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Resuscitation of Asphyxiated Fetal Rats with Room Air or Oxygen: Changes of Cerebral Intra- and Extra-cellular Calcium

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Abstract

Objective: To compare the effects of resuscitation using room air or oxygen on hypoxic damage of fetal rat brains. **Methods:** Thirty-five fetal rats of 20-day gestational age were randomly divided into three groups: sham operation (control, n=11), room-air resuscitation (n=10), and oxygen (concentration 92.8%) resuscitation (n=14). Fetal rats in the latter two groups suffered from ischemia and hypoxia in-utero resulting from interruption of placental circulation. After recirculation, intra- and extra-cellular concentrations of calcium, sodium, and potassium in the brains were measured in each group. **Results:** Intracellular free calcium concentration of fetal rat brains was similar between the room-air resuscitation group (552.08 ± 93.50 nmol/L) and the oxygen resuscitation group (520.61 ± 79.08 nmol/L), and both were significantly higher than that in the control (315.27 ± 86.88 nmol/L) (P<0.001). There was no difference in the total concentrations of calcium, sodium, or potassium among the three groups. **Conclusion:** Resuscitation with room air or 92.8% oxygen had a similar effect on the parameters measured, suggesting that resuscitation of asphyxiated neonates using room air might not be inferior to that using high-concentration oxygen.

Key words Fetal hypoxia; Oxygen; Resuscitation; Room air

Introduction

Newborn asphyxia is a common and serious problem worldwide. Every year thousands of newborn infants require some form of resuscitation immediately after birth.

Over the past decades, neonatal resuscitation programs have been well developed, but some of the procedures employed in these programs are not based on strong scientific evidence. One example is the use of pure oxygen in resuscitation.[1-3] However this practice met no challenge until recent years. There is more and more evidence indicating that resuscitation using room air might not be inferior to that using pure oxygen.[4-6] Compared to oxygen, resuscitation with room air is much more convenient, especially in the poorly equipped hospitals in the countryside. In this preliminary report we compare the effects of resuscitation using room air with that using 92.8% oxygen by measuring intra- and extra-cellular levels of calcium, sodium, and potassium in fetal rat brains in an animal model of perinatal asphyxia.

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Materials and Methods

Establishment of Hypoxic-ischemic Fetal Rat Model[7]

Pregnant Sprague-Dawley rats at 20 days of gestation age were anesthetized by administration of 0.1 ml/200 g body weight Rompun/Vetalar mixture (1:1 by volume, Parke-Davis). An abdominal midline incision was performed and the uterine horns exposed. Blood vessels arising from the branching point and turning into individual placenta in one horn were clamped for 15 minutes. Circulation was then restored for 30 minutes by removal of the clamps. Blood vessels of fetal rats in the control were not clamped after being exposed. Distressed and sham-operated fetuses were finally taken out for examination.

Randomization

Pregnant rats were randomized into three groups. Totally 35 fetal rats of 20-day gestational age were

included in the study: sham operation (Control, n=11), room-air resuscitation (Air, n=10), and oxygen-resuscitated group (Oxygen, n=14).

The control fetal rats were decapitated immediately after exposure of placental vessels for determining intracellular free calcium concentrations and total concentrations of calcium, sodium, and potassium in the fetal brains.

Pregnant rats in the room-air resuscitation group were given room air all the time. After circulation was restored for 30 minutes, their fetuses were decapitated to determine intracellular free calcium concentrations and total concentrations of calcium, sodium, and potassium in the fetal brains.

Pregnant rats in the oxygen-resuscitated group were allowed to breathe in 92.8% oxygen for 30 minutes after restoration of placental circulation. Subsequently their fetal rats were decapitated for the determination of intracellular free calcium concentrations and total concentrations of calcium, sodium, and potassium in the fetal brains.

Determination of Calcium, Sodium, and Potassium Levels

Intracellular free calcium concentrations ($[Ca^{2+}]_i$) in the fetal rat brains were analyzed from fluorescence images (RF-5000, Japan),[8] using calcium fluorescent indicator Fura-2AM from Sigma (St. Louis, MO, U.S.A.). Total concentrations of calcium (TCa), sodium (TNa), and potassium (TK) in the brain tissues were determined from atomic absorption spectrophotometer (Beckman-700, U.S.A.).

Data Analysis

Values of cerebral $[Ca^{2+}]_i$, TCa, TNa, and TK are expressed as mean ± standard deviation ($\bar{x} \pm s$). Multiple group comparisons were performed by ANOVA with the SPSS statistical package (SPSS 8.0, U.S.A.). Correlation and linear regression were assessed using SPSS 8.0 either. All tests were two-tailed. Probability levels less than 0.05 were considered significant.

Results

Table 1 shows the effects of resuscitation with room air or 92.8% oxygen on fetal rat brains. Intracellular free calcium concentrations in fetal rat brains in the room air group were similar to those in the oxygen group ($p>0.05$), and both were significantly higher than those in the control ($p<0.001$). There was no significant difference among the three groups in total levels of calcium, sodium, and potassium ($p>0.05$).

Correlation and linear regression of all parameters measured were studied within each group. Cerebral intracellular free calcium concentrations were inversely related to the total sodium levels in the control ($r= -0.6178$, $p<0.05$). Total sodium levels of brain tissue were positively related to total potassium levels in the control group and the room air group ($r=0.6556$ and 0.7457 respectively, $p<0.05$). Total calcium levels of brain tissue were found to be positively related to total potassium levels in the room air group ($r=0.8563$, $p<0.05$), and positively related to total sodium levels in the oxygen group ($r=0.6438$, $p<0.05$).

Discussion

Asphyxia of newborn babies is an important cause of mortality and morbidity worldwide, with an incidence of 1% in the western developed countries[9] and 3.5% to 9.5% in China.[10] Each year thousands of infants worldwide require some form of resuscitation immediately after birth. It is, therefore, important to formulate guidelines for neonatal resuscitation based on scientific evidences.

A wealth of research has proved that pathologic factors, such as ischemia, hypoxia and trauma, can cause an increase in intracellular free calcium concentration or calcium overload.[8,11,12] In our study, we had similar finding: intracellular free calcium concentrations of fetal rat brains increased significantly after ischemia and hypoxia. Calcium overload subsequently induces a series of changes in cellular function and structure, leading to suppression of mitochondrial function, degeneration of membrane phospholipid and decomposition of protein. Cell death ensues soon after. Altered calcium homeostasis is regarded as the "final common pathway" for hypoxia-ischemia and other forms of acute brain damages.[11,12] Therefore the maintenance of a stable calcium homeostasis is crucial in

Table 1 Effects of resuscitation with room air or oxygen on fetal rat brains

Treatment	n	Cerebral $[Ca^{2+}]_i$ (nmol/L)	TCa (mmol/kg brain)	TNa (mmol/kg brain)	TK (mmol/kg brain)
Control	11	315.27 ± 86.88	38.42 ± 17.16	559.12 ± 96.53	698.28 ± 118.24
Air	10	552.08 ± 93.50 △	30.62 ± 13.75	640.66 ± 155.02	742.92 ± 197.16
Oxygen	14	520.61 ± 79.08 △	40.73 ± 23.06	582.43 ± 186.77	755.38 ± 107.39(13)
F Value between groups		24.853 ▲	0.863	0.773	0.512

Values are expressed as mean ± SD. Figure in parenthesis indicates the number of fetal rats measured. When compared with control, △ $P<0.001$; When compared between groups, ▲ $P<0.001$. The rest are not significant when compared with each other.

Abbreviations: $[Ca^{2+}]_i$, intracellular free calcium concentration; TCa, total calcium concentration by mmol/kg dry brain; TNa, total sodium concentration by mmol/kg dry brain; TK, total potassium concentration by mmol/kg dry brain.

preventing or ameliorating brain damage of hypoxic-ischemic fetuses. Clinically the purpose of resuscitation is to interrupt the pathological process and to restore functions of the damaged cells. Up to now, there is little information available on whether resuscitation using room air is equal to, or even better than that using 100% oxygen. The selection of resuscitation program is still influenced by our past experience or intuition.

Over the past half-century resuscitation with pure oxygen has become a mainstream routine practice.[1-3] It seems obvious that cellular functions will be restored more quickly if extra oxygen is given to a patient who has suffered from oxygen deficiency for some time.

However, as in other areas of medicine, intuition is not a good way for introducing new therapeutic routines into neonatology however attractive they may seem. With the discovery of oxygen-free radicals by the end of the 1960's, the role of oxygen-free radicals in the pathogenesis of diseases has become increasingly recognized. The establishment of the theory of oxygen paradox or hypoxia-reoxygenation injury by the end of the 1980's has further expanded our knowledge of oxygen-free radicals. Oxygen-free radicals are generated during and after hypoxia-ischemia in several ways[12] and their production is positively related to the oxygen level in tissue with hypoxia-ischemia.[13-15] The higher the oxygen concentration in the tissues, the greater the amount of oxygen-free radicals produced and the more tissues destroyed. People are prompted to evaluate oxygen concentration at resuscitation once more with increasing interest.

In 1992, Rootwelt et al[4] compared the efficiency of room-air resuscitation with that of pure oxygen resuscitation for the first time, using 2- to 5-day old piglets for hypoxia-ischemia model. They demonstrated that the mean arterial blood pressure, heart rate, base deficit, pH, and plasma hypoxanthine normalized as quickly in the room air as in the 100% oxygen group. Morphologic examination of the brain four days after the hypoxic insult did not reveal any differences between the two groups. Subsequent studies[16-20] had similar findings: Cardiac output, cerebral blood flow, cerebral blood volume, cerebral vascular resistance, brain oxygenation, blood pressure, blood gas, and regional blood flow to organs such as the myocardium, spleen, kidney, muscles, skin, and intestine did not differ between the two groups. In contrast, some other studies have shown that when compared to animals resuscitated with 21% oxygen, those resuscitated with 100% oxygen had slower normalization of cerebral hypoxanthine,[21] slower restoration of Na^+, K^+-ATPase activity in the striatum,[22] higher levels of oxygen free radical[23] and nitric oxide[20,24] production in the cerebral cortex, and more severe myelin damage in several brain regions.[25] Although a high arterial oxygen tension is obtained more quickly when resuscitation is performed with 100% oxygen, resuscitating with room air lowered the pulmonary vascular resistance with an identical velocity and pattern as in the 100% oxygen group.[26,27]

In our study, we investigated the efficiency of resuscitation with room air or 92.8% oxygen, using fetal rats at 20 days of gestational age. Similar intra- and extra-cellular concentrations of calcium, sodium, and potassium in fetal rat brains were found in the room-air group and the oxygen group. The results indicate that room air is as efficient as 92.8% oxygen for fetal rat resuscitation.

Clinical resuscitation trials have provided evidence supporting our findings. Ramji et al.[5] resuscitated 42 newborn infants with room air and 42 infants with 100% oxygen. There were no differences between the two groups in heart rates, apgar scores at one minute, blood gases (except $PaCO_2$ at 30 minutes), and the outcomes concerning survival and neurologic status after 28 days. Neonates in the room air group had significantly higher 5-minute Apgar scores and lower arterial $PaCO_2$ at 30 minutes. An international multicenter study[6] have showed that there were no differences between the room-air and the 100%-oxygen group regarding pH, base deficit, or heart rate during the observation period of 30 minutes. The interval between birth and the first cry or first breath was significantly shorter in the room-air group. Apgar scores at one minute were also higher in the room-air group compared to the 100%-oxygen group. The incidence of hypoxic-ischemic encephalopathy stage 2 or 3 as well as mortality was identical in the two groups within seven days. Overall neonatal mortality showed a tendency to be significantly lower in the room-air group. In a study on term neonates with moderate asphyxia, Vento et al.[28] found that babies resuscitated with oxygen had a more severe oxidative stress and a significantly higher damage profile parameters (such as oxidative derivatives of DNA in urine) compared with those resuscitated with room air.

To sum up, we conclude that 100% oxygen is not superior to room air for newborn resuscitation. On the contrary, room air might be better than 100% oxygen.

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