

Brain damage in preterm infants: etiological pathways

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Summary. - Preterm newborns represent a high-risk population for brain damage, primarily affecting the white matter, and for related neurodevelopmental disabilities. Determinants of brain damage have been extensively investigated, but there are still many controversies on how these factors can influence the developing brain and provoke damage. The concept of etiological pathway, instead of a single determinant, appears to better explain pathogenetic mechanisms: the brain damage may represent the final outcome of exposure to several combinations of risk factors in the same pathway or in different pathways and can change according to the gestational age. The aim of this article is to review the current knowledge on the pathogenesis of brain damage in preterm infants, within the frame of two main theoretical models, the ischemic and the inflammatory pathway. The relationship between the two pathways and the contribution of genetic susceptibility to ischemic and/or inflammatory insult, in modulating the extent and severity of brain damage, is also discussed.

Key words: preterm, hypoxia, ischemia, inflammation, genetic susceptibility, brain damage.

Riassunto (*Il danno cerebrale nei neonati pretermine: pathway eziologici*). - I neonati pretermine rappresentano una popolazione ad alto rischio per danno cerebrale, che interessa prevalentemente la sostanza bianca sottocorticale, e per successive disabilità di sviluppo. Sebbene esistano diversi studi sui determinanti di danno cerebrale, permangono tuttavia dubbi su come questi fattori possano influenzare lo sviluppo cerebrale e provocare una lesione. Piuttosto che il ruolo di un singolo determinante, il concetto di *pathway* eziologico può spiegare con maggiore accuratezza la patogenesi del danno: questo rappresenterebbe, infatti, il risultato dell'esposizione a diversi fattori, situati sullo stesso *pathway* o su *pathway* diversi. Obiettivo di questo articolo è rivedere le attuali conoscenze sulla patogenesi del danno cerebrale nel neonato pretermine, all'interno dei due principali modelli teorici: il *pathway* ischemico e quello infiammatorio. Vengono inoltre discusse le relazioni tra i due *pathway* considerati e il contributo della suscettibilità genetica nel modulare l'estensione e la severità clinica delle lesioni.

Parole chiave: pretermine, ipossia, ischemia, infiammazione, suscettibilità genetica, danno cerebrale.

Introduction

Preterm newborns are approximately 10-12% of all live newborns in developed countries [1] and represent a high-risk population for brain damage which may result in neurodevelopmental disabilities of variable severity. The rate of cerebral palsy (CP) is about 2 per 1000 in children born at term or near term and about 6 per 100 in children born at less than 32 weeks [2]. Comorbidity for mental retardation (MR) is present in the 82% of preterm children with tetraplegia and 44%

with diplegia [3]. Preterms, without CP and/or MR, show a lower IQ score at school-age compared with full term peers [4, 5]. Furthermore 9% of preterms, with birth weight lower than 1000 grams, present a visual impairment while 11% show some hearing impairment [6]. Learning disabilities are considered common in the preterm population that is 3 to 5 times more likely to be affected by reading, spelling, mathematics, or writing disorders [4]. Therefore preterms are more likely to be hyperactive and inattentive than term schoolmates [5]. Brain damage in

preterm infants primarily affects the white matter [7, 8]; however, grey matter, basal ganglia, cerebellum, and brainstem can also be affected though to a lesser extent [9]. The most common type of damage, even though at variable rates in different studies, is represented by periventricular leukomalacia (PVL) and intraventricular hemorrhage (IVH) (Table 1).

Determinants of brain damage have been extensively investigated, and several factors - including bleeding, abnormal weight gain during pregnancy, *abruptio placentae*, premature rupture of membranes, respiratory distress syndrome, and chorioamnionitis - have been frequently reported to be associated with brain damage in preterm newborns [10-18]. However, there are still many controversies on how these factors can influence the developing brain and provoke damage. Furthermore, whether the combined diagnosis of cerebral palsy, mental retardation, and epilepsy, as a common consequence of brain damage in preterm newborns, reflects more severe manifestations of a single exposure or overlapping outcomes related to different exposure, remains an open question [14, 19].

The concept of etiological pathway, instead of a single determinant, has become increasingly more important and appears to better explain pathogenetic mechanisms [20]. In the pathway leading to brain damage, preterm birth might represent either a consequence of a cascade of events acting earlier, and directly responsible for damage, or an independent and sufficient cause of brain damage. In other words, whether brain damage in preterm newborns is part of a series of developmental adverse events leading also to preterm labor, or is merely the consequence of organ immaturity due to preterm birth, or is the final result of a combination of both mechanisms, is still unclear and needs further investigations. A better knowledge of pathogenesis of brain damage is crucial in order to prevent it and reduce its *sequelae* in preterm infants.

As far as current knowledge goes, the pathogenesis of cerebral damage is discussed mainly within the frame of two theoretical models: the ischemic and the inflammatory pathway [21-24]. The aim of this article is to review the current knowledge on the pathogenesis of brain damage in preterm infants with particular attention to the mechanisms underlying the two main proposed pathways.

The ischemic pathway

The ischemic theory focuses on the role of hypoxia-ischemia resulting from perinatal complication, often present in preterm newborns, as a determinant of a breakdown of neuronal metabolism and the subsequent cerebral damage [25-27]. Evidence supporting this model stems from several studies which demonstrated

that: a) a severe and acute loss of oxygen in cerebral tissue leads to a reduced protein synthesis and neuronal death within minutes from the insult [28] b) anoxia also acts as a triggering factor for an uncontrolled and elevated release of excitatory neurotransmitters, which contribute to brain damage even with long-time kinesis [29] c) apoptosis could influence the result of ischemia when this is not sufficiently severe to determine a tissue necrosis [30]. These evidences are based on the results of several experiments using animal models: the reduction of glucose supply and the lower availability of ATP in neuronal cells produce a Ca^{+2} overload in the cytoplasmic fluids that activates several lytic enzymes and, at the same time, reduce the production of antioxidant molecules and structural proteins which are useful for cellular homeostasis [31-33]. One of the consequences of the "calcium overflow", provoked by anoxia in affected cells, is represented by a release of excitatory neurotransmitters which, in turn, hyperstimulate postsynaptic neurons and oligodendroglia through the opening of specific receptors, so allowing a further entrance of calcium within these cells [34]. Cell damage is enhanced by the production of free radicals and nitric oxide that attack the structural components of the neurons [35, 36]. Free radicals, together with other toxic factors such as histamine and serotonin, could be produced by the activation of mast cells in the brain. The role of these cells in brain damage has been supported by the observation that Interleukin (IL)-9, a cytokine that binds to the receptor of these cells, exacerbates the excitotoxic damage induced by ibotenate in mice [37]. Apoptosis as a result of ischemia has been hypothesized on the basis of the apoptotic bodies found in the brain of asphyxiated animals. Apoptosis is thought to be provoked by mild or moderate ischemia sufficient to determine the lesion of vital components, such as mitochondria, that release pro-apoptotic molecules (i.e. cytochrome c) in the cytosol [38].

Although these observations, demonstrate that hypoxia can produce brain damage, they do not completely explain why asphyxiated preterm newborns present a site specificity of damage, slightly different from that of term infants and adults suffering of stroke following an acute hypoxic-ischemic event [39-43].

Peculiarities in the immature brain arterial bed, and in particular the presence of ventriculofugal arteries, have been taken into consideration to explain why preterm newborns suffer leukomalacic lesions predominantly around the occipital and frontal horns of the lateral ventricles [44]. However, not all authors agree on the extent and distribution of the anatomical vascular substrates in human newborns [45, 46]. Then, the insufficient autoregulation of cerebral blood flow, firstly demonstrated in animal models, has been subsequently indicated as an adjunctive factor that

Table 1. - Brain damage by ultrasound (US) scans by gestational age (GA)

Brain damage by US scans	Kuban K <i>et al.</i> [8] no. (%)	Vermeulen <i>et al.</i> [98] no. (%)	Inder <i>et al.</i> [99] no. (%)	Larroque <i>et al.</i> [100] no. (%)	De Vries <i>et al.</i> [101] no. (%)
GA ≤ 32 weeks					
PVL (all grades)	129 (8.0)	10 (5.4)	19 (19.8)	560 (21)	368 (22.5)
IVH (all grades)	295 (18.4)	17 (9.1)	NR	613 (23)	128 (7.8)
Other types / unknown ^(*)	-	-	20 (20.8) TE	80 (3) PHI	18 (1.1) FI + NR
Died	-	7 (3.8)	NR	NR	176 (10.8)
Total newborns	1605	185 (100)	96 (100)	2667 (100)	1636 (100)
GA > 32 weeks					
PVL (all grades)	-	-	-	-	45 (8.9)
IVH (all grades)	-	-	-	-	10 (2.0)
Other types / unknown ^(*)	-	-	-	-	6 (1.2)
Died	-	-	-	-	34 (6.8)
Total newborns	-	-	-	-	503 (100)

(*) including: TE, PHI, FI and NR. TE: transient echodensities; PHI: periventricular parenchymal hemorrhagic involvement; FI: focal infarction; NR: not reported; PVL: periventricular leukomalacia; IVH: intraventricular hemorrhage.

contributes, along with peculiarity of brain vessels, to provoke hypoxia-ischemia in selected areas such subcortical white matter, but not in other areas such deep forebrain and brainstem [26, 47].

More recently, experiments on the selective susceptibility of immature oligodendroglia and subplate neurons to hypoxic insults and the glutamate excitotoxicity in white matter following ischemia have been indicated as possible cofactors for the variable selective injury to this area in preterm newborns [26, 29, 34, 48].

The inflammatory pathway

According to the inflammatory theory both brain damage and preterm birth may be caused by the microbial infection of the fetal membranes and by the secondary inflammatory response of mother/fetus [49, 50].

A large body of experimental evidence indicates that preterm labor in the setting of intrauterine infection is triggered by an uncontrolled maternal-fetal inflammatory reaction to the presence of invading microorganisms [50]. The demonstration of an increased rate of preterm birth after chorioamnionitis, either in the presence or in the absence of a premature

rupture of membranes (pPROM), has suggested the possibility of a strong relationship between infection and preterm birth [50]. The pathogenesis of this event might consist in the local release of large amounts of inflammatory cytokines, particularly IL-1, IL-6 and tumor necrosis factor α (TNF- α), from intrauterine tissues; this release is considerably higher than that observed during normal labor at term gestation and could lead to preterm labor through a massive generation of prostaglandins [51, 52]. In fact, these cytokines, have been shown to stimulate the prostaglandin release by fetal membranes and uterine deciduas [53].

Moreover, cytokines, as inflammatory mediators, are also supposed to take part directly in the brain damage of preterm newborns. Inflammation of the chorion-amnion has been associated with PVL by Wu and co-workers who found that preterms born to mothers with a histological chorioamnionitis have 60% increased risk of developing a cystic periventricular leukomalacia (cPVL) and this risk increases more than twice in preterms born to mothers with a clinical chorioamnionitis [54]. High levels of pro-inflammatory cytokines in umbilical blood have been associated with an increased risk of developing CP [55, 56]. The association between IL-1, -6, -8, TNF α in fetal blood and CP has also been confirmed in term infants [57].

The site of cytokine production is still not clear. These molecules in fact, are believed capable of reaching the fetal brain from the amnio-chorionic membranes, where the infection is supposed to occur, by crossing the placenta and the blood brain barrier [58, 59]. Alternatively, cytokines could be produced directly in the fetal brain, as demonstrated by the fact that microglia can be stimulated to produce IL-1 which, in turn, promotes the production of TNF- α and IL-6 by astrocytes [60]. Furthermore, IL-18, produced by astrocytes directly in the white matter, could lead to the secondary release of other cytokines (Interferon- γ , IL-2, IL-8, IL-6), expression of adhesion molecules, induction of nitric oxide synthase and activation of cyclooxygenase-2, all of which may contribute to a proinflammatory response [61].

Autoptoc studies have demonstrated the presence of high amounts of TNF α and IL-1 β in neocortex, hippocampus, basal ganglia, and thalamus of dead infants with signs of PVL compared with infants without these pathological findings [62]. More recently, a higher expression of Interferon- γ (IFN γ) has been demonstrated in macrophages and astrocytes present in the lesional and perilesional PVL area compared with brains of newborns without this finding [63].

Cytokines are present in the CNS not only as mediators of inflammation, but also as physiologic and trophic factors, essential in all stages of nervous system development, from the neurulation to the neuronal migration and differentiation, to synaptogenesis [64-66] (Fig. 1). The ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), sustain, in fact,

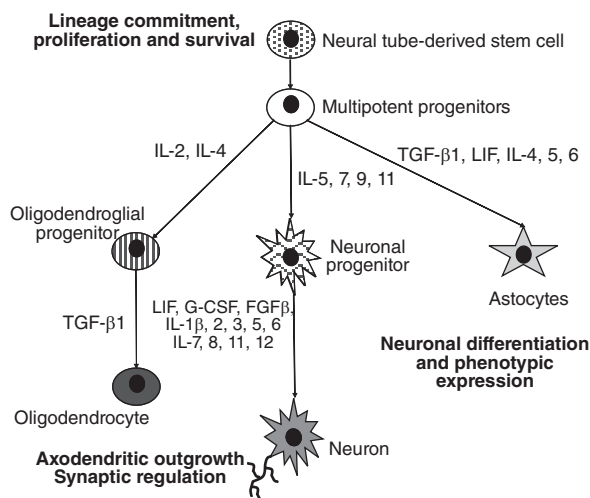


Fig. 1. - Simplified representation of cytokine involvement in the development of the SNC. Modified from (Mehler *et al.*, 1997) [64].

the growth of the radial glia which, in turn, is a required anatomical support for the cortical neuronal migration [67]. IL-2 and IL-4 promote the proliferation of oligodendrocyte progenitors, while IL-5, IL-7, IL-9 and IL-11 promote the differentiation of the neuronal progenitors [64]. Cytokines in preterm infants, in the context of inflammation, could act not only as mediators of damage but also as a dysregulated network of neurotrophic factors that alter the fragile mechanisms of CNS development.

The association between inflammation and brain damage has not been confirmed by some authors [57, 68, 69]. With regard to this lack of association, Hagberg argued that cytokines could be produced in a near-delivery period of time, i.e. following an acute uterine inflammation or later, in the neonatal period, due to the invasive procedures in the neonatal intensive units [70]. Then, in order to study the role of inflammation in brain damage and to clarify whether cytokines act as true risk factors or as indicators, it is crucial to measure the concentration of these molecules in the right "time-window". The lack of the confirmation that the antecedents, i.e. inflammation's markers, really occur before the onset of the outcome, does not provide definitive evidence on the temporal sequence necessary to assert the validity of this causal pathway [23]. Sequential measurements of indicators of infection/inflammation and damage markers will be helpful in elucidating timing issues.

Ischemic and inflammatory pathway

Ischemic and inflammatory pathways can, to some extent, coexist (Fig. 2). Cytokine involvement in a presumed ischemic brain damage might be explained in two ways: 1) cytokine concentration in biological fluids arises after a hypoxic insult and contributes to disruption of neuronal cells by the activation of the inflammatory cascade or 2) cytokines could cause a blood flow reduction in cerebral areas with an immuno-induced mechanism.

Several studies have shown increased concentrations of cytokines after an hypoxic/anoxic insult, both in adult and newborn brain. [71-80]. In the adults, circulating levels of IL-6 and IL-8 in these patients seem to correlate with stroke severity and long-term clinical outcome [80, 81].

More controversial appears the evidence that in the brain the local reduction of blood flow, eventually causing an hypoxia/ischemia, could occur after an inflammatory insult [82]. However, the evidence of a decreased systolic, mean and diastolic blood pressure in premature newborns delivered after chorioamnionitis and showing increased levels of IL-6 and IL-1 β in cord blood, suggests that an important hemodynamic

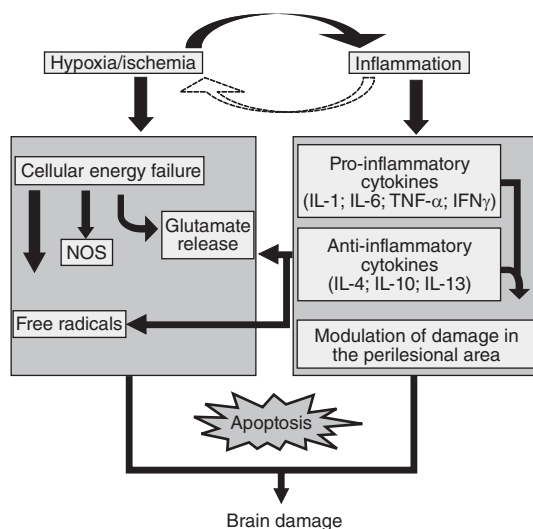


Fig. 2. - Schematic representation of the relationship between ischemic and inflammatory pathways.

disturbance could be associated with the release of high amounts of circulating cytokines that might significantly contribute to brain damage in these newborn [83].

The exact pathogenetic mechanism that leads to the cytokine release in the extracellular space is not completely known. Recently, it has been demonstrated that necrotic cells and damaged tissues during ischemia release molecules (i.e.: fibrin, fibrinogen, heparin sulfate, fibronectin, Heat Shock Protein, RNA, DNA) that could activate the inflammatory pathway through the binding with Toll-like Receptors (TLRs). Then, the activation of TLRs leads simultaneously to the release in the extracellular matrix of pro-inflammatory cytokines, as $\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-6 and anti-inflammatory cytokines such as IL-4 , IL-10 , IL-13 ; the activation of both, pro- and anti-inflammatory molecules, by the same initial signal is probably able to modulate the extension and severity of inflammatory damage [84-86]. Furthermore, the activation of the pro-inflammatory pathway leads to the increasing expression of adhesion molecules which in turn could lead to an increased reperfusion injury [87].

Another model of activation of the inflammatory pathway after induced ischemia, refers to the release of cathepsin B from lysosome into the cytoplasm, where this endopeptidase is able to activate the caspase cascade by the processing of caspase-1 and -11 (in mouse, -4 in human) which are involved in the maturation of the functional forms of cytokines $\text{IL-1}\beta$ and IL-18 [88]. According to this model, the activation of the pro-inflammatory cytokines seems to play a key role not only in promoting the inflammatory response to an ischemic insult, but also in the modulation of the post-ischemic apoptosis [89].

Genetic susceptibility to ischemic and inflammatory pathways in preterm newborns

In preterm newborns, the role of the genetic susceptibility to the ischemic and/or inflammatory insults, in modulating the extent and severity of brain damage, is still poorly understood. Recently, the cord blood concentration of IL-6 in full-term neonates has been found higher in those with a CC-genotype in the promoter region of this cytokine (-174), compared with newborn with a GG-genotype. The same polymorphism has been associated with more severe WMD and higher prevalence rate of developmental disabilities as cerebral palsy, sensorineural deafness, vision loss and cognitive impairment in preterm newborns [90]. The Factor V Leiden mutation (Arg506Gln), supposed to be involved in adverse vascular events in utero, early postnatal life or in childhood, has been proposed as a possible inherited risk factor for cerebral palsy in both, term and preterm infants [91, 92]. The role of other prothrombotic polymorphisms in ischemic stroke of children, as Factor II G20210A and C677T methylenetetrahydrofolate reductase (MTHFR) enzyme, is still controversial [92, 93].

Nevertheless, evidences supporting the importance of genetic factors in the response to ischemic and inflammatory insults, come from studies on cerebral ischemia in adults, in which the genetic predisposition has been demonstrated as a result of an additive effect of several genes and as a gene-environment interaction. In a recent case-control study including young adults with stroke, not only a significant increase of stroke risk was associated with the $\epsilon 4$ -carriership of the apolipoprotein gene and with the TT677 genotype of the MTHFR gene, but also an effect modification between prothrombotic and proatherogenic gene variants of these polymorphisms and modifiable risk factors, such as smoking and hypertension, in the pathogenesis of cerebral ischemia was also found [94].

With regard to genetic predisposition to inflammatory insults, an association between polymorphisms of IL-1R antagonist (intron 2 variable numbers of an 86 base pair tandem repeat) and $\text{TNF}\alpha$ (-308 G/A), and increased risk of ischemic stroke [95], and between the IL-6 A174G/C genotype of pro-inflammatory cytokine and lacunar infarcts [96] has been reported. Finally, as in other complex multifactorial disorders, it is likely that the risk of stroke is modulated by the interaction between gene polymorphisms which increases the risk and gene polymorphisms that have a protective effect on stroke. This is the case of the polymorphism of $\text{IL-1}\beta$ (-511TT) which has been reported to have a protective effect by modulating the inflammatory response to the ischemic event. [97].

Conclusions

Etiological pathways for brain damage in preterm newborns are complex and difficult to be explored. It is likely that different patterns of risk factors may act in different "susceptibility windows of pregnancy". The resulting brain damage in the newborn could represent the final outcome of exposure to several combinations of risk factors in the same pathway or in different pathways, and can change according to the gestational age.

Further studies are necessary in order to better understand the relationship between inflammatory and hypoxic-ischemic events. Genetic susceptibility could modify the final result in term of severity of brain damage.

Increasing the knowledge on the pathogenesis of brain damage is essential in order to implement effective prevention strategies.

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