean ± SEM. Statistical significance ($P < 0.05$) for each variable was estimated by analysis of variance (ANOVA) followed by a Dunnett’s post hoc analysis.

**Results**

*Effect of intracerebral hemorrhage on ICAM-1, IκB-α, tissue factor, TNF-α and IL-1β in brain and lung tissues*

Fig. 1A indicates that intracerebral hemorrhage acutely upregulated ICAM-1 expression in brain and lung tissues within 6 h of injury. The elevated ICAM-1 levels were sustained through 24 h following brain injury. Expression of IκB-α, the negative regulator of NFκB, remained unchanged in both brain and lungs throughout the 24 h post-injury duration examined (Fig. 1B). Expression of pro-coagulant tissue factor and cytokine TNF-α started to rise in brain within 6 h of injury. Brain IL-1β levels became significantly elevated after 6 h post-injury. Interestingly, the rise of tissue factor in lung tissues was delayed till 24 h after the brain injury. TNF-α levels were not significantly altered in the lung tissues following intracerebral hemorrhage during our 24 h post-injury frame. There was only a transient increase in the levels of lung IL-1β at 6 h post-injury time, which was quickly dropped back to normal range (Fig. 2).

**Histological examination in the lung tissues**

Lung tissue samples from control rats demonstrated normal intra-alveolar structure with no sign of intra-alveolar and interstitial neutrophil filtration (Fig. 3A). While there are...
some degrees of neutrophil aggregation and disruption in the intra-alveolar structure at 6 h after intracerebral hemorrhage (Fig. 3B), neutrophil infiltration and disruption of intra-alveolar structure becomes progressively intensified by 12 h (Fig. 3C) and 24 h (Fig. 3D) following intracerebral hemorrhage. The microscopic images reveal that most of the intra-alveolar spaces and capillary beds were filled with debris of red blood cells and heavy neutrophil aggregates at 12 h and 24 h post-injury time points.

Discussion

The major finding of our present study is that intracerebral hemorrhage triggers acute inflammatory response in both brain and lung tissues. We observed elevated ICAM-1 and tissue factor in both brain and lung tissues following brain injury. However, rise in the pro-coagulant tissue factor exhibited a significant delay in lung tissues compared with that in the brain. Although the levels of the cytokines TNF-α and IL-1β were significantly elevated in the brain, they remained unchanged in lung tissues (with the exception of a transient rise of IL-1β at 6 h) following intracerebral hemorrhage. Our histological microscopic examination of lung tissues further confirmed progressively increased neutrophil infiltration and intra-alveolar structural disruption. Collectively, these data strongly support our hypothesis that a secondary pulmonary inflammatory response may develop immediately following intracerebral hemorrhage.

Up-to-date, acute pulmonary dysfunction in patients with brain injury is believed to be attributed by either direct hemodynamic/hydrostatic insult (the “blast theory”) or increased vascular permeability (the “permeability theory”) as a result of increased sympathetic outflow (Fisher et al., 1999; Chen, 1995; Pyeron, 2001). This “neurogenic” root of pulmonary complication coincides with the neurogenic theory for intracerebral hemorrhage-induced electrocardiographic dysfunction of the hearts (Goel et al., 2005). Nonetheless, pulmonary complication of intracerebral hemorrhage is rather complex and may involve multi-factorial insults including disruption of blood–brain barrier, alteration in cerebrospinal fluid ubiquitin, and hypoxemia (Earle et al., 2005; Orliaguet et al., 2000; Lampl et al., 2005). Since acute intracerebral hemorrhage is often accompanied with a massive inflammatory response in the brain (Gong et al., 2000), it is natural to speculate that circulating inflammatory factor(s) originated from brain may enter the circulation, pass through the disrupted blood–brain barrier (Lampl et al., 2005), and mediate the cytotoxic and apoptotic effect in the lung. Our current study supports the idea that an acute inflammatory response may play an integral role in the development of pulmonary injury by initiating neutrophil infiltration into the intra-alveolar and interstitial space in the lung tissues en route to a massive tissue injury. Severe brain injury has been demonstrated to stimulate release of potent pro-inflammatory mediators including TNF-α, interleukin, and ICAM-1 into the circulation (Gong et al., 2000; Donnelly et al., 1996), which is supported by our current observation. These intracerebral hemorrhage-initiated inflammatory cytokines may pass through the blood–brain barrier to trigger neutrophil infiltration and a cascade of inflammatory response in pulmonary tissues. Absence of overt change in pulmonary TNF-α, IκB, and IL-1β levels (although IL-1β rose transiently at 6 h) during the first 24 h following intracerebral hemorrhage does not favor any significant role of TNF-α, IκB, and IL-1β in mediating pulmonary inflammatory response although it is premature to rule out

Fig. 3. Hematoxylin and eosin [H&E] staining displaying microscopic structure of lung from control (panel A), 6 h (panel B), 12 h (panel C), and 24 h (panel D) post-intracerebral hemorrhage groups. Intra-alveolar structure is normal with absence of intra-alveolar and interstitial neutrophil filtration in control. There is a progressively increased neutrophil aggregation and disrupted intra-alveolar structure from 6 h to 24 h following intracerebral hemorrhage.

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their possible contribution in delayed inflammatory response (>24 h). The delay of the significant rise in lung tissue factor level (24 h post-injury compared to 6 h in the brain) may reflect the time required for transmission of the inflammatory mediator from brain into the pulmonary system. It should be noted that several other circulating cytokine mediators such as thrombin and complement are also upregulated during central nervous system injury-initiated cerebral inflammatory response (Nishino et al., 1994; Pasinetti et al., 1992). Further studies are warranted to examine their potential contribution to the progression of pulmonary inflammatory injury. Last but not the least, other mediators from the blood clotting cascade following cerebral hemorrhage may also participate in the secondary pulmonary inflammatory response and deserve in-depth investigation.

Intracerebral hemorrhage and respiratory distress are common in premature neonates and newborns who suffered from birth injury. In addition to an infant’s high susceptibility to hemorrhage “courtesy of” the fragile cerebral vasculature, in utero exposure to infection plays an important role in the genesis of fetal or neonatal injury leading to cerebral palsy and chronic lung disease. Fetal inflammatory response has been shown to link antenatal infection with brain white matter damage and subsequent motor handicap (Garnier et al., 2003; Owens, 2005). Consistent with our current findings, cytokines released during intrauterine infection have been indicated to be directly responsible for injury to the immature brain and cardiopulmonary systems (Garnier et al., 2003). Although cerebral hemorrhage and associated respiratory distress are major problems in premature infants or newborns with birth injury, these problems are equally important health care issues for the elderly likely due to a significantly longer rehabilitation period following intracerebral hemorrhage (Cifu et al., 1996). At present, treatment for intracerebral hemorrhage-initiated respiratory problem is mainly supportive with the use of mechanical ventilator and α-adrenergic blockers while controlling intracranial pressure (Pyeron, 2001).

In summary, our findings revealed that a likelihood secondary pulmonary inflammatory response following intracerebral hemorrhage through a mechanism(s) related to ICAM-1 and tissue factor release. Given the high prevalence and severity of pulmonary tissue injury following intracerebral hemorrhage, a thorough understanding of the pathophysiological mechanisms behind intracerebral hemorrhage-and ischemia stroke-initiated lung damage should aid to the management of patients with cerebral injury to prevent further respiratory complications.

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