Scanning Electron Microscopy of cochlea in new-born rats exposed to hyperbaric oxygen: preliminary report

Microscopia elettronica a scansione su coclee di ratti neonati esposti a ossigenoterapia iperbarica: dati preliminari

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Summary

The aetiology of hearing loss in new-borns in neonatal intensive care is still debated. While the physiopathology of brain, lung and retina damage related to oxygen supplementation has been widely described, no studies have been carried out to define the relationship between hearing loss and supplementation of oxygen in new-borns. In the present investigation, the cochlear morphology of new-born rats was evaluated by means of scanning electron microscopy in order to assess morphological changes after supplemental oxygen administration. After treatment, electron microscopy revealed many changes in the morphology of outer hair cells, if compared to normal rats of the same age. The results suggest that cochlear changes are similar to those previously observed in other regions and may be related to a vascular mechanism of hypoxia-ischaemia and neovascularization and/or an oxidative stress.

Riassunto

L'eziologia delle ipoacusie neonatali successive a ricovero presso reparti di terapia intensiva neonatale è attualmente oggetto di controversie. Mentre la fisiopatologia dei danni cerebrali, polmonari e retinici è stata ampiamente descritta in letteratura, non esistono studi sperimentali che definiscano la relazione fra sordità ed esposizione ad alte supplementazioni di ossigeno nei neonati. In questo studio è stata effettuata, con microscopia elettronica, una valutazione delle caratteristiche morfologiche cocleari in ratti neonati sottoposti a ossigenoterapia in iperbarismo. La microscopia elettronica ha evidenziato notevoli alterazioni a livello delle cellule ciliate esterne degli animali trattati con ossigeno paragonati ad animali di controllo della stessa età. I risultati da noi ottenuti suggeriscono che le modificazioni cocleari evidenziate sono paragonabili alle ben note alterazioni osservate in altri distretti e correlate a diversi meccanismi: l'uno vascolare legato all'ipossia-ischemia e neovascolarizzazione, l'altro legato allo stress ossidativo.

Introduction

Sensorineural hearing impairment and deafness occur in 2-4% of newborns in the neonatal intensive care unit (ICU). Even if the incidence of hearing loss in newborns in the neonatal ICU is greater than in normal newborns the aetiology of the deafness is still debated [1].

Postulated causes include: hypoxic-ischaemic injury to the brainstem, haemorrhage within the inner ear, bilirubin or aminoglycoside antibiotic toxicity, cytomegalovirus infection, acoustic trauma or a combination of these factors [2].

A recent histopathological study on temporal bones from 15 premature infants in the neonatal ICU showed bilateral selective outer hair cell loss in 2 patients, bilateral selective inner hair cell loss in 3 cases and a combination of both outer and inner hair cell loss in 2 patients [3].

As far as concerns hyperbaric oxygenation Kovshenkova demonstrated that when this treatment is performed, combined with other metabolic and antioxidant treatment, a decrease in the auditory threshold occurs in 10% of newborns [4].

Many studies have been carried out in order to clarify the role of supplementation of oxygen in hyperbarism on the pathogenesis of Retinopathy of prematurity (ROP). Moreover, experimental animal models have been validated to study this pathological condition and it is now widely accepted that ROP is iatrogenic [5].

On the other hand, to our knowledge, no studies have been carried out, so far, to define the relationship between hearing loss and supplementation of oxygen in newborns.

In this study, we used a well-known experimental animal model of hyperbaric oxygen treatment in order to evaluate morphological changes in the organ of

Corti by means of scanning electron microscopy (SEM) after hyperbarism in new-born rats.
The aim of the investigation is to establish the mechanism of hearing loss in newborns in neonatal intensive care.

Material and methods

The care and use of animals followed the animal welfare guidelines of the Catholic University of the Sacred Heart. Three groups of new-born Wistar rats, of either sex, were divided into 3 groups as follows:
Group A (n = 5): normal controls, sacrificed on the 60th day of life.
Group B (n = 5): controls, rats exposed, with the mother, to a room-air environment, under normobaric conditions, for the first 10 days of life and then sacrificed.
Group C (n = 5): immediately after birth the newborn rats and their mothers were kept inside a pressure chamber and treated for the first 5 days of life. The animals received a continuous flow of oxygen at 80%, at a compression pressure of + 81 kPa. After treatment animals were exposed to a room-air environment under normobaric conditions for 5 days before sacrifice.
The animals were deeply anaesthetised, via Metedomidine and Ketamine i.m. (0.1 ml and 0.2 ml, respectively). The chest and pericardium were opened, an incision was made in the right atrium and an in vivo perfusion was delivered by Karnovsky intracardiac injection. Following decapitation, both temporal bones were removed and perfused with 3% glutaraldehyde in 0.1 M phosphate buffer, immersed in the same fixative for 24 hours at 4°C and rinsed in 0.1 M phosphate buffered saline (PBS). The temporal bones were decalcified in a 10% ethylenediaminetetra-acetic acid (EDTA) solution (pH 7.0) for > 10 days at 4°C.
The specimens were prepared by removing the bone cochlear capsules, *stria vascularis* and Reissner membrane to expose the organ of Corti, and then immersed in OsO4 solution NaCacodylate 0.1 M pH 7.4, at 4°C, for 2 hours. After dehydrating by a series of ethanol solutions (70 to 100%), specimens were dried in a critical-point dryer. The samples were mounted on stubs and coated with gold. SEM was performed using a Philips 505 (Eindhoven, NL) SEM apparatus.

Results

Significant differences were observed in cochlear morphology between the 3 groups.
SEM of the cochlea showed:
Group A and group B: these animals, showed normal morphology of the organ of Corti, no differences having been observed between these groups (data not shown).
Group C: in this group of rats, the cochlea showed a low ossification score, compared with the control groups (A and B). Moreover, the most important differences have been demonstrated between rats in groups A-B and C in the morphology of outer hair cells. In fact, after hyperbarism, the basal turn of the cochlea shows an increase in height of the outer hair cells. These cells appear to be more flexible and weak, but the inner hair cells are normal and there are no linkages between the hair cells and stereocilia (Figs. 1, 2). In the middle and apical cochlear turns, a morphological modification is evident, in the outer hair cells: a linkage of the hair cells in the apical pole and stereocilia has been demonstrated (Figs. 3, 4). No modifications are present in the inner hair cells (data not shown).

Fig. 1. Outer hair cell of the cochlear basal turn (group C). These cells appear more flexible and weak.

Fig. 2. Magnification of Figure 1 shows apical pole of outer hair cell.

Fig. 3. Outer hair cells of middle cochlear turn (group C) show morphological modification of outer hair cells: links between different hair cells.

Fig. 4. High magnification of outer hair cells of middle cochlear turn; links between the stereocilia, in outer hair cells, are clearly visible (group C).

Discussion

Supplying oxygen to animals is known to produce tissue damage, toxicity increasing with the increase in oxygen concentrations and exposure pressures [6].
Prolonged exposure to hyperbaric oxygen causes central nervous system damage, as demonstrated by Yoles et al. in dogs [7].
Pulmonary toxicity is also related to the high concentrations of oxygen administered with increased airway pressure to most pre-term neonates presenting respiratory distress syndrome. The pathogenesis of this lung disorder may include tissue damage caused by the superoxide anion (O2-) and other free oxygen radicals [8].
Retinal changes following high levels of oxygen have been widely demonstrated. Damage is preceded by changes in the vessels of the retina [5]. In fact, vessels are irreversibly closed during hyperoxia and, when the new-born resumes normal breathing, a proliferative revascularisation occurs. This new vascularisation may return to normal or may be followed by a blinding fibrous invasion [9]. ROP has a multifactorial aetiology: prematurity itself and oxygen supplementation play predominant roles [5].
In the present study, we have evaluated whether simple oxygen therapy could determine morphological changes in the inner ear, as in the retina.
Our results showed cochlear damage following supplementation of oxygen in hyperbarism. Indeed, a clear difference is observed between the cochlear morphology of rats exposed to hyperbarism and those not exposed. The main modifications consist in an increase in height of the outer hair cells, the presence of linkages in outer hair cells and their stereocilia. No differences have been demonstrated between normal controls, sacrificed at the 60th day of life, and controls rats exposed to a room-air environment under normobaric conditions and sacrificed on the 10th day of life. In fact, even if the rat cochlea is immature at birth, the onset of hearing starts 9-10 days after birth [10].
The pathophysiology of cochlear damage is not easy to study since analysis of the cochlear microcirculatory system is more difficult than that of the retina, due to the complexity and occult location in the temporal bone of the inner ear.
On the basis of data reported in the literature, two different mechanisms can be hypothesised to occur in the cochlea of newborns, after oxygen exposure. First, a vascular mechanism: the vasoconstriction during hyperoxia could be followed by a renewed vascularisation and a blinding fibrous invasion [9]. Second, an oxidative stress mechanism: newborns are particularly vulnerable to oxygen toxicity as they have poor antioxidant protection against the free oxygen radicals [11].
As far as concerns the relationship between morphological and functional changes in the cochlea, Amatuzzi et al. showed, by means of histopathological evaluation, outer and hair cell loss in premature infants from neonatal ICUs; these modifications being related to a sensorineural hearing loss, evaluated by Auditory Brainstem Responses [3].
In accordance with these Authors [3], we may conclude that, in newborn, not premature, normal rats, hyperbaric oxygen treatment could determine important morphological and functional modifications in the cochlea, even if much work is still needed in order to elucidate the mechanism of action of hyperbaric oxygen.
As a future evolution, measurement of compound action potential in rats might be hypothesized in the attempt to establish a relationship between the present

preliminary morphological results and functional electrophysiological data.

Conclusions

Hyperbaric oxygen therapy could determine cochlear modifications in newborn rats. Outer hair cell damage would justify the presence of hearing loss in newborns in neonatal intensive care.
The morphological data obtained will be useful in further functional studies in order to better define the physiopathology of hearing loss in newborns in neonatal intensive care exposed to hyperbaric oxygen.

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