0031-3998/01/4906-0812
PEDIATRIC RESEARCH
Vol. 49, No. 6, 2001
Printed in U.S.A.

Impaired Early Neurologic Outcome in Newborn Piglets Reoxygenated with 100% Oxygen Compared with Room Air after Pneumothorax-Induced Asphyxia

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ABSTRACT

Birth asphyxia is a serious problem worldwide, resulting in 1 million deaths and an equal number of neurologic sequelae annually. It is therefore important to develop new and better ways to treat asphyxia. In the present study we tested the effects of reoxygenation with room air or with 100% oxygen (O_2) after experimental pneumothorax-induced asphyxia on the blood oxidative stress indicators, early neurologic outcome, and cerebral histopathology of newborn piglets. Twenty-six animals were studied in three experimental groups: *1*) sham-operated animals (SHAM, $n = 6$), *2*) animals reoxygenated with room air after pneumothorax (R21, $n = 10$), and *3*) animals reoxygenated with 100% O_2 after pneumothorax (R100, $n = 10$). In groups R21 and R100, asphyxia was induced under anesthesia with bilateral intrapleural room air insufflation. Gasping, bradyarrhythmia, arterial hypotension, hypoxemia, hypercarbia, and combined acidosis occurred 62 ± 6 min (R21) or 65 ± 7 min (R100; mean ± SD) after the start of the experiments; then pneumothorax was relieved, and a 10-min reoxygenation period was started with mechanical ventilation with room air (R21) or with 100% O_2 (R100). The newborn piglets then breathed room air spontaneously during the next 3 h. Blood oxidative stress indicators (oxidized and reduced glutathione, plasma Hb, and malondialdehyde concentrations) were measured at different stages of the experiments. Early neurologic outcome examinations (neurologic score of 20 indicates normal, 5 indicates brain-dead) were performed at the end of the study. The brains were next fixed, and various regions were stained for cerebral histopathology. In the SHAM group, the blood gas and acid-base status differed significantly from those measured in groups R21 and R100. In group R100, arterial Po_2 was significantly higher after 5 (13.8 ± 5.6 kPa) and 10 min (13.2 ± 6.3 kPa) of reoxygenation than in group R21 (8.7 ± 2.8 kPa and 9.2 ± 3.1 kPa). The levels of all oxidative stress indicators remained unchanged in the study groups (SHAM, R21, and R100). The neurologic examination score in the SHAM group was 18 ± 0, in group R21 it was 13.5 ± 3.1, and in group R100 it was 9.5 ± 4.1 (significant differences between SHAM and R21 or R100, and between R21 and R100). Cerebral histopathology revealed marked damage of similar severity in both asphyxiated groups. We conclude that the blood oxidative stress indicators and cerebral histopathology did not differ significantly after a 10-min period of reoxygenation with room air or with 100% O_2 after pneumothorax-induced asphyxia, but reoxygenation with 100% O_2 might impair the early neurologic outcome of newborn piglets. **(*Pediatr Res* 49: 812–819, 2001)**

Abbreviations:
R21, reoxygenation with room air
R100, reoxygenation with 100% oxygen
SHAM, sham-operated
Fio_2, fraction of O_2 in the inspired gas

Birth asphyxia is a serious clinical problem worldwide. Each year approximately 4 million babies are born asphyxiated, which results in 1 million deaths and an equal number of serious neurologic sequelae, such as cerebral palsy, mental retardation, and epilepsy (1). It is therefore clinically important to develop new and better ways to treat asphyxia. The human infants affected are usually resuscitated with a high concentration of oxygen (2). The guidelines of the American Heart Association from 1992 for the resuscitation of newborns recommend ventilation *via* bags that can give as close to 100% O_2 as possible (3). The possible toxic effects of hyperoxia have

Received April 25, 2000; accepted October 20, 2000.
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Supported by the Hungarian Ministry of Health (Budapest, Hungary, grant ETT-80-4/1998), the Hungarian Research Council for Science (Budapest, Hungary, grant T 017111 and T 026295), and the Alexander von Humboldt Foundation (Bonn, Germany, grant V-8100).

been known for many years (4). The sudden reintroduction of a high concentration of oxygen to hypoxic tissues may result in a burst of oxygen free radical formation (5–7), which may increase the hypoxic tissue damage. Reoxygenation and reperfusion after severe hypoxia and ischemia may contribute substantially to birth asphyxia-related brain injury (8). In a number of animal studies, resuscitation with room air has been shown to be as efficient as (9, 10), or even superior to (11–13), resuscitation with 100% O_2. Finally, the gradual reintroduction of oxygen after ischemia has been shown to improve the functional and metabolic recovery of the CNS (14, 15).

A recently published clinical study indicates no major differences in outcome when resuscitation is performed with room air as compared with 100% O_2 (16). However, the time to the first breath and the time to the first cry were significantly shorter in the room air group than in the oxygen group. Studies on newborn animals have shown that room air is as effective as 100% O_2 in normalizing both regional and cerebral blood flows and also the evoked potentials after hypoxemia (10, 17).

In the present study we tested the hypothesis that asphyxiated newborn piglets can be successfully resuscitated with room air. We additionally tested the effects of reoxygenation after pneumothorax-evoked asphyxia with 100% O_2 on blood oxidative stress indicators, early neurologic outcome, and cerebral histopathology by using our newborn piglet asphyxia model (18–20). We further hypothesized that reoxygenation with 100% O_2 after asphyxia would affect the blood oxidative stress indicators, early neurologic outcome, and cerebral histopathology of the animals, and the data were therefore compared with those obtained on SHAM and R21 newborn piglets.

METHODS

Surgical preparation and experimental protocol. The present study was performed on 26 piglets from the Agriculturist Cooperative, Szeged, Hungary, which were between 3 and 6 h old at the beginning of the experiments and weighed 1.12 to 1.42 kg. The animals were anesthetized with ketamine hydrochloride (Ketanest, Parke-Davis, Morris Plains, NJ, U.S.A.; 10 mg/kg body weight intramuscularly). Figure 1 provides an outline of the experimental protocol. The animals were immobilized in the supine position, a tracheotomy was performed (local anesthesia, lidocaine hydrochloride, EGIS Ltd., Budapest, Hungary; 1.0 mL, 1.0 vol% s.c.), the animals were intubated (2.5–3.0 tubes, Portex Ltd, Hythe, Kent, U.K.), and one of the umbilical arteries was cannulated. The newborn piglets were divided into three experimental groups. SHAM newborn piglets ($n = 6$, three males and three females) served as controls: only Ketanest and local chest skin anesthesia (lidocaine hydrochloride, EGIS Ltd, to both sides) were applied, and no intrapleural air was insufflated. In 20 newborn piglets, after skin anesthesia, room air was insufflated through chest tubes inserted into the pleural cavities, and pneumothorax was produced by a constant air flow from a high-pressure room air cylinder (21). The clinical status of the animals worsened continuously, and by the end of 1 h after the induction of pneumothorax, gasping, bradyarrhythmia, arterial hypotension, hypoxemia, and severe combined acidosis had developed. The pneumothorax was then relieved, and the response to therapy was controlled by transillumination (Wild M4000 fiberscope, Heerburgg, Switzerland) of the thorax (18, 22, 23). Asphyxiated animals were randomly divided into two groups. Ten piglets (five males and five females) were artificially ventilated and reoxygenated with room air (Fio_2 0.21) for 10 min (group R21), whereas the others ($n = 10$, five males and five females) were ventilated with 100% O_2 (Fio_2 1.0) for 10 min (group R100). A conventional, constant-volume, pressure-limited infant respirator (MTA-KUTESZ, Budapest, Hungary) was used to ventilate the involved animals on the basis of our previous laboratory practice (24). Ventilator settings were as follows: tidal volume, 10–16 mL; frequency, 40 breaths/min; inspiratory time, 0.75 s; peak inspiratory pressure, 1.18–1.48 kPa (12–15 cm H_2O); and, with the avoidance of adverse effects (*e.g.* increased intracranial pressure and production of antidiuretic hormone), end-expiratory pressure, 0 kPa. The spontaneously breathing animals subsequently remained in room air (recovery phase) and were followed up to the end (240 min from the start) of the experiments. Rectal temperature was kept between 38 and 39°C; heart rate and mean arterial blood pressure were monitored continuously.

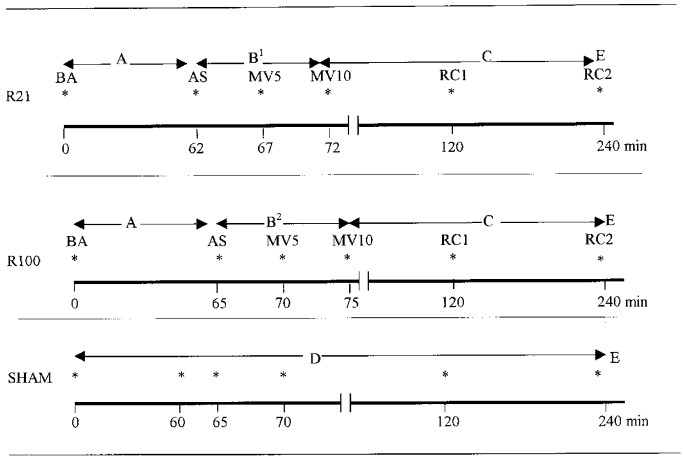

Figure 1. Experimental protocol of pneumothorax-evoked asphyxia with reoxygenation either with room air—R21 group—or 100% O_2—R100 group—and SHAM group animals. *A*, bilateral pneumothorax, spontaneous breathing in room air; B^1, mechanical ventilation with 21% O_2; B^2, mechanical ventilation with 100% O_2; *C*, recovery, spontaneous breathing in room air; *D*, spontaneous breathing in room air; *E*, neurologic and cerebral histopathologic examination; *BA*, baseline; *AS*, asphyxia; *MV5*, 5 min of mechanical ventilation; *MV10*, 10 min of mechanical ventilation; *RC1*, 120 min after BA; *RC2*, 240 min after BA; * indicates time points of blood sampling.

Blood samples and measurements of blood oxidative stress indicators. Arterial blood samples for gas, acid-base, and oxidative stress indicator analyses were drawn at different times (baseline, asphyxial, during mechanical ventilation, and recovery) as specified in Figure 1, according to standard methods. The withdrawn blood was replaced with a double volume of 0.9 vol% NaCl (maximal saline vol, 12 mL/animal). The laboratory methods used (briefly) were as follows. *Glutathione assay*: A highly sensitive and specific method was used (25). Twenty-five microliters of whole blood anticoagulated with EDTA was immediately hemolyzed in 2.5 mL of cold (4°C) 0.01 M potassium phosphate buffer containing 5 mM EDTA

(pH 7.5) and stored at −70°C until further use. For analysis, 25 μL of hemolysate was added to the standard glutathione assay mixture (final volume = 1.0 mL) in the following sequence: 5,5'-dithio-bis-2-nitrobenzoic acid (0.6 μM), glutathione reductase (10 μg), and NADPH (0.2 μM). The combined action of 5,5'-dithio-bis-2-nitrobenzoic acid and NADPH in the presence of glutathione reductase results in a reaction cycle, the rate of which depends on the total concentration of reduced glutathione and oxidized glutathione recorded spectrophotometrically at 412 nm during the first 6 min. The concentrations of thiols were expressed as the oxidized glutathione to reduced glutathione ratio with reference to Hb determined by the cyanmethemoglobin method. *Plasma Hb assay*: Heparinized plasma samples were diluted 1:40 (vol/vol) with 5 mM PBS (pH 7.4) and measured spectrophotometrically (26). *Plasma malondialdehyde assay*: Heparinized plasma samples were hydrolyzed in dilute phosphoric acid (0.22 M). Malondialdehyde was reacted with thiobarbituric acid (7 mM) to form malondialdehyde-thiobarbituric acid adduct. After methanol precipitation of proteins, the amount of adduct formed was quantified by HPLC (Waters-Millipore Corp., Milford, MA, U.S.A.) and by spectrophotometric detection at 532 nm (Variable Wavelength Monitor, Pharmacia LKB, Uppsala, Sweden) (27).

Piglet neurologic examination. Neurologic examinations were performed by a blinded observer at 240 min from the start of the experiments (Fig. 1). The results were recorded and scored from 5 to 20, with 20 corresponding to normal and 5 to brain-dead, according to a standard scoring system (28) (Table 1).

Cerebral histopathology. Newborn piglets were again anesthetized with Ketanest (20 mg/kg body weight, intramuscularly), the right atrium was punctured with a 12-gauge needle, and brains were perfused and fixed through the arterial catheter with 0.9 vol% NaCl (0.25 L/kg body weight), followed by phosphate-buffered formaldehyde (4.0 vol%, 0.15 L/kg body weight). The brain was next removed and preserved for later pathologic examination. Different paraffin sections were taken, stained with hematoxylin and eosin, and examined by light microscopy. The following regions were examined by one of the authors (I.B.), who was not informed of the mode of treatment or of the clinical information: frontal and temporal cortex, cerebellum, hippocampus, basal ganglia, and pons. The extent of damage for each region was graded as described previously (28), with minor modifications (Table 2). The hypoxic cellular change primarily involved largely shrunken hyperchromatic neurons with pyknotic nuclei; the scores were 5 to 1, depending on the proportions of damaged to spared cells.

Statistics. All values are given as mean ± SD. The results of the neurologic and cerebral histopathologic examinations are also expressed as median values (25th and 75th percentiles). The examinations on the SHAM group were performed to investigate the stability of the preparation, and the results were included in the statistical analysis. The nonparametric Kruskal-Wallis analysis was followed by the Mann-Whitney *U* test to determine significant differences among groups. All analyses were performed with a statistical computer program (Statgraphics, Statistical Graphics Co., Englewood Cliffs, NJ, U.S.A.); and p values <0.05 were considered to be significant.

Table 1. *Piglet neurologic examination results*

Examination	Finding	Score*
Mental status (subtract 1 for seizures)	Coma, no responsiveness	1
	Stupor, responsiveness to vigorous stimulation only with posturing	2
	Lethargy, drowsiness or delirium	3
	Awake	4
	Subtotal	—
	Subscore (= subtotal)	—
Cranial nerves		
Pupils	Unreactive	1
	Sluggish	2
	Normal	3
Corneals	Absent	1
	Present	4
Oculovestibular	Absent	1
	Present	4
Suck	Absent	1
	Present	4
	Subtotal	—
	Subscore (= subtotal/4)	—
Reflexes		
Deep tendon reflexes	Absent	1
	Hyper	2
	Normal	4
Stepping	Absent	1
	Present	4
Righting	Absent	1
	Present	4
	Subtotal	—
	Subscore (= subtotal/3)	—
Motor	Unable to stand	1
	Bears weight with abnormal posture	2
	Gets to standing with difficulty	3
	Stands normally	4
	Subtotal	—
	Subscore (= subtotal)	—
Coordination	No attempt to walk	1
	Attempts to walk but cannot	2
	Walks but falls	3
	Walks normally	4
	Subtotal	—
	Subscore (= subtotal)	—
	Score (= sum of subscores)	—

* Score of 20 is considered normal, score of 5, brain-dead.

Table 2. *Pathologic scoring system*

Score	Cellular change	% Ratio (damaged/spared neurons)
5	None	None
4	Hypoxic	Scattered cells
3	Hypoxic	<33%
2	Hypoxic	33–66%
1	Hypoxic	66–100%

Approval. The experimental procedures were performed in accordance with the prescriptions of the National Institutes of Health for the care and use of laboratory animals, and were approved by the Ethical Committee on Animal Investigation, Medical University, Szeged, Hungary (ATB 90).

RESULTS

There were no significant differences in age (4.5 ± 0.3, 4.2 ± 0.3, and 4.6 ± 0.2 h) or in body weight (1283 ± 56, 1326 ± 46, and 1329 ± 37 g) among the SHAM and the asphyxiated groups (R21 and R100, respectively), and no difference in pneumothorax time (62 ± 6 and 65 ± 7 min) between R21 and R100 groups, respectively. The three groups were also comparable with regard to the measures at baseline.

Cardiovascular, blood gas, and acid-base measurements. Mean arterial blood pressure was decreased significantly in both pneumothorax groups (R21 and R100) at asphyxia, but was then significantly elevated both at 5 min and at 10 min during mechanical ventilation. Later, during the 180-min recovery phase (120 and 240 min after baseline), significant hypotension was observed (R21 and R100) as compared with both the baseline values and the data measured in the SHAM group (Fig. 2). Heart rate was decreased significantly at asphyxia in both pneumothorax groups (R21 and R100); it increased temporarily during mechanical ventilation, but remained significantly decreased during recovery as compared with both the baseline values and the data from the SHAM group (Fig. 3). Arterial pH was decreased significantly at asphyxia in both pneumothorax groups (R21 and R100); it then increased continuously during recovery, but it remained significantly decreased as compared with both the baseline values and those of the SHAM group (Fig. 4). Arterial P_{CO_2} was increased significantly at asphyxia in both pneumothorax groups (R21 and R100), decreased significantly during mechanical ventilation, but remained significantly higher as compared with both the baseline values and those of the SHAM group during recovery (Fig. 5). Base deficit was increased significantly at asphyxia in both pneumothorax groups (R21 and R100), decreased significantly during mechanical ventilation and recovery, but remained significantly higher as compared with both the baseline values and the data from the SHAM group (Fig. 6). Arterial P_{O_2} was decreased significantly at asphyxia in both pneumothorax groups (R21 and R100), and then increased significantly during mechanical ventilation. The values measured in the R100 group were significantly higher after both 5 and 10 min of mechanical ventilation as compared with the data from the R21 group. During recovery, arterial P_{O_2} decreased significantly again as compared with both the baseline values and those during mechanical ventilation and the data from the SHAM group (Fig. 7). There were no significant differences between the two pneumothorax groups (R21 and R100) as regards any of the measurements followed during the study (except arterial P_{O_2} during mechanical ventilation).

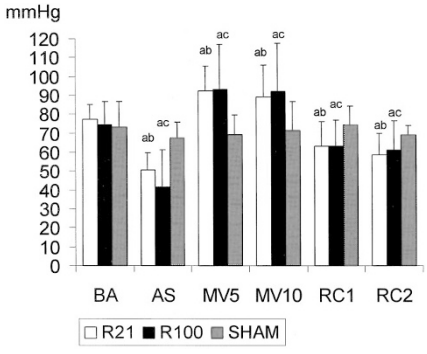

Figure 2. Mean arterial blood pressure (mm Hg) of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

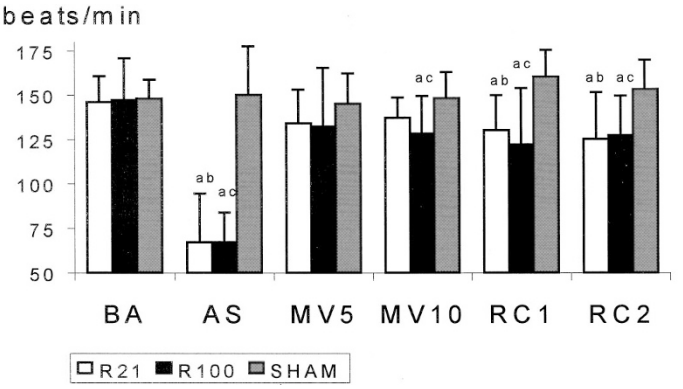

Figure 3. Heart rate (beats/min) of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

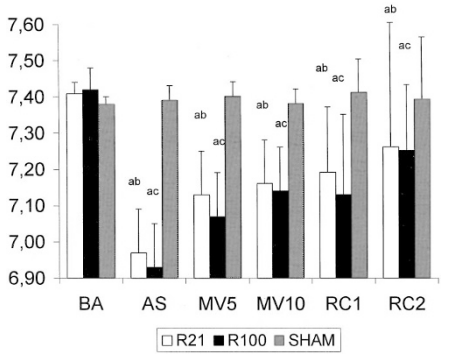

Figure 4. Arterial blood pH of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

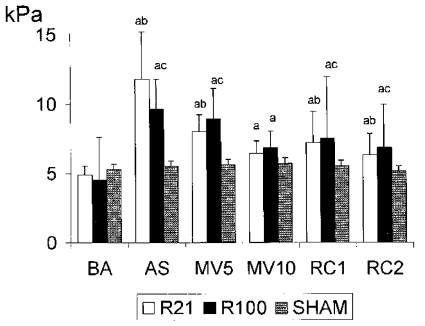

Figure 5. Arterial blood P_{CO_2} (kPa) of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

Blood oxidative stress measurements. There were no significant differences either within or among the three groups during the study (Table 3).

Piglet neurologic examination. Results of the neurologic examinations are shown in Table 4. Neurologic examination scores were significantly lower in both pneumothorax groups

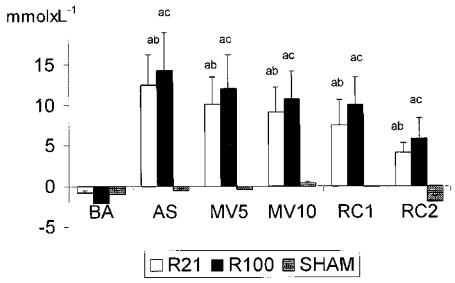

Figure 6. Arterial blood base deficit (mM) of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

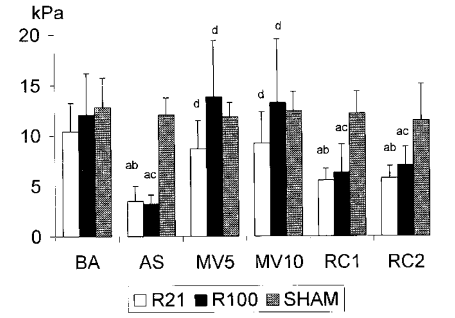

Figure 7. Arterial blood Po_2 (kPa) of the experimental animals. *a*, $p < 0.05$ *vs* baseline within the group; *b*, $p < 0.05$ *vs* R21 and SHAM; *c*, $p < 0.05$ *vs* R100 and SHAM. Values are mean ± SD. Abbreviations as in Figure 1.

(R21 and R100) as compared with the score for the SHAM group. Moreover, the score for the R100 group was significantly lower than that for the R21 group.

Cerebral histopathology. Histopathologic examination scores are shown in Table 5. The results on the SHAM piglets (not subjected to pneumothorax) were significantly higher (except for the region of the pons) than those on the asphyxiated groups (R21 and R100). Marked microscopic neuronal damage (Fig. 8) was seen in the newborn piglets with asphyxia, without any significant differences between the two groups (R21 and R100). No significant damage was observed in the pons without any significant differences between the two groups (R21 and R100). No significant damage was found in the pons.

DISCUSSION

The present study on a clinically relevant animal model confirms previous findings relating to piglets (9) and human newborns (16, 29) that room air and 100% O_2 are equally effective in normalizing cardiorespiratory, blood gas, and acid-base abnormalities during early recovery in newborns with asphyxia. The measured cardiovascular, blood gas, and acid-base values revealed the well-known characteristics (bradyarrhythmia, arterial hypotension followed by significant hypertension, then mild hypotension, hypoxemia, and combined acidosis) routinely seen in clinical practice during or after asphyxia in human newborns. Animals treated with 100% O_2 had significantly higher, although clinically still normoxic arterial Po_2 values during postasphyxial mechanical ventilation as compared with the data from the room air-treated, relatively hypoxemic group. Furthermore, it emerged that a brief period (10 min) of reoxygenation with 100% O_2 might have some influence on cerebral damage, as piglets treated with room air had a significantly better neurologic score, predicting a more favorable early neurologic outcome, as compared with animals receiving 100% O_2 ventilation. It seems that normoxia after cerebral hypoxia augments brain injury in the present model, whereas a lower arterial Po_2 is neuroprotective. Meanwhile, higher arterial Po_2 values during mechanical ventilation had no significant effects on the blood oxidative stress indicator values measured during the study and led to microscopic cerebral histopathologic alterations of similar severity in both asphyxiated groups at the end point of the experiments.

The experimental model of pneumothorax was chosen to evoke severe asphyxia because both laboratory (7, 18–20) and clinical (30, 31) data published previously confirmed a strong association between the neonatal air-leak syndrome and cerebral damage. Therefore, it is not surprising that we observed significant cerebral damage in both groups of asphyxiated animals. Moreover, pneumothorax is far more frequent in newborns than in any other period of life (23, 32, 33), and the brain of the newborn piglet is comparable both histologically (34) and electrophysiologically (35) with that of human infants at 36–38 wk of gestation.

The experimental protocol followed in this study was set up on the basis of our extensive laboratory practice during a period of 20 y, documenting evidence that pneumothorax-asphyxiated piglets are able to survive the asphyxiation load (for up to 4 h) without further mechanical respiratory support (7, 36). Newborns should be ventilated as long as necessary to obtain reasonable blood gas values, avoiding lung and other organ injury as much as possible. As the arterial gas values measured 10 min postasphyxially were very close to those reported to be clinically acceptable (37), the piglets were weaned from the respirator and reventilation was not introduced. The study substance oxygen was not further supplemented. As the blood glucose levels proved to be sufficiently maintained postasphyxially (38) and as most routinely used drugs, *e.g.* sodium bicarbonate, colloids, vasopressors, or atropine, could have potential deleterious effects on the CNS in newborns (39, 40), no parenteral medication was given, except normal saline. Similarly, as in another study published recently (41), no additional attempt was made to correct the systemic hypotension or the metabolic acidosis in postasphyxic piglets. In spite of the random allocation, mean arterial blood pressure measured at the start of mechanical ventilation in piglets reoxygenated with 100% O_2 was slightly lower than that in animals treated with room air; however, all of them had values higher than 35 mm Hg, which is the critical level for well-maintained cerebral perfusion in newborn piglets (42, 43). It is worth mentioning that there was no correlation between mean arterial blood pressure and neurologic score in any of the groups. This is not surprising, as cerebral blood flow was found to be independent of mean arterial blood pressure in preterm infants undergoing intensive care, when they were able to maintain adequate cerebral perfusion at a mean arterial blood pressure in the range 23.7–39.3 mm Hg (39). As a widely accepted drug (44–46) with more favorable cardiovascular

Table 3. *Blood oxidative stress indicators*

	◀ PTX→		← MV →		← RC →	
	BA	AS	MV5	MV10	RC1	RC2
R21						
Blood						
GSSG/GSH	0.29 ± 0.06	0.27 ± 0.15	0.36 ± 0.18	0.34 ± 0.12	0.23 ± 0.18	0.22 ± 0.09
Plasma						
Hb (μM)	21.2 ± 11.0	17.4 ± 6.3	19.9 ± 9.1	17.4 ± 8.8	20.1 ± 12.6	23.8 ± 17.0
MDA (μM)	0.99 ± 0.53	0.93 ± 0.56	0.96 ± 0.66	0.95 ± 0.50	0.84 ± 0.47	0.98 ± 0.63
R100						
Blood						
GSSG/GSH	0.30 ± 0.25	0.30 ± 0.12	0.25 ± 0.18	0.22 ± 0.28	0.20 ± 0.28	0.15 ± 0.18
Plasma						
Hb (μM)	23.4 ± 12.0	25.5 ± 10.1	22.3 ± 7.9	24.5 ± 9.7	19.6 ± 9.8	21.4 ± 21.8
MDA (μM)	1.24 ± 0.72	1.24 ± 0.63	1.08 ± 0.56	1.04 ± 0.60	0.92 ± 0.47	1.31 ± 0.75
SHAM	BA	60 min	65 min	70 min	120 min	240 min
Blood						
GSSG/GSH	0.18 ± 0.17	0.37 ± 0.17	n.m.	n.m.	0.29 ± 0.22	0.32 ± 0.31
Plasma						
Hb (μM)	29.0 ± 15.1	29.9 ± 14.2	n.m.	n.m.	20.7 ± 13.4	30.9 ± 19.1
MDA (μM)	1.55 ± 0.63	1.30 ± 0.80	n.m.	n.m.	1.24 ± 0.78	1.14 ± 0.75

Values are mean ± SD.

AS, asphyxia; BA, baseline; GSSG/GSH, oxidized/reduced glutathione; MDA, malondialdehyde; MV, mechanical ventilation; MV5, 5 min of mechanical ventilation; MV10, 10 min of mechanical ventilation; n.m., not measured; PTX, pneumothorax; RC, recovery; RC1, 120 min after BA; RC2, 240 min after BA.

effects than those of inhalation drugs in piglets (35), ketamine was used in our experiments as part of the anesthesia. Although it is known from the literature that ketamine could offer some neuroprotective effects as a noncompetitive antagonist of the *N*-methyl-D-aspartate receptor, all animals received the same anesthetic, which makes all the results comparable.

As the major finding of a better early neurologic outcome after room air reoxygenation is of important clinical relevance, this observation requires further explanation. Increasing Fio_2 levels facilitates posthypoxic cerebral cortical hyperoxia (47) and results not only in cerebral, but also in systemic, oxidative damage (48). Although the blood oxidative stress indicator levels in our study did not reveal any significant liberation of potentially highly damaging mediators, on the basis of previously published observations (12, 49) (as there were no other significant differences between the two asphyxiated groups apart from the arterial Po_2 values) we presume the deleterious effects of hyperoxia on the neuronal cell membranes were caused either by increasing cerebral dopamine concentration (50, 51) or by other lipid peroxidation products (52–55) not measured here. It also seems likely that the oxidative stress remained localized within the CNS, and the elevations in the biochemical markers become lost on dilution in the plasma (56). These substances might disturb the most complex neuronal membrane transport processes, which are regulated by the very sensitive enzyme Na^+/K^+ ATPase. The function of the enzyme is critical in maintaining intra- and extracellular ion concentrations in the brain. A significant decrease in its activity, leading to an influx of sodium and calcium into the cell, accompanied by water, resulting in cytotoxic cellular edema, was experienced in both the cortex (49) and the striatum (12) in piglets treated with 100% O_2. Moreover, a highly significant production of reactive oxygen species was recently demonstrated intravitally, in asphyxiated newborn piglets likewise resuscitated with 100% O_2 for 10 min (57), and concentrations of both conjugated dienes and fluorescent compounds were significantly elevated in the cerebral cortex of asphyxiated piglets (52, 58) with almost identical arterial Po_2 values (approximately 13.0 kPa) to those we measured in animals ventilated with 100% O_2. Although the arterial Po_2 values in our R100 group did not significantly exceed the values measured in the SHAM group, both systemic xanthine oxidoreductase conversion (54) and the accumulation of purine metabolites (5–7, 9, 13, 19, 54) must have taken place in the R100 animals during the asphyxic period with global hypoxia-ischemia, which was not present in the SHAM animals without asphyxia. It is also worth emphasizing that both the cardiac morphology (59) and the cerebral circulation (60) are almost identical in human newborns and piglets. The preductal (cerebral) arterial Po_2 might therefore have been significantly higher, which we were able to measure in blood taken from the umbilical artery, owing to a functional right-to-left shunt through the patent ductus arteriosus. The observed phenomenon that the Po_2 values measured in R100 animals were not different from those for the SHAM animals could have resulted from both intrapulmonary and intracardiac right-to-left shunts caused by severe pneumothorax-evoked pulmonary hypertension (61). Microscopic neuronal lesions might therefore have developed during the profound hypoxemia-ischemia, which were very similar in severity in both groups with asphyxia, whereas functional

Table 4. *Neurologic examination scores*

Neurologic examination score*	R21	R100	SHAM
Mean ± SD	13.5 ± 3.1(a,c)	9.5 ± 4.1 (b)	18.0 ± 0
Median	15.0 (a,c)	10.0 (b)	18.0
(25th, 75th percentiles)	(12.0, 15.75)	(5.75, 12.0)	(18.0, 18.0)

a, $p < 0.05$ *vs* R21 and SHAM; *b*, $p < 0.05$ *vs* R100 and SHAM; *c*, $p < 0.05$ *vs* R21 and R100.

* Score of 20 is considered normal, score of 5, brain-dead.

Table 5. *Piglet cerebral pathologic examination results**

Group	Frontal cortex	Temporal cortex	Cerebellum	Basal ganglia	Hippocampus	Pons
R21	3.8 ± 0.41(a)	3.9 ± 0.31(a)	3.8 ± 0.41(a)	3.8 ± 0.41(a)	4.0 ± 0.0(a)	5.0 ± 0.0
	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	5.0 (5.0, 5.0)
R100	3.6 ± 0.85(b)	3.6 ± 0.69(b)	3.6 ± 0.69(b)	3.7 ± 0.66(b)	3.9 ± 0.31(b)	5.0 ± 0.0
	4.0 (4.0, 4.0)	4.0 (3.25, 4.0)	4.0 (3.25, 4.0)	4.0 (4.0, 4.0)	4.0 (4.0, 4.0)	5.0 (5.0, 5.0)
SHAM	4.83 ± 0.31	4.83 ± 0.31	4.83 ± 0.31	5.0 ± 0.0	4.83 ± 0.31	5.0 ± 0.0
	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)

* Scores are expressed as mean ± SD and median (25th, 75th percentiles).
a, $p < 0.05$ *vs* R21 and SHAM; *b,* $p < 0.05$ *vs* R100 and SHAM.

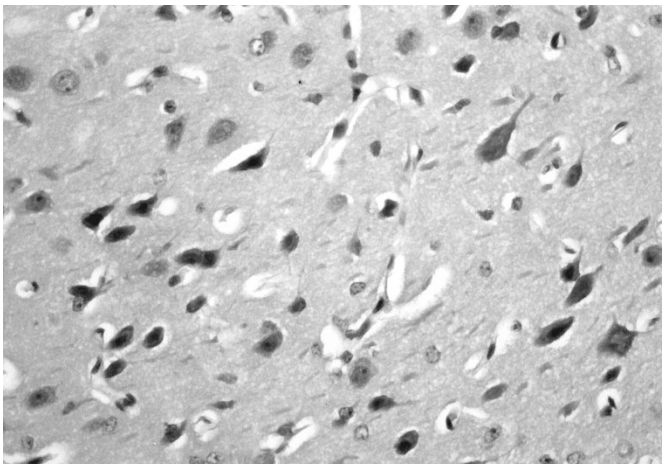

Figure 8. High power view of Grade 2 damage (ratio of damaged to spared neurons = 33–66%) in the temporal cortex of an asphyxiated animal reoxygenated with 100% O_2 (R100 group). The damaged neurons appear as shrunken, hyperchromatic and eosinophilic-staining cells in contrast to the lightly stained normal neurons.

neurologic disturbances might have originated from postasphyxial events, with less serious handicaps in R21 piglets. The lower degree of damage observed in the pons in the asphyxiated animals might have originated from the better-maintained blood flow and oxygenation of the brain stem structures as compared with those of other brain regions during asphyxia. Moreover, a cerebral intraparenchymal sodium accumulation was demonstrated earlier in our neonatal pneumothorax model both *in vitro* (18, 62) and *in vivo* (19, 63).

As an exceptional laboratory finding, pial-arachnoidal arterial micro-air embolization was previously demonstrated in our piglet model. Because this cerebral vascular damage was in each case accompanied by rapidly occurring visible morphologic alterations, *i.e.* diffuse cortical bleeding (64), it can be excluded with certainty from taking part in the present set of experiments, as the cerebral histopathology in numerous regions of the perfused and fixed piglet brains did not reveal any concomitant alterations anywhere within the CNS.

In conclusion, this study suggests that normalization of the neonatal cardiorespiratory status after pneumothorax-evoked asphyxia is just as efficient when reoxygenation is performed with room air as it is with the often-recommended 100% O_2. Although there were no significant differences as regards blood oxidative stress measuremtns and cerebral histopathologic damage between the two reoxygenation modalities (room air or 100% O_2), reoxygenation with 100% O_2 seems to impair the early neurologic outcome, whereas relative hypoxemia is neuroprotective in asphyxiated newborns. Further research is necessary to reveal the causative mechanisms. We suggest that during the treatment of newborn infants, especially after an asphyxic episode, inspiratory oxygen levels should be minimized by using the lowest necessary increase in Fio_2 above that in normal air.

Acknowledgments. The authors thank Ágota Fábián Nagy, Ilona Szécsi, and Ildikó Wellinger for their skillful technical assistance and for the analysis of blood samples, and Dr. Róbert Zánthó for revising the manuscript.

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