The following three projects were developed and studied by Dr. Bettina Gerstner (Department of Neonatology, Charité-Virchow Clinic) in the framework of the NBL3 project.

**Project 1**

*Glutaraciduria type I: Pathomechanism of white matter degeneration in glutaryl-CoA dehydrogenase deficiency.*

**Background:** Glutaryl-CoA dehydrogenase deficiency (GDD) is an inherited metabolic disease that was first described by Goodman et al. in 1975. The prevalence is 1:100,000 worldwide (Lindner et al. 2004). It is characterized by elevated concentrations of glutaric acid (GA) and its metabolites glutaconic acid (GC) and 3-hydroxy-glutaric acid (3-OH-GA). Its hallmarks are striatal and cortical degeneration, which have been linked to excitotoxic neuronal cell death. However, magnetic resonance imaging studies have also revealed widespread white matter disease. Correspondingly, we decided to investigate the effects of GA, GC, and 3-OH-GA on the rat immature oligodendroglia cell line, OLN-93. For comparison, we also exposed the neuroblastoma line SH-SY5Y and the microglia line BV-2 to GA, GC, and 3-OH-GA.

OLN-93 cells are incubated with GA, GC and 3-OH-GA at different dosages (0.1, 1, and 10 mM). Cell viability of immature oligodendrocytes (cell line, OLN-93) is measured by metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium. Flow cytometry is used to assess apoptosis via externalization of phosphatidylserine, activation of caspase-3, and DNA fragmentation. NMDA and AMPA receptor mRNA expression of immature OLN-93 is examined by RT-PCR. Apoptosis is blocked by incubation with the pan-caspase inhibitor z-VAD-fmk (40 µM).

**Results:** Apoptosis but not necrosis is detected at various stages (early: annexin-V, effector: caspase-3) after 24-48 h incubation with GA, GC, or 3-OH-GA in OLN-93 but not in neuroblastoma or microglia cells. GA, GC, and 3-OH-GA induce OLN-93 cell death in a dose-dependent fashion. OLN-93 lack expression of NMDA receptors, making classical glutamatergic excitotoxicity an unlikely explanation for the selective toxicity of GA, GC, and 3-OH-GA for OLN-93 cells.

**Conclusion:** GA, GC, and 3-OH-GA directly initiate the apoptotic cascade in oligodendroglia cells. This mechanism may contribute to the white matter damage observed in glutaryl-CoA dehydrogenase deficiency.

**Project 2:**

*Influence of hyperoxia on the developing white matter in the neonatal brain.*

**Project 2:**

Advances in neonatal intensive care have markedly improved survival rates of premature infants. Unfortunately, a substantial proportion of very low birth weight infant survivors have neurologic deficits affecting motor and cognitive function (Hack et al. 2002; Ment et al. 2003; Vohr et al. 2000; Wood et al. 2000). Long term neurocognitive impairment constitutes a major personal burden for affected individuals and their families and poses a considerable socioeconomic problem. In the immature human brain, periventricular leukomalacia (PVL) is the predominant white matter injury underlying the development of cerebral palsy (Volpe 2001). PVL has its peak incidence during a well-defined period in human brain development (23-32 weeks postconceptional age) characterized by extensive oligodendrocyte migration and maturation. Their progenitor cells are present in the subventricular zone and migrate into the intermediate zone and cortical plate where they differentiate into immature and then mature oligodendrocytes, which myelinate axons (Levison and Goldman 1993). It is known that hyperoxia may induce damage in immature lungs and retina, and has been implicated in the pathogenesis of bronchopulmonary dysplasia and retinopathy of prematurity (Saugstad 2001). The influence of oxygen on the human brain is still unknown. We hypothesize that the dramatic rise of oxygen tissue tension associated with mammalian birth and additional oxygen exposure of the preterm
infant during intensive care may be harmful to immature oligodendrocytes (OLs). This study aims to investigate the effects of hyperoxia on oligodendroglial cells in vitro in the immature rat brain in vivo.

Our group has recently shown that oxygen causes widespread neuronal death in the developing rat brain (Felderhoff-Mueser et al. 2004). Hyperoxia increased the density of degenerating cells in various brain regions of seven-day-old Wistar rats in comparison to unexposed littermates (Felderhoff-Mueser et al. 2004). This cell death is associated with oxidative stress, decreased expression of neurotrophins, decreased activation of neurotrophin-regulated pathways, and increased levels of pro-inflammatory cytokines (Felderhoff-Mueser et al. 2004 and 2005).

In this project we want to investigate whether oxygen is toxic to developing oligodendrocytes in vitro by using primary oligodendrocytes and the OLN-93 cells (immature OL cell line). The oligodenndrocytes are induced to develop into different stages of maturation by the use of specific growth factors, such as PDGF-AA, FGF, CNTF, and T3. Furthermore the effect of hyperoxia will also be examined in vivo by using P0-P14 day-old Wistar rat pups. 80% oxygen is chosen for the experiments to be consistent with the conditions of the former animal experiments focussing on neuronal. Furthermore we want to mimic the rapid 3-4 fold increase in oxygen tissue tension experienced after mammalian birth. Immature OLs (OLN-93), their progenitors (pre-oligodendrocytes, pre-OL) and mature OLs are subjected to 80% hyperoxia (0-96 hrs). Cell viability of primary OLs and OLN-93 cells is measured by metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT), Alamar blue, LDH-release, or cell count. Stage specific maturation is shown by immunohistochemistry of the surface marker O4, O1, and MBP. To elucidate the downstream pathway that is activated by hyperoxia, flow cytometry is used in the OLN-93 cell line to assess apoptotic signals (externalization of phosphatidylserine, cleaved caspase-3, fragmented DNA). Expression of FAS and FAS-ligand, phosphorylation of cell survival promoting proteins (ERK, AKT, p38, CREB) is examined in Western blot analysis. In addition, P1 to P14 rat pups are subjected to 80% oxygen for 24 hrs, sacrificed and their brains processed for immunofluorescence staining (cleaved caspase-3, deOlmos silver staining, TUNEL, HE, MBP, OLIG2, GFAP, CD68) to detect (apoptotic) cell death, microglia and astroglia activation, and possible differences in white matter maturation.

**Project 3:**

*Estrogen and Estrogen-Receptor: Influence on cell death and maturation processes in the developing white matter in a model of hyperoxia-induced brain injury.*

**Project 3:**

Substantial neurologic morbidity occurs in infants born prematurely with higher mortality and morbidity rates observed in male survivors. Oxygen, which is widely used in neonatal medicine, constitutes one possible contributing neurotoxic factor, as it can trigger neuronal apoptosis in the developing brain of newborns or mice. Since in preterm infants periventricular leukomalacia (PVL) is the predominant white matter injury underlying the development of motor and cognitive deficits, the effect of hyperoxia on oligodendroglial cells is of major scientific interest.

Estrogen (E2) and estrogen-receptors (ER) play an important role in brain development and function. Both, pro- and antiapoptotic effects of E2 are described in the immature brain. However, the effect on maturational processes and myelination is unclear. This project aims at studying the effects and mechanisms of E2 and ER on developing oligodendrocytes in a recently established experimental model of hyperoxia-induced infant brain damage. The long-term goal is to establish potential interventions, that are safe and easy to apply in premature infants, to protect neuronal development and function.

The goal of our study is

1. To demonstrate that hyperoxia leads to loss of oligodendrocytes in vitro and in vivo possibly associated with delayed or incomplete myelination.
2. To analyze the effect of E2 in a model of oxygen-induced injury to the immature white matter.
3. To investigate whether vulnerability of oligodendrocytes to injury and response to estrogen is maturity dependent, subject to expression of ER-α or ER-β.
4. To analyze the interaction of estrogen with members of the apoptotic cascade (FAS/FASL, caspase–3 and -8, MAP-kinase, Bcl-2) and identify possible interaction/protection pathways triggered by estrogen and specific ER-α /-β activation.

5. To investigate the influence of E2 on OL-development by selective ER-activation. Secondly, to demonstrate that hyperoxia leads to delayed and incomplete myelination and secondary neuronal cell loss that can be prevented by E2 or selective ER-agonists.

6. To analyze myelination patterns and cell survival after hyperoxia +/- estrogen treatment using ER-α and -β gene-disrupted (ERKO) mice.

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Publikations/Abstracts


Gerstner B, Gratopp A, Marcinkowski, Obladen M, Bührer C. Oligodendrocyte apoptosis caused by glutaric acid and its metabolites. 44. Annual Meeting of the European Society of Pediatric Research, 26.-30.9.03, Bilbao, Spanien.


