Neuropathology of cerebral palsy

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Hypoxia/ischemia related brain damage is a major factor for morbidity and mortality not only in the adult, but also in the pre- and perinatal period where it mainly presents as cerebral palsy *(CP)*. The insult on a developing system has its own particular risk factors, pathophysiology and morphology.

The morphological description of the different lesions encountered in cerebral palsy like periventricular leukomalacia, ulegyria, marbled state etc. has been supplemented in recent years by pathophysiological and immunohistochemical data and a more detailed picture has been drawn concerning the alterations of different cell types and the time course of events.

In the following, the pathophysiology and morphological alterations related to cerebral palsy are summarised.

Pathophysiology of CP

Developmental aspects

The *vascularisation* of the brain starts around Day 28 of gestation at the time of the closure of the neural tube. The primordial vessels present as an indistinct meshwork, also called the head plexus. One to 2 days later the internal carotid arteries can be recognised which join at their caudal devisions to form the posterior communicating artery at Day 29. By Day 32 the basilar and vertebral arteries are formed and at Day 35 the anterior cerebral arteries become distinguishable at the anterior devisions of the internal carotid arteries and the medial cerebral arteries evolve as lateral branches of the proximal anterior cerebral arteries. As dorsal branches of the mesencephalic arteries extend they take over the territories of the posterior cerebral arteries which are supplied by the internal carotid arteries at earlier stages of development. Between Day 44 and 52 the mature pattern of vascularisation with the circle of Willis and the cerebral arteries is completed [Raybaud 2010].

The intrinsic vasculature of the brain develops around Week 5 of gestation with large penetrating arteries running as branches of the middle cerebral arteries from the base of the brain to the basal ganglia and diencephalon as well as to the germinal matrix of the subependymal periventricular zones. The vascularisation of the basal ganglia and the diencephalon is completed by 24 to 28 weeks of gestation. From the surface

of the convexity thin vessels, the long penetrating arteries descend to the periventricular white matter at 24 weeks of gestation followed between 24 and 28 weeks by short penetrating arteries that end in the subcortical white matter. The development of the long and short penetrating vessels accompanies the rapid cortical organization, axonal outgrowth and formation of synapses that takes place in this period [du Plessis 2009]. The short cortical arteries are not fully developed until post-term period resulting in relatively low blood supply of the white matter where the short penetrating arteries end [Volpe 2001]. This area is typically affected in the diffuse type of periventricular leukomalacia (PVL) whereas the regions corresponding to the endpoints of the long penetrating arteries match with the focal type of PVL (see below).

In addition to the border zones of short and long penetrators the "watershed regions" between the basal cerebral arteries play an important role in hypotensive brain injury occurring around term [Nikas et al. 2008]. On the surface of the brain these border zones form a parasagittal line whereas in the parenchyma the borders of the anterior and middle cerebral arteries run anterior to the frontal horns of the lateral ventricles and the borders between middle and posterior arteries are located in the white matter around the occipital horns [Rezaie and Dean 2002]. Parasagittal cortical border zone infarctions in term infants may appear as so called ulegyria [Nikas et al. 2008; see below]. Injury of the cerebral cortex and in the white matter has a subsequent impact on the development and maturation of the tissue.

In regard to the maturation of the vascular walls, an arterial muscular layer appears at 20 weeks in striatal vessels, at 24 weeks in the putamen and at 26 weeks in the caudate nucleus. A clear arteriovenous differentiation of extrastriatal parenchymal vessels is apparent only in the last weeks of gestation [Raybaud 2010]. Hence, the penetrators ending in the germinal matrix only consist of a single layer of endothelium. Direct damage to the endothelium due to hypoxia possibly aggravated by hypercarbia-induced increase in blood flow has been proposed as pathogenesis for periventricular and intraventricular haemorrhage [Folkerth 2005].

The *vulnerability* of cells to hypoxia/ischemia and other stressors differs depending on cell type and stage of development. The deep white matter comprises growing axonal pathways, neurons of the adjacent subplate zone and oligodendroglial progenitors.

The transient subplate zone is located between the intermediate zone and the cortical plate and is involved in the guidance of cortical afferents from the thalamus and corticocortical callosal fibres as well as the development of the cortical neuronal circuitry. It consists of a loose meshwork of axons and well-differentiated neurons which show a spontaneous activity and are connected to the cortical plate. The rich extracellular matrix of the subplate zone harbours a variety of axonal guidance molecules making it both, a substrate and a gradient zone for the navigation of thalamocortical axons [Kostović and Judaš 2006].

Injury to the fetal white matter may affect any of its cellular elements. Damage to oligodendroglial precursors between Week 24 and 34 of gestation results in periventricular leukomalacia. In vitro studies demonstrated that the precursors are vulnerable to free radicals, oxygen/glucose deprivation and cytokines [Babcock et al. 2009]. In vitro experiments demonstrated margination of chromatin, nuclear condensation and DNA fragmentation in injured oligodendroglial precursors consistent with apoptosis as the mode of death [Volpe 2001]. Focal hypoxic-ischemic lesions in periventricular regions supplied by long penetrating arteries affect crossroads of projection, associative, and commissural fibres whereas injury of the "watershed" regions between short and long penetrators may also strike the subplate zone and disturb the formation of cortical connections.

Pontosubicular neuronal necrosis is observed between Week 30 of gestation until 2 months post partum occurring consecutively to hypoxia/ischemia or hypoglycaemia. Presumably it relates to an increased perinatal vulnerability of pontine and subicular neurons. However, the pyramidal cells of the subiculum as well as in CA1 and CA2 also belong to the most vulnerable neurons concerning hypoxia/ischemia in the adult. The affected neurons in pontosubicular necrosis show the typical picture of apoptosis including apoptotic bodies and DNA fragmentation. In addition, the Fas/Fas ligand system and also caspase 3 was revealed to contribute to form pontosubicular necrosis [Burke and Gobe 2005; Takizawa et al. 2006].

In addition to lack of oxygen and nutrients developing cells may show a vulnerability to a variety of potentially toxic substances. Oligodendroglial precursors express lower levels of antioxidant enzyme manganese-containing superoxide dismutase which catalyses the dismutation of superoxide to hydrogen peroxide and oxygen. The enzyme only reaches its adult expression levels at 40 weeks of gestation or later. Further, oligodendroglial precursors are more vulnerable to excitotoxic injury by kainate than mature oligodendroglia because they express α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-kainate receptors. Lastly, cytokines like interferon-γ which are released in the context of intrauterine infections may affect oligodendroglial precursors (for review see [Folkerth 2005]).

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Animal models

Animal models play an important role in *CP* research since they allow studying the pathophysiology of this heterogeneous disorder systematically in living organisms. However, the relevance of the results may – just as in vitro experiments – be of limited value to human pathology. Mammals with a gyrencephalic brain and a gestation time of several months like rabbits, sheep and primates resemble the human situation better than small rodents such as mice and rats. In the following paragraph some recent data obtained from animal experiments are presented.

Gunn and Bennet [2009] reviewed data from hypoxia in fetal sheep point-

ing out that the brain can fully adapt to a moderate reduced oxygen supply down to 10 – 12 mmHg as long as substrate delivery is assured and no hypotension occurs. In this situation vasoconstriction redirects blood flow to the heart and brain, oxygen extraction from the blood increases and the brain switches to lower EEG frequencies. Energy consumption of the cells may be reduced by reduction of non-obligatory energy consumption via inhibitory neuro-modulators like adenosine. To some extent anaerobic glycolysis can become a source of energy.

Asphyxia without hypotension is associated with only modest brain damage. Neuronal loss in fetal sheep was only seen if in addition to prolonged severe partial asphyxia (partial occlusion of the uterine artery) episodes of acute hypotension occurred. Therefore, acidosis resulting from asphyxia is by itself a clinical measure with only limited predictive value.

The predominant mechanism of brain injury is severe hypotension which after a short phase of compensative vasoconstriction leads to progressive deterioration of brain function. In the course of events the blood flow is diverted from the cortex to the basal ganglia and brain stem. The initial vasoconstriction ceases and finally a profound systemic hypotension develops.

In transient (peripartal) hypotension (successful resuscitation) a short period of clinical recovery may be seen after normalisation of blood and oxygen supply, both in animal models and in newborn infants. Nevertheless, if the brain damage initiated a cascade of biochemical reactions leading to delayed cell death a second phase of deterioration will follow 6 – 15 hours after birth.

Due to redirection of blood flow to the brain stem and the basal ganglia in hypotension, these areas are often spared from injury. A single ischemic insult of 30 minutes mainly resulted in neuronal loss in the parasagittal cortex (watershed regions) in near-term fetal sheep. However, after three 10-minute episodes of hypotension at 1-hour intervals the striatal damage prevailed the cortical injury. Since the injury affected primarily inhibitory striatal neurons Gunn and Bennet [2009] speculated that the damage might in part result from abnormal excitatory inputs to these neurons.

Lastly the authors underlined the neuro-protective effect of hypothermia which is disproportionate to the changes in metabolism associated with the temperature change. This correlation also applies to an increase in body temperature. Hyperthermia of 1 – 2 °C markedly worsens brain damage.

Inder et al. [2005] presented an animal model of periventricular leukomalacia in the baboon which differed from other models in that there was no direct insult other than the standard neonatal intensive care situation. A white matter injury occurred in 50% of the animals and was mostly located in the parietal and occipital lobes. In addition, haemorrhages were seen in the subarachnoidal space (38%), in the ventricles (5%), germinal matrix (9%), white matter (28%) and cerebellum (9%). Although the handling of the animals closely reflects the clinical situation the model is costly because it requires a special facility for nonhuman primates.

A model for peri- and intraventricular haemorrhage was presented by Chua et al. [2009] using rabbit pups. The risk of spontaneous germinal matrix haemorrhage in premature rabbits could be increased form 10% to 80% by intraperitoneal administration of glycerol. This procedure resulted in dehydration and high osmolarity of the serum and arterial hypotension leading to haemorrhage. Posthaemorrhagically 70% of the pubs survived for 14 days or longer developing ventriculomegaly, motor dysfunction with increased muscle tone or complete paralysis. Upon autopsy gliosis und reduced myelination of the white matter was noted. Hence, the model mimics many of the clinical and histopathological alterations found in germinal matrix haemorrhage in pre-term infants. However, the authors also pointed out some limitations of the model. Since the pups were delivered by caesarean section and hand-fed the model is somewhat laborious. Furthermore, glycerol may potentially open the blood-brain barrier which would lead to metabolic changes. Like Inder et al. [2005] the work of Chua [2009] mainly focused on the reproduction of certain clinical and morphological aspects of perinatal brain injury, new data on the pathophysiology and biochemical signal cascades of the disorder was not presented.

A recent study focused on the activation of the inflammatory cascade in chronic fetal hypoxia in the guinea pig [Rong Guo et al. 2010]. The authors confirmed earlier data of alterations in mRNA levels of P53, Bax and Bcl-2 involved in proliferation and apoptosis. Further, a significant neuronal loss in the hippocampus was demonstrated as well as an up-regulation of inflammation cytokine genes by means of quantitative RT-PCR. A cohort of 6 animals each was held in chambers with normoxia or 12.5% and 10.5% oxygen, respectively. Of 22 cytokines that showed changes in expression levels under hypoxic conditions TNF-α and IL-1β were up-regulated under hypoxia in a dose dependent manner.

Morphology of CP

Haemorrhage

Subependymal, intraventricular or leptomeningeal haemorrhages are the most common autoptic pathological finding in brains of premature asphyctic infants. The lesions develop pre-, peri- or postpartum and clinically impress as fluctuating deterioration, tetraparesis, respiratory arrest or coma. Most haemorrhages were found between 5 and 35 hours postpartum for gestational ages between 27 and 31 weeks [Emerson et al. 1977]. In an autoptic study by Leech et al. [1979] on 170 infants with respiratory distress syndrome the most common sites of intracranial haemorrhage were found to be the subependymal tissue (60%), the ventricles (68%), the subarachnoidal space (44%) and the dura (intradural 48%, subdural 3%).

Subependymal and intraventricular haemorrhages originate from the germinal layer in the vicinity of the terminal vein between thalamus and caudate nucleus. The bleeding spreads within the germinal tissue and may disrupt the ependyma resulting in intraventricular haematoma. Massive haematomas may occlude the aqueduct and obstruct the

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flow of CSF. Cases in which the sub-ependymal parenchyma is destructed may later suffer from cerebral palsy [Aida et al. 1998].

On autopsy fresh lesions are readily identified macroscopically and impress as masses of erythrocytes on histological examination (Figure 1a). Haematomas are resolved by macrophages, some of which remain within the residual cystic defect as haemosiderophages. According to Sherwood et al. [1978] macrophages are present in the lesion by Day 4, whereas an astrocytic reaction is minimal and considerably delayed and only seen after 14 days.

In regard to the pathogenesis of subependymal and intraventricular hemorrhage, injections into the carotid artery of pre-term infants resulted in leakage of injected material into the capillary bed of the germinal layer, suggesting that the capillaries within the germinal layer which is supplied with blood by Heubner's artery may rupture by a rise in arterial pressure, particularly in conditions of hypercapnia and hypoxia [Hambleton and Wigglesworth 1976]. Minor maternal trauma was proposed as possible co-factor for the development of subependymal and intraventricular haemorrhage [Strigini et al. 2001]. Tissue necrosis as possible cause of the haemorrhage was suggested by Towbin's [1968], which is in accordance with the observation that the two conditions may coexist in up to one third of patients, although in different locations [Larroche 1964; Ross and Dimette 1965].

Subarachnoidal haemorrhage may result from spreading of an intraventricular bleeding to the brain surface but may also arise independently in as-

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Figure 1. Pathology of pre- and perinatal brain lesions.
a: Fresh haemorrhage in the germinal layer in a female fetus with a malformation of the heard, 25th gestational week (H&E, original magnification × 5);
b: Fresh neuronal necrosis presenting as eosinophilic neurons with karyorrhectic nuclei in the subiculum in a male fetus, intrauterine death at the 38th gestational week (H&E, original magnification × 250);
c: Old absorbed ischemic periventricular leukomalacia in an asphyctic female infant suffering from a tumour of the heard, born at the 38th gestational week, survival 24 days (H&E, original magnification × 25);
d: Same case as in c, numerous macrophages and foam cells are present at the borders of the lesion (H&E, original magnification × 100);
e: Macroscopic findings in multicystic encephalopathy of a female full-term infant with perinatal asphyxia born by caesarian section;
f: Same case as in e, histological overview showing gliotic remnants of the cortex (H&E, original magnification × 5);
g: Immunohistochemical labelling of reactive astroglia at the border of the necrosis, same case as in e (GFAP, counterstain hemalum, original magnification × 100);
h: Immunohistochemical labelling of residual axons and axonal swelling in the cortex of multicystic encephalopathy, same case as in e (neurofilament, counterstain hemalum, original magnification × 100).