Anesthesiology 2005; 102:761–7 

# S-100 Protein and Neurohistopathologic Changes in a Porcine Model of Acute Lung Injury

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***Background:*** **Survivors of acute respiratory distress syndrome exhibit neuropsychological sequelae that might be attributable to hippocampal damage. The authors sought to determine the effects of hypoxemia in a pig model of acute lung injury on the hippocampal region and the release of S-100 protein in comparison to a control group in which hypoxemia was induced by reducing the inspired oxygen fraction.**

***Methods:*** **Hypoxemia was induced in 14 female pigs by repeated lung lavages (lung injury group; n = 7) or by reducing the inspired oxygen fraction (hypoxia-only group; n = 7). Hemodynamic variables, gas exchange, and serum concentrations of S-100 protein were measured at baseline, after induction of acute lung injury, and subsequently for 12 h. Animals were euthanized, and the brains were removed for histopathologic examination.**

***Results:*** **Comparable blood gases were seen in both groups. Serum S-100 protein concentrations were comparable for both groups at baseline. At all other time points, S-100 concentrations were significantly higher in the lung injury group. Neuropathologic examination showed basophilic and shrunken neurons of the pyramidal cell layer in the hippocampal CA1 subregion of all pigs in the lung injury group. Few abnormalities were seen in the hypoxia-only group.**

***Conclusions:*** **The same degree of hypoxemia induced in a lavage model of acute lung injury results in greater brain damage assessed by S-100 protein and histopathologic findings when compared to a group in which hypoxemia at the same degree was induced by reducing the inspired oxygen fraction. This suggests that acute lung injury leads to neuropathologic changes independent of hypoxemia.**

DURING the past decade, survival rates of acute respiratory distress syndrome (ARDS) are improving,[1–3] and therefore, several studies have focused on the long-term effects of this life-threatening disease. Most of these investigations documented pulmonary function abnormalities[4–6] as well as general physical function abnormalities.[6] More recently, several other studies showed that survivors of severe ARDS had psychosocial problems or generalized cognitive decline.[6–12] One group reported a significant correlation between the amount of time that patients with ARDS were under a certain oximetric saturation threshold value and their processing speed, attention, and memory skills.[10] Memory impairments are known to be associated with hypoxic damage to the hippocampus, especially the CA1 and CA2 subregions.[13–15] Because ARDS is characterized by profound arterial hypoxemia, which is often resistant to increases in inspired oxygen fraction ($F_{IO_2}$),[16] it might be possible that this condition results in damage of this vulnerable brain region. However, no study has examined the effects of hypoxemia resulting from ARDS on the hippocampal CA1 and CA2 subregions. In a clinical setting, adequate assessment of the above-mentioned regions is difficult to achieve. Furthermore, the results might be affected by other pathophysiologic processes, such as inflammation, drug toxicity, or thromboembolic events. Therefore, we designed a laboratory animal study in an established acute lung injury (ALI) model to investigate the effects of hypoxemia at a degree that might frequently occur in the clinical setting of ARDS on the hippocampal CA1 and CA2 subregions. Moreover, we included serial measurements of the brain-specific marker S-100 protein, which has shown to be a valuable diagnostic tool after focal, global, and traumatic brain injury,[17–20] and compared these findings to those of a control group in which the same degree of hypoxemia was induced by reducing the $F_{IO_2}$.

This article is accompanied by an Editorial View. Please see: Raabe A, Wissing H, Zwissler B: Brain cell damage and S-100B increase after acute lung injury. ANESTHESIOLOGY 2005; 102:713–4.

## Materials and Methods

### *Animal Preparation*

The experimental protocol was approved by Bezirksregierung Köln (Köln, Germany), and the study was performed according to the Helsinki convention for the use and care of animals. After intramuscular premedication with 6 mg/kg azaperone, an intravenous line was obtained, and anesthesia was induced with 5 mg/kg thiopental in 14 female pigs weighing 29 ± 2 kg. Muscle relaxation was achieved by administration of 0.2–0.4 mg · $kg^{-1}$ · $h^{-1}$ pancuronium. Anesthesia was maintained with continuous infusion of 5–10 mg · $kg^{-1}$ · $h^{-1}$ thiopental and 8–12 $\mu g \cdot kg^{-1} \cdot h^{-1}$ fentanyl. Animals were positioned supinely and intubated with an 8.0- to 9.0-ID endotracheal tube (Mallinkrodt, Athlone, Ireland). Controlled mechanical ventilation was adjusted with a respiratory rate of 20 $min^{-1}$, a tidal volume of 10 ml/kg, an inspiratory:expiratory time ratio of 1:2, and a positive end-expiratory pressure of 5 cm $H_2O$. The inspiratory

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Received from the Department of Anesthesiology, University Hospital of Rheinisch-Westfälische Technische Hochschule Aachen, Aachen, Germany. Submitted for publication September 15, 2004. Accepted for publication November 17, 2004. Support was provided solely from institutional and/or departmental sources.

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oxygen fraction was kept at 1.0. A 16-gauge arterial catheter (Vygon, Ecouen, France) and a 8.5-French venous sheath (Arrow Deutschland GmbH, Erding, Germany) were inserted percutaneously into femoral vessels. A pulmonary artery catheter (7.5 French, model AH-05050; Arrow Deutschland GmbH) was positioned under transduced pressure guidance. The blood temperature, as determined by the pulmonary artery catheter, was maintained at 36.7° ± 0.9°C during the experiment by using an infrared warming lamp and a warming pad. A continuous infusion of 4–5 ml · $kg^{-1}$ · $h^{-1}$ balanced electrolyte solution was administered for adequate hydration in both groups.

*Data Acquisition*

All hemodynamic measurements were taken in the supine position with zero reference level at the midchest. Mean arterial pressure and central venous pressure were transduced (pvb Medizintechnik, Kirchseeon, Germany) and recorded (AS/3 Compact; Datex-Ohmeda, Achim, Germany). Heart rate was traced from the blood pressure curve. Cardiac output was measured by using standard thermodilution techniques and expressed as the mean of three consecutive measurements. Blood samples were collected in duplicate, and analysis of arterial oxygen tension ($Pa_{O_2}$), arterial carbon dioxide tension ($Pa_{CO_2}$), and hemoglobin were performed immediately. Blood gases were determined by using standard blood gas electrodes (ABL 510; Radiometer, Copenhagen, Denmark). Hemoglobin was measured *via* species-specific spectroscopy (OSM 3; Radiometer). Oxygen delivery ($D_{O_2}$) and consumption ($\dot{V}_{O_2}$) were determined using the conventional formulas $D_{O_2} = CO \cdot Ca_{O_2} \cdot 10$ and $\dot{V}_{O_2} = CO \cdot (Ca_{O_2} - Cv_{O_2}) \cdot 10$, where CO is cardiac output, $Ca_{O_2}$ is arterial oxygen content, and $Cv_{O_2}$ is mixed venous oxygen content. Blood samples for the determination of S-100 were drawn from the arterial line and allowed to clot for 30 min at room temperature. Tubes were then centrifuged with 3,000*g* for 10 min. The supernatant was stored in aliquots at −80°C until analysis. Serum concentrations of S-100 were measured with the commercially available, automated immunoluminometric assays at the Liaison (Byk-Sangtec Diagnostica, Dietzenbach, Germany).

*Experimental Protocol*

Baseline measurements for S-100 protein, hemodynamics, and blood gas analysis were performed when the animal preparation was completed. We then established two experimental groups in which hypoxemia was achieved by different interventions. In the first group (lung injury group [LIG]), ALI was induced by a commonly used model of surfactant depletion.[21–23] This model produces arterial blood gas, radiographic, and histologic changes of the lung similar to those seen in patients with ARDS.[21,24] Briefly, after disconnecting the animals from the ventilatory circuit, the lungs were filled with 30 ml/kg prewarmed saline through the endotracheal tube. Removal of the lavage fluid was achieved by tilting the animals head down at an angle of approximately 45° using the gravitational gradient. This procedure was repeated until a stable ratio of $Pa_{O_2}$ to $F_{IO_2}$ of less than 100 mmHg was reached for 1 h. This point was set as ALI. During the lavages, continuous oxygen saturation data were documented every 15 s by pulse oximetry (AS/3 Compact). In the second group (hypoxia-only group [HOG]), hypoxemia was induced by changing the $F_{IO_2}$ and ventilator settings. To simulate the decrease in arterial oxygen saturation to the same degree as during the lavage procedures, the animals in the second group were ventilated for 180 s with a mixture of 10% oxygen in 90%. This procedure was repeated six times during a period of approximately 30 min, which was analogous to the induction procedure in the LIG. After that, animals were subjected to ventilation with a gas mixture of oxygen in nitrogen that achieved comparable $Pa_{O_2}$ and $Pa_{CO_2}$ values as in the LIG. Figure 1 depicts the study protocol in a flow diagram. Repeated measurements for S-100 protein, hemodynamics, and gas exchange were performed after the induction of ALI and after 2, 4, 8, and 12 h. At the end of the experiment, the sedated animals were killed by an intravenous bolus injection of potassium chloride.

*Histopathology*

All animal brains were carefully removed 5–15 min after decapitation. Fixation was done in a solution of 4% paraformaldehyde in sodium phospate buffer (pH 7.4). Ten days after fixation, coronar slides were made and the hippocampus, neocortex, basal ganglia, cerebellum, and brain stem as regions of interest were removed for light microscopy. Tissue samples were paraffin embedded, and 4-$\mu$m sections were taken and stained with hematoxylin and eosin. Light microscopy enabled determination of cell morphology in the regions of interest. We focused on argyrophilic dark neurons (DNs) because they reflect the early histopathologic state of damaged neurons after various brain injuries.[25] Neurons that showed all of the following three signs were classified as DNs: (1) irregular cellular outlines, (2) increased amounts of chromatin throughout the nucleoplasm and cytoplasm, and (3) intensely and homogeneously stained nucleoplasm with almost indiscernible heterochromatin. The amount of damaged tissue in any given region was quantitated by counting the DNs and relating them to the total number of neurons in this region.

*Statistical Analysis*

All values are expressed as mean ± SD. Normal distribution of the variables was determined by use of the Kolmogorov-Smirnov test. Group comparisons at all time points were performed by an independent *t* test. Statistical analysis was performed using standard software

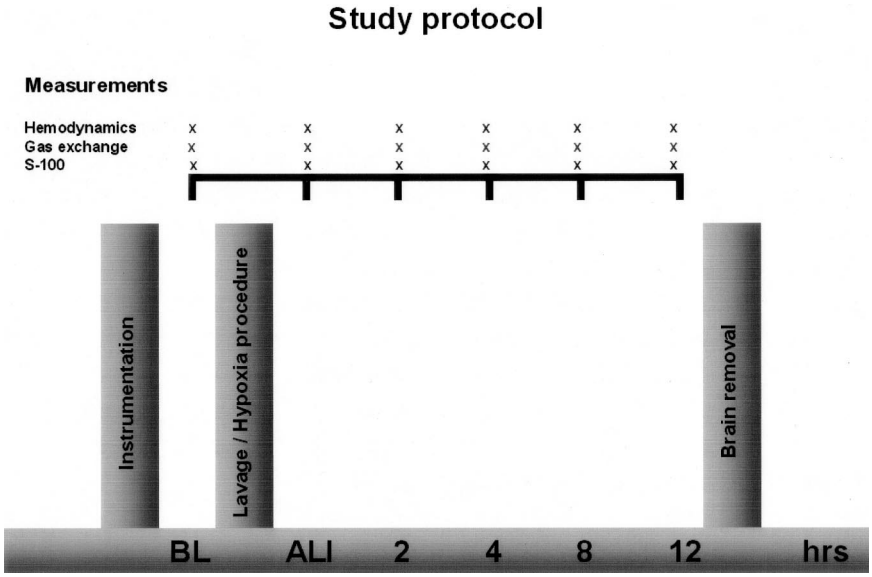


**Fig. 1. Flow diagram of the study protocol. ALI = acute lung injury; BL = baseline.**

(SPSS 11.0 for Windows; SPSS Inc., Chicago, IL). *P* values of less than 0.05 were considered significant.

## Results

All animals survived the entire study period. In the LIG, a mean of 10 ± 4 lavages had to be performed to obtain stable ALI with a decrease of $Pao_2$ from 396.7 ± 28.4 to 60.4 ± 7.1 mmHg. Grossly, comparable blood gases and hemodynamics were observed in both groups. The $Pao_2$ for the LIG was higher compared with the HOG after establishing ALI (60 ± 7 *vs.* 46 ± 8 mmHg; $P \leq 0.05$). After 8 h, $Paco_2$ was significantly higher in the HOG. Notably, over time, mean arterial pressure and oxygen

**Table 1. Hemodynamic Variables**

| | Baseline | ALI | 2 h | 4 h | 8 h | 12 h |
|---|---|---|---|---|---|---|
| HR, $min^{-1}$ | | | | | | |
| LIG | 86 ± 5 | 92 ± 16 | 95 ± 21 | 93 ± 12 | 97 ± 13 | 93 ± 18 |
| HOG | 88 ± 12 | 103 ± 24 | 86 ± 18 | 83 ± 16 | 76 ± 16* | 81 ± 10 |
| Hemoglobin, g/dl | | | | | | |
| LIG | 8.0 ± 0.6 | 7.6 ± 1.2 | 7.3 ± 0.6 | 6.8 ± 0.6 | 6.9 ± 0.7 | 6.6 ± 1.0 |
| HOG | 7.0 ± 0.7* | 6.5 ± 0.9 | 7.2 ± 0.7 | 7.3 ± 1.1 | 7.6 ± 1.0 | 7.2 ± 0.7 |
| MAP, mmHg | | | | | | |
| LIG | 99.3 ± 2.0 | 107.3 ± 7.9 | 102.1 ± 7.5 | 95.3 ± 9.8 | 98.4 ± 9.9 | 82.0 ± 5.4 |
| HOG | 90.9 ± 14.2 | 80.0 ± 12.2* | 85.7 ± 10.3* | 78.3 ± 7.6* | 77.6 ± 13.6* | 78.0 ± 8.9 |
| CVP, mmHg | | | | | | |
| LIG | 10.9 ± 1.2 | 13.0 ± 4.0 | 13.4 ± 2.2 | 14.4 ± 2.6 | 12.4 ± 1.9 | 13.3 ± 4.1 |
| HOG | 9.6 ± 3.4 | 11.1 ± 6.6 | 8.6 ± 2.4* | 8.9 ± 1.7* | 8.1 ± 1.6* | 8.4 ± 1.0* |
| CO, l/min | | | | | | |
| LIG | 6.0 ± 0.9 | 5.5 ± 1.1 | 5.9 ± 1.7 | 6.0 ± 1.7 | 6.1 ± 1.9 | 5.8 ± 1.7 |
| HOG | 4.7 ± 0.8* | 5.2 ± 1.3 | 4.6 ± 1.6 | 4.3 ± 1.5 | 3.4 ± 0.5* | 4.1 ± 0.8 |
| MPAP, mmHg | | | | | | |
| LIG | 21.1 ± 1.3 | 35.3 ± 6.0 | 35.3 ± 3.8 | 36.0 ± 3.6 | 35.6 ± 7.2 | 33.3 ± 4.8 |
| HOG | 19.2 ± 4.6* | 29.0 ± 4.6 | 21.7 ± 5.9* | 21.0 ± 3.2* | 21.0 ± 3.9* | 19.7 ± 2.3* |
| PCWP, mmHg | | | | | | |
| LIG | 11.7 ± 1.4 | 12.0 ± 2.0 | 12.0 ± 1.3 | 11.7 ± 2.4 | 10.9 ± 1.7 | 11.5 ± 2.2 |
| HOG | 10.1 ± 3.1 | 11.8 ± 4.0 | 9.9 ± 4.1 | 10.0 ± 1.7 | 9.7 ± 1.9 | 9.9 ± 1.8 |
| SVR, dyn · s · $cm^{-1}$ | | | | | | |
| LIG | 1,421 ± 326 | 1,431 ± 398 | 1,291 ± 404 | 1,152 ± 315 | 1,196 ± 313 | 1,023 ± 287 |
| HOG | 1,374 ± 61 | 1,090 ± 233 | 1,469 ± 652 | 1,376 ± 450 | 1,637 ± 315* | 1,329 ± 104* |
| PVR, dyn · s · $cm^{-1}$ | | | | | | |
| LIG | 416 ± 67 | 345 ± 102 | 334 ± 101 | 341 ± 103 | 335 ± 124 | 329 ± 123 |
| HOG | 152 ± 42* | 300 ± 78 | 229 ± 114 | 231 ± 95 | 281 ± 101 | 279 ± 72 |

Data are presented as mean ± SD.

* $P < 0.05$ between groups.

ALI = acute lung injury; CO = cardiac output; CVP = central venous pressure; HOG = hypoxia-only group; HR = heart rate; LIG = lung injury group; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

**Table 2. Variables of Gas Exchange**

| | Baseline | ALI | 2 h | 4 h | 8 h | 12 h |
|---|---|---|---|---|---|---|
| $Pa_{O_2}$, mmHg | | | | | | |
| LIG | 397 ± 28 | 60 ± 7 | 155 ± 78 | 129 ± 53 | 191 ± 108 | 232 ± 160 |
| HOG | 467 ± 95 | 46 ± 8* | 214 ± 62 | 191 ± 40* | 202 ± 50* | 233 ± 44 |
| $Pa_{CO_2}$, mmHg | | | | | | |
| LIG | 35.4 ± 0.5 | 45.1 ± 6.2 | 56.6 ± 6.6 | 53.3 ± 6.2 | 49.4 ± 5.8 | 52.5 ± 7.5 |
| HOG | 39.6 ± 10.0 | 53.6 ± 1.0 | 57.7 ± 9.3 | 57.6 ± 6.8 | 61.9 ± 5.3* | 54.7 ± 2.8 |
| $D_{O_2}$, ml/min | | | | | | |
| LIG | 506 ± 39 | 499 ± 61 | 550 ± 106 | 534 ± 99 | 577 ± 177 | 510 ± 103 |
| HOG | 492 ± 117 | 335 ± 119* | 461 ± 162 | 418 ± 138 | 354 ± 75* | 365 ± 104* |
| $\dot{V}_{O_2}$, ml/min | | | | | | |
| LIG | 172 ± 11 | 172 ± 18 | 152 ± 24 | 174 ± 12 | 167 ± 23 | 170 ± 18 |
| HOG | 166 ± 101 | 145 ± 28 | 144 ± 64 | 119 ± 23* | 132 ± 19* | 164 ± 45 |
| pH | | | | | | |
| LIG | 7.36 ± 0.01 | 7.38 ± 0.06 | 7.29 ± 0.06 | 7.30 ± 0.03 | 7.33 ± 0.05 | 7.28 ± 0.05 |
| HOG | 7.51 ± 0.13* | 7.34 ± 0.05 | 7.34 ± 0.1* | 7.30 ± 0.08* | 7.26 ± 0.05* | 7.34 ± 0.08 |

Data are presented as mean ± SD.

* $P < 0.05$ between groups.

ALI = acute lung injury; $D_{O_2}$ = oxygen delivery; HOG = hypoxia-only group; LIG = lung injury group; $Pa_{CO_2}$ = arterial carbon dioxide partial pressure; $Pa_{O_2}$ = arterial oxygen partial pressure; $\dot{V}_{O_2}$ = oxygen consumption.

delivery were also higher in the LIG compared with the HOG (tables 1 and 2). Lavage and hypoxia procedures resulted in a biphasic decrease of arterial oxygen saturation. However, the total time of desaturation below an oxygen saturation measured by pulse oximetry ($Spo_2$) of 85% was 713 ± 63 s for the LIG and 702 ± 76 s for the HOG ($P$ = not significant) and therefore comparable for both groups. Figure 2 shows the decrease of $Spo_2$ and the equivalent time points during lavage and hypoxia, respectively. Serum concentrations of S-100 protein were not significantly different between the two groups at baseline measurement. After induction of ALI, serum concentrations in the LIG increased approximately fivefold, whereas in the HOG, they increased only twofold (0.42 ± 0.15 *vs.* 0.19 ± 0.54 μg/l; $P < 0.003$). Furthermore, a significant difference in S-100 was found between the two groups at all other time points (fig. 3). In five of seven pigs in the LIG, a secondary increase in S-100 serum concentrations was observed. The postmortem examination showed macroscopic absence of hemorrhage, ischemic stroke, and thromboembolic signs. Ten days after fixation and slicing in coronar orientation, gross sections confirmed the postmortem examination. In light microscopy of all seven pigs of the LIG, the pyramidal cell layer of the hippocampus showed segmental changes, especially representation of DNs. These damaged pyramidal cells were preferentially seen in the CA1 subregion adjacent to the lateral ventricle (figs. 4A–C). In four of seven cases, the CA2 subregion was involved, whereas DNs were not observed in regions distant from the hippocampus, *i.e.*, the CA3-subregion, dentate gyrus, or entorhinal cortex. In the HOG, all seven pigs showed DNs in the CA1 subregion, and two of seven pigs showed DNs in the CA2 subregion. Similar to the LIG, no damage was observed in regions distant from the hippocampus. The percentage of damaged neurons was significantly greater in the LIG than in the HOG (40 ± 12 DNs of 768 ± 24 neurons *vs.* 13 ± 7 of 770 ± 19; $P \leq 0.05$). No damage was seen in any of the other regions of interest.

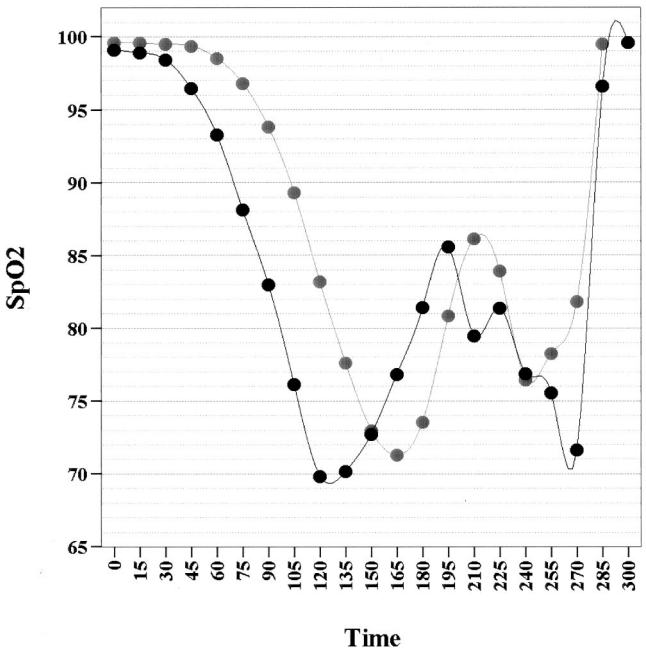


**Fig. 2. Continuous pulse oximetry saturation ($Spo_2$, in percent) of all pigs during a single lavage procedure showing the equal amount of hypoxemia for both groups (time in seconds). *Black line* = lung injury group; *gray line* = hypoxia-only group.**

## Discussion

The purpose of the current study was to investigate the effects of hypoxemia on different brain regions of pigs in an established experimental model of ALI at a

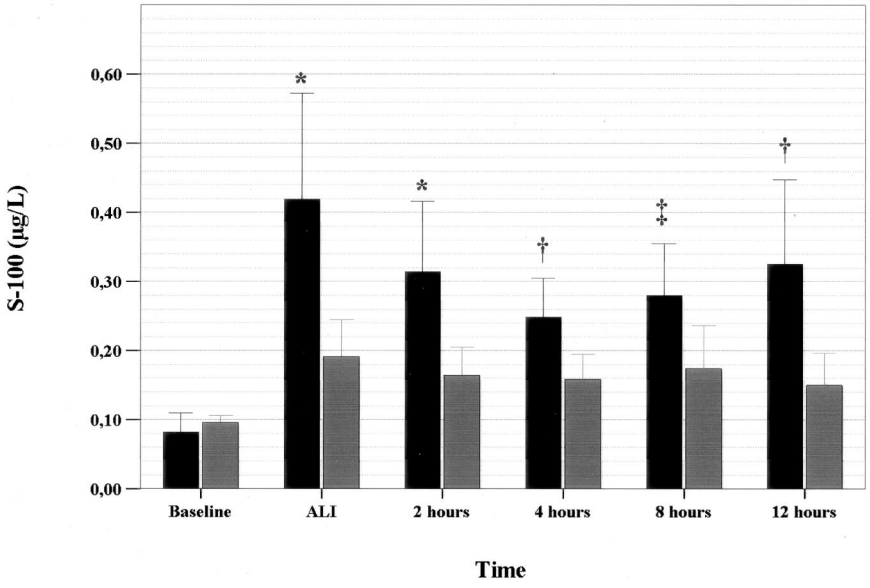


**Fig. 3. Time course of S-100 protein. *Black boxes* = lung injury group; *gray boxes* = hypoxia-only group. * $P < 0.003$ between groups; † $P < 0.005$ between groups; ‡ $P < 0.02$ between groups.**

degree that might frequently occur in the clinical setting of ARDS. There are three major results from our data: First, the peak serum concentrations of S-100 protein in the LIG increase approximately fivefold shortly after induction of ALI, probably reflecting damage to brain tissue. Second, these biochemical changes of neuronal damage are confirmed by histopathologic changes in the vulnerable CA1 subregion of the hippocampus of all pigs. Third, the same degree of hypoxemia without the induction of lung injury results in minor brain damage and S-100 liberation. This suggests that ALI leads to neuropathologic changes independent of hypoxemia.

The role of the central nervous system in severe ARDS has been poorly investigated in the past, although there is clinical evidence that nearly all survivors of severe ARDS show neuropsychological impairments at the time of hospital discharge, including impaired memory, attention, and concentration deficits or decreased mental processing speed. These findings are persistent after 1 yr in at least a third of these patients.[6,7,10] To what extent hypoxemia contributes to this phenomenon is at best unclear because the clinical course of patients with ARDS is often complicated by several confounding factors such as thromboembolism, long-term analgosedation, and sepsis. A simple way to identify patients with neuronal damage is the determination of serum concentrations of S-100 protein. S-100, predominantly existing in astroglial cells of the central nervous system of all vertebrates, has proved to be a fast-responding, sensitive, and specific marker, representing brain tissue damage and disintegration of the blood–brain barrier.[17–20] In our study, serum concentrations of S-100 protein peaked

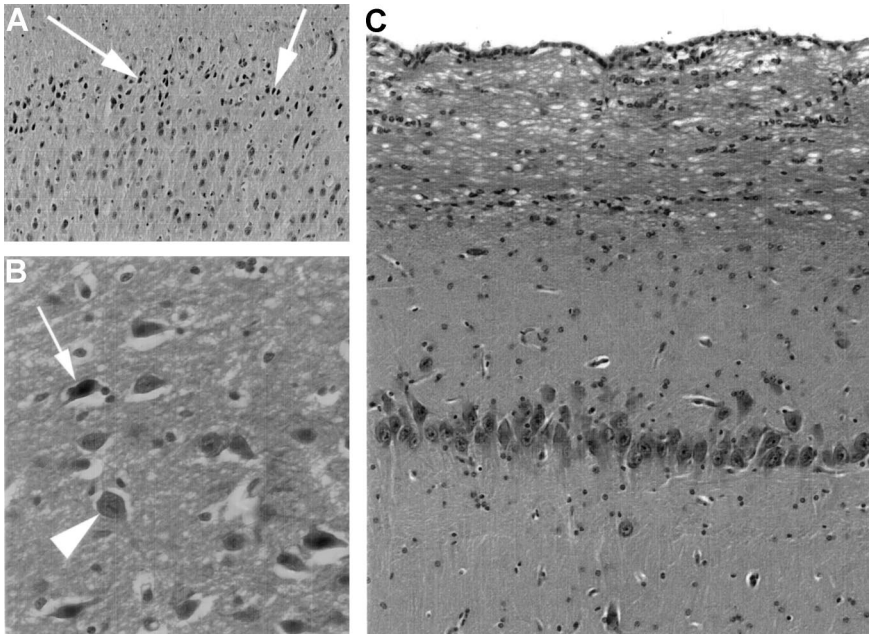


**Fig. 4. (*A*) Pyramidal cell layer of the CA1 subregion of a pig from the lung injury group showing a layer of shrunken, so-called dark neurons (*arrows*), whereas neighboring layers of neurons seem to be intact. (*B*) Parahippocampal region of the same pig representing disseminated dark neurons, focally accompanied by satellite cells (*arrow*) adjacent to intact neurons (*arrowhead*). (*C*) CA1 subregion of a pig from the hypoxia-only group showing intact neurons in the pyramidal cell layer.**

at a value of 0.42 ± 0.15 μg/l. These concentrations are comparable to those obtained in other porcine models leading to brain damage after cardiocirculatory arrest or cardiopulmonary bypass.[26,27] Interestingly, very high concentrations of S-100 protein have been found to induce apoptotic changes and stimulate the expression of proinflammatory cytokines.[28]

Animal studies performed in the 1980s in which profound arterial hypoxemia was established by decreasing the arterial oxygen tension to 25 mmHg for approximately 30 min showed only discrete neuronal damage assessed by clinical and histopathologic examinations. These results worsened by decreasing the arterial pressure.[29,30] A recent investigation using different types of mechanisms inducing cerebral damage confirmed that hypoxemia alone is less threatening for the central nervous system than hypoxemia combined with severe arterial hypotension.[31] This is in accordance with our findings in the HOG. In our investigation, mean arterial pressure, cardiac output, hemoglobin, and oxygen delivery remained within physiologic ranges in all investigated pigs during the entire experiment. In the LIG, these variables were at some time points even significantly higher. Nevertheless, there is evidence for brain damage in this animal model assessed by biochemical alterations of S-100 protein and histopathologic changes in vulnerable regions of the hippocampus, *i.e.*, CA1 and CA2 sectors.

The hippocampus is known to be associated with cognitive functions, especially memory processing. Neuronal damage in this part of the brain leads to obvious impairment in humans[32,33] as well as in animals.[13,14] Volpe *et al.*[14] showed in a rat model of ischemia that the loss of neurons in the CA1 subregion is significantly correlated with memory performance in these animals. Using the same model, Deshpande *et al.*[34] proved that ultrastructural changes in the CA1 subregion occur as early as 6 h after a 10-min period of common carotid artery occlusion. More recently, Gale and Hopkins[35] documented significant correlations between hippocampal volume and performance on measures related to nonverbal information processing in patients with intermittent hypoxia due to obstructive sleep apnea syndrome. Hopkins *et al.*[10] reported that survivors of severe ARDS have impaired processing speed, attention, and memory skills, which were correlated with the amount of time these patients were under a certain oximetric threshold value. Although this investigation was not designed to observe brain structural changes directly, it suggested that the CA1 subregion may be damaged in patients with ARDS, because all of the reported neurologic findings are mediated by the hippocampus. The histopathologic and biochemical alterations in our porcine model of ALI might be the link between this finding and our laboratory investigation.

Because the duration and amount of hypoxemia and hypercapnia in both groups are grossly comparable and no signs of thromboembolism were present, the question arises as to why S-100 concentrations and neuropathologic findings deteriorate in the LIG. This might be explained at least in part by the lavage procedure used to achieve a stable lung injury. Several studies showed that ALI is associated with the increase of different proinflammatory and antiinflammatory cytokines such as interleukin 1β, interleukin 6, tumor necrosis factor α, and neutrophils.[36,37] This immunologic response to the induction of ALI could be a possible explanation for our observations because it is well known that inflammation *per se* contributes to neuronal damage, especially in hippocampal neurons.[38,39] This ongoing inflammatory process might also explain the secondary increase in S-100 concentrations in five of seven pigs.

Our investigation has certain limitations that must be addressed. The animals were ventilated with relatively large tidal volumes when compared with the results of the ARDS Network Study. However, because lung-protective ventilation was not an endpoint of the experimental design and the tidal volumes were used in both groups, we only see a minor problem hereon. Using different mechanisms to induce hypoxemia was followed by different responses in gas exchange and hemodynamics, making the two groups not completely comparable at all investigated time points. The concept that an inflammatory response is responsible for the observed differences remains speculative because we did not measure any cytokine serum concentrations.

Nevertheless, our findings allow new insights in the pathophysiology of brain damage after ALI. In conclusion, we demonstrated for the first time that an established porcine model of ALI shows brain-related cell damage in the vulnerable CA1 and CA 2 subregions of the hippocampus and increased S-100 protein serum concentrations when compared to a porcine model of hypoxia alone.

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