Peertechz

Archives of Community Medicine and Public Health
submitpaper@peertechz.com

ARCHIVES OF Community Medicine and Public Health OPEN ACCESS JOURNAL

ISSN: 2455-5479 DOI: https://dx.doi.org/10.17352/acmph

Research Article

Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment?

Olivia Galipo[1], Luisa Scucces[1], Maria Cristina Morganti-Kossmann[2] and Salvatore Musumeci[3]*

[1]Department of Pediatrics, University of Catania, Catania, Italy

[2]Department of Epidemiology and Preventive Medicine, Australian and New Zealand Intensive Care Research Centre, Monash University, Melbourne, VIC, Australia

[3]Department of Biomolecular Chemistry, National Research Council (CNR), Catania, Italy

Received: 04 November, 2024
Accepted: 18 November, 2024
Published: 19 November, 2024

***Corresponding author:** Salvatore Musumeci, Professor, Department of Biomolecular Chemistry, National Research Council (CNR), 95125 Catania, Italy, E-mail: smusumec@dipchi.unict.it

Keywords: Quinolinic acid; Cytokines; Neuroinflammation; Newborn; Asphyxia; Chitotriosidase

Abstract

In children, perinatal asphyxia remains a frequent cause of disability and death. Increased catabolism of tryptophan through the kynurenine pathway, occurs in the human brain and systemic tissues alongside immune activation. The aim of this study was to determine the interaction between changes in the tryptophan pathway as well as cerebral and systemic inflammation triggered in asphyxic neonates and correlate these molecular changes with clinical parameters of asphyxia. The levels of the tryptophan catabolites, kynurenine, and quinolinic acid, as well as cytokines, were quantified in CSF and plasma of asphyxic neonates at 0 and 7 days after birth. Since macrophages and microglial cells are the source of quinolinic acid, we also measured chitotriosidase activity, which is a marker for monocytic activation.

Significantly higher concentrations of IL-4, IL-6, IL-8, and IL-10 and a non-significant increase of TNF, and IFN-γ were found in CSF of asphyxiated infants at day 1 compared to day 7. Most of the inflammatory parameters normalized at 7 days, but chitotriosidase activity remained elevated. The children were followed up for an average of a 4-5 years period, and only in one case, the evaluation of general movements showed an absent fidgety.

Introduction

Severe asphyxia during birth is a common event in both term and pre-term neonates. This insult causes irreversible brain damage with long-lasting physical disabilities and mental illnesses [1]. Neuroinflammation is one of the major responses elicited by perinatal asphyxia likely contributing to delayed and aggravated neural injury [2-5].

The inflammatory cascade comprises the secretion of several immune mediators, which can alter other molecular pathways. Recent work has shown that pro-inflammatory cytokines enhance the metabolism of the amino acid tryptophan (TRP), via the Kynurenine Pathway (KP). Interferon (IFN)-γ in particular, has been reported to be a key regulator of the KP [6]. Conversely, studies have shown that TRP plays an important role in regulating immune activation. In fact, a decrease in TRP levels suppresses the proliferation of blood mononuclear cells [7] and inhibits allogeneic T-cell responses [8,9]. Tryptophan is an essential amino acid required for protein synthesis in all living cells. In blood, TRP is normally transported across the BBB into the brain by a sodium-independent amino acid transport system. TRP is metabolised into kynurenine (KYN) by indoleamine 2,3-dioxygenase (IDO-1) in astrocytes, infiltrated macrophages, microglia, dendritic and mast cells. KYN is then further processed into other neuroactive metabolites such as Kynurenine Acid (KYNA), Anthranilic Acid (AA), 3-Hydroxykynurenine (3HK), 3-Hydroxyanthranilic acid (3HAA), Quinolinic Acid (QUIN) and Nicotinic Acid (NA) [10]. QUIN is a key end product of the KP that acts as an

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

N-methyl-D-aspartate (NMDA) receptor agonist, a known mediator of excitotoxic injury and neuronal death, inducing lipid peroxidation, axonal damage, and astrocytic death via various mechanisms [11]. In the brain, QUIN is highly produced by infiltrating macrophages and activated microglia and is neurotoxic at concentrations as low as 100 nM. Because QUIN depolarises neurons and astrocytes, it also plays a pivotal role in the onset of post-traumatic seizures, which can arise as a consequence of brain hypoxia [12,13]. These findings have implicated QUIN in a variety of Central Nervous System (CNS) disorders [14-18] with its elevation being associated with rapid death in subjects with brain injury [19].

Another important product of the KP is the neuroprotective molecule KYNA. By being an NMDA receptor antagonist, QUIN and KYNA have opposite actions. Under normal conditions, the levels of QUIN and KYNA are found in an equilibrium state that does not exhibit any detrimental effects on brain function. However, in CNS diseases QUIN is produced at a higher concentration than KYNA. This excess in QUIN drives TRP metabolism toward neurotoxicity [20]. Studies have shown that macrophages are one of the major sources of QUIN production in the brain [21]. Abundant macrophage infiltration in the brain is a critical element of immune activation following asphyxia [22]. The accumulation of macrophages is easy to detect in the laboratory environment by standard histology, but it is more challenging to investigate in the in vivo settings, in particular in humans. Therefore, specific products of macrophage activation can function as surrogate markers of their brain accumulation. The human chitotriosidase (Chit) belongs to the glycoside hydrolase family 18 and is highly secreted by fully activated mononuclear cells in all tissues and, to a lesser extent, by peripheral polymorphonuclear leukocytes [19,23]. Chit activity has been identified as a marker for macrophage activation in infectious diseases [24], neuroinflammatory diseases [25] and stroke [26].

The aim of this study was to determine the interaction between the neurotoxic changes elicited by the alteration of the KP and immune activation occurring in the brains of neonates suffering from perinatal asphyxia. Levels of TRP metabolites as well as immune mediators and parameters of macrophage activation were measured in matching CSF and plasma samples at 1 and 7 days after birth, and their levels correlated with biochemical parameters of asphyxia. The results were also correlated with long-term neurological scores to explore the potential of QUIN as a prognostic marker of brain damage and poor outcomes in asphyxic children.

Material and methods

Patients and sample collection

This study included 6 neonates admitted to the NICU (Neonatal Intensive Care Unit) of the University of Catania Hospital, Italy. All neonates were singleton and born via vaginal delivery at term gestation (37 to 41 weeks). The body weights at birth were between 2400 to 3900 grams.

All 6 babies have shown clear evidence of severe birth asphyxia and required resuscitation (IPPV (Intermittent Positive Pressure Ventilation) and nCPAP (nasal Continuous Positive Airway Pressure)) for more than 1 min immediately after birth coinciding with Apgar scores < 5 at 5 min after birth.

Systemic blood pressure and heart rate were monitored throughout the admission at the NICU. Within 6 h following admission, arterial blood was taken for measurement of pH, lactic acid, Ht (haematocrit), pCO_2, O_2 saturation, and $FiCO_2$ (fraction of inspired CO_2). A lumbar puncture was performed to obtain CSF for screening of meningitis infection. The leftover CSF samples following clinical tests were kept at -70 °C until the measurement of cytokines, Chit activity, and TRP metabolites. Lumbar puncture was repeated at day 7 and measurements were conducted as indicated above. This study had received prior approval from the institutional human ethics committee and written consents were obtained from the parents.

Multiplex assay for cytokines

The concentration of IL-6, IL-8, IL-10, IFN-γ, and TNF was determined using a Bio-Plex Cytokine Assay System (Bio-Rad, Sydney, Australia) in CSF according to the manufacturer's instructions. In brief, samples (50 µl) or standard and a mixture of cytokine antibody-coupled beads (50 µl) were added into a 96-well filter plate in duplicates, mixed, and incubated overnight at 4 °C. After removal of the incubation buffer and washing, a cocktail of biotinylated antibodies (against IL-6, IL-8, IL-10, IFN-γ, and TNF) was added to each well to react with cytokines. Fluorescent-tagged streptavidin (streptavidin-phycoerythrin) was then added to form a complex of bead-cytokine-antibody-fluorescent. The concentration of each cytokine was determined by a Bio-Plex Suspension Array System (Bio-Rad) that quantifies cytokines based on the different colour of beads representing different cytokines and the presence of streptavidin-phycoerythrin. Cytokine concentrations were automatically calculated by the Bio-Plex Manager software (Bio-Rad) using a standard curve derived from recombinant cytokine standards.

Chitotriosidase activity assay

Measurement of Chit activity was performed using the enzyme activity assay described previously [27]. Briefly, 5 µl of undiluted plasma or 30 µl of CSF were diluted with 100 µl solution containing 22 mM chitotriosidase substrate 4-methylumbelliferyl-beta-D-N,N,N-triacetyl-chitotriose (Sigma Chemical Co.) and 0.5 M citrate phosphate buffer pH 5.2 for 15 min at 37 ºC, as originally described by Hollak et al [28]. The reaction was stopped with the addition of 2 ml of 0.5 M Na_2CO_3-$NaHCO_3$ buffer pH 10.7, and the product 4-methylumbelliferone was read on a Hitachi 2500 fluorimeter at excitation 365 nm and emissions 450 nm. Chit activity was expressed as nanomoles of substrate hydrolyzed per millilitre per hour (nmol/ml/h) against standards. Chit activity was repeated in at least two assays for each sample to obtain media/mean values.

Measurement of tryptophan metabolites

TRY, KYN, KYNA, and 3HAA were quantified using high-performance liquid chromatography (HPLC). CSF and

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

serum samples were extracted using equal volumes of 5% trichloroacetic acid and centrifuged at 2000xg for 15 min to pellet the precipitates. The supernatants were then removed, and filtered before being injected into the HPLC system. The HPLC consists of a solvent delivery pump (GBC LC1110, Australia), an auto-sampler (Shimadzu SIL-10AD, Japan), a solvent degasser (Shimadzu DGU-14A), and a WinChrom chromatography data acquisition system (GBC, Australia). For TRY and KYN, the mobile phase consisted of sodium acetate (40 mM) and citric acid (40 mM) adjusted to pH 5 before the addition of acetonitrile to a final concentration of 2.5%. This was passed through a 0.45 µm filter (Millipore) using a pump at a flow rate of 1 ml/min. Fifty µl of extracted supernatant or standard were injected into the HPLC, the metabolites separated by a C18 column (Onyx Monolothic, Phenomenex, Australia) and quantified using a photodiode array detector (Shimadzu SPD-M10A) at an absorbance of 278 and 363 nm for TRP and KYN, respectively. KYNA was measured using the same HPLC setup described above except that the detection was performed by a fluorescence detector (GBC) at excitation of 344 nm and emission at 388 nm, a polymeric column (20 × 3.2 mm; 5 µm particle size; Polymer Lab) and the mobile phase consisted of sodium acetate (50 mM), zinc acetate (0.25 M) and 5% acetonitrile with pH 6.3 at the flow rate of 1 ml/min. 3HAA was measured by a Synergi Hydro column (250 × 4.60 mm; 4 µm particle size; Phenomenex) connected to a fluorescent detector (GBC, Ex = 320 nm, Em = 420 nm) with mobile phase containing 25 mM sodium acetate and 2.5% acetonitrile, pH 5.5 and a C18 column.

QUIN was quantified by Gas Chromatography-Mass Spectrometry (GC-MS). The extracted supernatant of CSF was prepared following the procedures described previously [29]. GC-MS was carried out by an Agilent Technologies 6890 gas chromatograph, an Agilent 5973 mass selective detector (Agilent Technologies, Sydney, Australia), a HP-5MS capillary column, a GC oven, and a 7683 autosampler (Agilent Technologies). Finally, QUIN concentration was determined as dihexafluoroisopropyl ester, a derivative of QUIN.

Assessment of metabolic status

The concentrations of glucose, lactate, glycerol, and glutamate in CSF were measured by an ISCUS analyser (CMA Microdialysis, Sweden) against the standards provided by the manufacturer.

Data analysis

Since the concentration of cytokines, TRP metabolites, glucose, lactate, glycerol, glutamate, and the activity Chit were not normally distributed. A Mann-Whitney U Test was used for graphic representation and statistical analysis. Data were shown as dot plots for each individual value and presented as median with interquartile range for comparison, $p < 0.05$ considered significant.

Results

Clinical physiological data

The clinical observation data of these asphyxic neonates enrolled in the study are summarised in Table 1. The children were followed up for an average of 4-5 years period (range 2.5-8 years). Patient #2 showed an IPV in the basal nuclei by echography (data not shown) while the evaluation of general movements showed an absent fidgety. In this neonate, the sitting was observed at 11 months and the walking at 24 months. Bayley Scales of Infant Development was determined at 65 at 24 months. While the general movement in some infants showed a normal pattern (Patients #1, #4, #5), in other newborns (Patients #2, #3, and #6) the general movement demonstrated a poor repertoire with absent fidgety. The ability to sit occurred at 8 months in five asphyxic newborns (#1, #3, #4, and #6), while in one case (#2) it was first manifest at 11 months. All children presented a marked delay in walking ability namely at 14 months (#5), 17 months (#1, #4, #6), and 19 and 24 months (#3 and #2 respectively). Only one newborn received breast milk and his Q.I ranged between 62-100 according to the other development parameters.

Biochemical parameters reflecting anaerobic metabolism were confirmed by low blood O_2 saturation (< 90%) and pH (< 7.00) and high lactate (> 9 mM) in blood. These and other parameters are summarized in Table 2.

Cytokine concentration in CSF of asphyxic neonates

Following birth asphyxia, six cytokines (IL-4, IL-6, IL-8, IL-10, IFN-γ, and TNF) were quantified in CSF at 1 and 7 days after birth (Table 3). Amongst them, only IL-8 displayed marked changes in all infants with a significant elevation at day 1 followed by a substantial reduction at day 7 (median: 594.1 pg/ml, IQR: 256.2-707.5 pg/ml; median 41.68 pg/ml, IQR: 31.74-70.51 pg/ml; respectively; $p < 0.05$). IL-6, TNF, and IFN-γ were not increased in CSF after asphyxia on either day

Table 1: Clinical data and neurological follow-up of asphyctic patients.

Patients	Gestational age at delivery (weeks)	Birth weight (g)	Breast Milk	Bilirubin Day 1	Bilirubin Day 2	Sitting (months)	Walking (months)	Q.I. (Bayley)	Echography
#1	38	2800	yes	1	0.6	8	17	87	IPV diffuse
#2	37	2400	no	1.3	4	11	24	65	IPV basal nuclei
#3	39	3900	no	1.4	3.2	8	19	62	IPV diffuse
#4	39	2660	no	3.9	8.4	8	17	68	Normal
#5	41	3360	no	3	5.3	9	14	100	IPV moderate
#6	40	3200	no	1.3	4	8	17	80	Normal

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

with the exception of patient #1 who had much higher levels of cytokine concentrations than other patients.

Evaluation of CSF Energy Metabolism in asphyxic neonates

Table 4 reports values of different metabolic molecules evaluated in CSF and serum of all asphyxic neonates. Glucose had similar concentrations in CSF between days 1 and 7, and these values are similar to glucose levels in the serum of normal babies [30]. In contrast, lactate was significantly higher in the CSF at day 1 (median: 6654 µM, IQR: 1825-7959 µM) as compared with day 7 (median: 1126 µM, IQR: 932.6-1255 µM). Instead, significantly higher glycerol concentration was observed in the CSF at day 1 after birth (median: 192 µM, IQR: 74-233 µM), then decreased significantly at day 7 (median: 20 µM, IQR: 15.5-56.5 µM) indicating higher brain cell membrane damage at day 1. There was no change in CSF glutamate levels between days 1 and 7, which has no association with potential cell membrane damage. CSF glycerol concentration was high on day 1 (195 ± 41 µM) and returned to almost normal level by day 7 (32.8 ± 10.2 µM). CSF glutamate increased on day 1 after birth as compared with day 7. There was no difference in serum glycerol levels between days 1 and 7, while serum glutamate showed elevated concentration on day 1 after birth. CSF and serum lactate and glucose appeared no changes between the samples from 2 days.

Tryptophan metabolism

The median concentration of TRP in CSF on day 1 was lower than on day 7 (0.92 µM *vs.* 3.60 µM), however, the variability of the TRP concentration was quite large among different infants (IQR: 0.74-7.21 µM), as compared with day 7 (0.49-4.67 µM). The significant decrease of KYN in CSF at day 7 as compared with day 1 was not associated with changes in other downstream metabolites (i.e. 3HAA, KYNA, and QUIN) over time (Table 5).

Chitotriosidase activity in CSF

The median Chit plasma level in the asphyxial newborn was 9.86 nmol/mL/h (IQR: 6.61-13.88 nmol/mL/h) and 5.57 nmol/mL/h at day 7 (IQR: 4.39-8.24 nmol/mL/h), the difference did not reach the significant level (Mann Whitney U test, $p = 0.082$; Table 6).

Table 2: Clinical and biochemical data of 6 neonates considered asphyctic at birth.

Patients	Day	pH	Lactate (mM)	Hematocrit (%)	$PaCO_2$ (mmHg)	Sat O_2 (%)	$FiCO_2$ (%)	HR (beats/min)	Arterial Pressure (mmHg)
#1	0	7.1	9.7	61.3	35.3	91	50	108	65/44
	7	7.4	1.4	54	46	95	21	130	64/35
#2	0	7.0	11.8	61	66.7	91	50	154	84/58
	7	7.4	1.2	42	38	95	21	130	64/35
#3	0	6.8	14	60	127	65	90	75	68/29
	7	7.4	1.2	42	38	95	21	130	64/35
#4	0	6.9	9	66	70	65	90	75	68/29
	7	7.4	1.9	49	38	95	21	130	64/35
#5	0	6.9	9.4	52.6	48.2	91	50	120	56/27
	7	7.4	1.1	54	35	94	21	140	56/35
#6	0	7.26	2.3	64.3	58.9	96	30	160	56/24
	7	7.4	1.4	54	46	95	21	130	64/35

pCO_2: Partial Pressure of Carbon Dioxide; sO_2: Arterial O_2 saturation; $FiCO_2$: Fractional Concentration of Inspired CO_2; HR: Heart Rate

Table 3: Cytokine concentrations in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Cytokines (pg/ml)	CSF		Serum	
	Day 1	Day 7	Day 1	Day 7
IL-4	0.068 ± 0.063 (0.926)	n.d.	0.030 ± 0.016 (0.533)	0.183 ± 0.155 (0.846)
IL-6	242.58 ± 177.68 (0.732)	10.32 ± 5.43 (0.526)	182.34 ± 29.39 (0.161)	34.88 ± 15.59 (TT0.446)
IL-8	632.03 ± 153.43 (0.242)	49.23 ± 10.30 (0.209)	169.03 ± 92.55 (0.546)	24.74 ± 5.50 (0.222)
IL-10	22.47 ± 10.83 (0.481)	0.90 ± 0.45 (0.5)	1697.50 ± 579.8 (0.341)	62.95 ± 33.54 (0.532)
IFN-g	18.58 ± 9.97 (0.536)	2.47 ± 0.55 (0.222)	16.33 ± 3.78 (0.231)	11.90 ±7.03 (0.590)
TNF-a	3.59 ± 2.51 (0.699)	0.62 ± 0.12 (0.193)	4.10 ± 0.79 (0.192)	6.15 ± 4.06 (0.660)

coefficient of variation σ/µ in brackets; n.d.: not detectable

Table 4: Metabolite concentrations in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Metabolites (µM)	CSF		Serum	
	Day 1	Day 7	Day 1	Day 7
Glucose	2647.8 ± 455.7 (0.172)	1914.3 ± 448.4 (0.234)	3043.3 ± 683.0 (0.224)	3599.7 ± 217.0 (0.060)
Lactate	6287.7 ± 1238.9 (0.197)	1100.2 ± 71.3 (0.647)	7194.0 ± 1357.7 (0.188)	2425.6 ± 824.9 (0.340)
Pyruvate	18.2 ± 5.3 (0.291)	8.0 ± 1.8 (0.225)	77.8 ± 10.4 (0.133)	40.7 ± 18.2 (0.447)
Glycerol	195.0 ± 41.9 (0.214)	32.8 ± 10.2 (0.310)	93.2 ± 11.9 (0.127)	83.8 ± 21.1 (0.251)
Glutamate	5.6 ± 0.9 (0.160)	1.7 ± 0.4 (0.235)	147.3 ± 32.6 (0.221)	164.1 ± 39.7 (0.241)
L/P ratio	552.7 ± 269.6 (0.487)	666.0 ± 442.5 (0.664)	89.8 ± 14.0 (0.155)	77.7 ±16.6 (0.213)

Coefficient of variation σ/µ in brackets.

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

Table 5: Tryptophan, and kynurenine metabolite concentrations in Cerebrospinal Fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Tryptophan and Kynurenine Metabolites (nM)	CSf		Serum	
	Day 1	Day 7	Day 1	Day 7
TRY	3657.2 ± 1521.1 (0.416)	2741.0 ± 864.9 (0.315)	5231.4 ± 620.1 (0.118)	5474.2 ± 524.0 (0.095)
KYN	553.8 ± 65.6 (0.118)	463.6 ± 65.6 (0.141)	5074.8 ± 1222.8 (0.240)	4900 ± 775.7 (0.158)
KYNA	95.7 ± 17.9 (0.187)	97.6 ± 38.3 (0.392)	743.7 ± 187.1 (0.251)	227.5 ± 69.7 (0.306)
3OH-AA	1176.3 ± 195.4 (0.166)	1098.8 ± 205.5 (0.187)	1425.5 ± 122.4 (0.085)	148.7 ± 97.7 (0.657)
QUIN	205.5 ± 64.4 (0.313)	132.2 ± 38.1 (0.288)	-	-

Coefficient of variation σ/μ in brackets.

Table 6: Chit activity in cerebrospinal fluid (CSF) and serum at days 1 and 7 of age in 6 post-asphyctic neonates.

Chit activity	CSF		Serum	
	Day 1	Day 7	Day 1	Day 7
Chit	9.19 ± 7.47	6.64 ± 2.67	11.21 ± 6.11	12.98 ±7.57

Discussion

The increasing evidence has demonstrated the significant role of cytokine in the brain as injury mediators in animal models [31-33], as predictive biomarkers in neurodegenerative diseases [34-37] and inflammation-driven depressive disease [15-18], in adult brain injury [38] as well as neonatal brain injury [39]. The metabolism of tryptophan plays a relevant role in the production of neurotoxic substances but at the same time neuroprotective substances [15]. This is a surprising fact but the different links of these two products to receptors of glutamate justify the bivalent function in the brain. There are some recent studies where the control of this mechanism was proposed utilizing an experimental model of the effect of adenosine A2A receptor antagonist, an inhibitor of quinolinic activity [40], or the effect kinurenine 3-hydroxylase inhibitor in rodents potentiate the expression of kinurenic acid [41], providing neuroprotection in neonatal rodents. However, the clinical use of these inhibitors did not receive sufficient guarantees to be proposed in conditions where the quinolinic acid is increased. One of these applications could be asphyctic newborns, subjects with brain trauma, stroke, and other degenerative brain injury. In this context, the vulnerability-stress-inflammation model has been supported by growing evidence [15-18]. According to the model, genetic landscape predisposes subjects to be easily affected by stress, which would show inflammatory responses later in life [16]. In a similar way, prenatal maternal stress (e.g., prenatal infection/inflammation, decreased fetal growth, hypoxia-related obstetric complications) has been linked to the development of CNS disease in offspring [42].

This study revealed that asphyxial newborns have a significant increase of proinflammatory cytokines in both CSF and plasma and that this increase was more significant in CSF likely due to the presence of activated macrophage and glial cells. The normalization of these immunological parameters was demonstrated in 7 days, but the levels of Chit activity remained high due to the persistence of macrophage and glial cell activation with the function of sweeper or scavenger of detrimental material. In relation to the macrophage activity, the quinolinic acid was found significantly increased in CSF respect plasma and this neurotoxic substance was maintained over the 7 days of observation especially in CSF. This observation is not surprising because a persistent increase of quinolinic acid was reported in the CSF of subjects with chronic brain trauma dating a year prior [43].

Within minutes of a traumatic impact, a robust inflammatory response is elicited in the injured brain. The complexity of this post-traumatic sequence involves a strong cellular response comprising the activation of resident microglia and astrocytes, as well as the infiltration of blood leukocytes. The second component regards the secretion of immune mediators, which can be divided into the following sub-groups: the archetypal pro-inflammatory cytokines (IL-1, TNF-α, IL-6), the anti-inflammatory cytokines (IL-4, IL-10, and TGF-β1), and the chemotactic cytokines or chemokines, which specifically drive the accumulation of parenchymal and peripheral immune cells in the injured brain regions [44]. Whilst the humoral immune response is particularly pronounced in the acute phase following Traumatic Brain Injury (TBI), the activation of glial cells seems to be a rather prolonged effect lasting for several months [45]. The interactions between cytokines/chemokines and the various cell types trigger a complex network of events, which subsequently intersect with adjacent pathological cascades including oxidative stress, excitotoxicity, or neuroprotective and reparative systems including angiogenesis, scarring, and neurogenesis [46]. It is well-accepted that neuroinflammation is responsible for both beneficial and detrimental effects, contributing to secondary brain damage but also facilitating neurorepair [47]. To what extent cytokines, can be defined as diagnostic factors reflecting brain injury or as predictors of long-term outcomes need to be further substantiated. In clinical studies, some groups have reported a correlation between cytokine production in either the cerebrospinal fluid or intraparenchymal tissue with initial brain damage, mortality, or poor outcome scores [46,48,49]. However, the validity of cytokines as biomarkers is not broadly accepted. This article discusses the evidence from both clinical and basic research exploring the validity of such immune molecules as potential prognostic markers to classify and predict the outcome following TBI.

Conclusion

The kynurenine pathway of tryptophan metabolism displays a Janus face as its derived metabolites can be neurotoxic or neuroprotective depending on which cell type is producing them. This is a surprising fact but the different links of these two products to receptors of glutamate justify the bivalent function in the brain.

This type of molecule would represent a relevant therapeutic strategy for limiting damages in asphyctic newborns, subjects

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

with brain trauma or stroke, and other degenerative brain injuries. However, the clinical use of this inhibitor did not receive sufficient guarantees to be proposed in conditions where the quinolinic acid is increased.

At this stage, the determination of cytokines expression profile and kynurenine pathway metabolites in brain damage requires sophisticated, time-consuming, and often expensive quantitative methods but in the future, the automation is likely to provide more information for the treatment of asphyctic newborns and subjects with TBI, preventing the long term disability and cognitive impairment and open a new way for the prevention and treatment of these two severe pathologies.

Acknowledgement

We thank Giuseppe Rapicavoli for his precious technical assistance in the laboratory determination of chitotriosidase.

Authorship contributions

Olivia Galipo' conceived the study; Luisa Scucces performed, analysed, and associated data; Maria Cristina Morganti Kossmann performed cytokines analysis and revised it critically for important intellectual content; Salvatore Musumeci participated in the coordination, analysis, and drafting of the manuscript.

References

1. Brew N, Walker D, Wong FY. Cerebral vascular regulation and brain injury in preterm infants. Am J Physiol Regul Integr Comp Physiol. 2014;306(11):R773-86.. Available from: https://doi.org/10.1152/ajpregu.00487.2013

2. Martín-Ancel A, García-Alix A, Pascual-Salcedo D, Cabanas F, Valcarce M, Quero J. Interleukin-6 in the cerebrospinal fluid after perinatal asphyxia is related to early and late neurological manifestations. Pediatrics. 1997;100(5):789-94. Available from: https://doi.org/10.1542/peds.100.5.789

3. Oygür N, Sönmez Ö, Saka O, Yeğin O. Predictive value of plasma and cerebrospinal fluid tumour necrosis factor-α and interleukin-1β concentrations on outcome of full term infants with hypoxic–ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 1998;79(3). Available from: https://doi.org/10.1136/fn.79.3.f190

4. Sävman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res. 1998;43(6):746-51. Available from: https://doi.org/10.1203/00006450-199806000-00006

5. Shalak L, Perlman JM. Hemorrhagic-ischemic cerebral injury in the preterm infant: current concepts. Clin Perinatol. 2002;29(4):745-63. Available from: https://doi.org/10.1016/s0095-5108(02)00048-9

6. Jones SP, Guillemin GJ, Brew BJ. The kynurenine pathway in stem cell biology. Int J Tryptophan Res. 2013;6:57-66. Available from: https://doi.org/10.4137/ijtr.s12626

7. Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta. 2006;364(1-2):82-90. Available from: https://doi.org/10.1016/j.cca.2005.06.013

8. Sarkhosh K, Tredget EE, Karami A, Uludag H, Iwashina T, Kilani RT, et al. Immune cell proliferation is suppressed by the interferon-γ-induced indoleamine 2,3-dioxygenase expression of fibroblasts populated in collagen gel (FPCG). J Cell Biochem. 2003;90(1):206-17. Available from: https://doi.org/10.1002/jcb.10593

9. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2, 3-dioxygenase–mediated tryptophan degradation. Blood. 2004;103(12):4619-21. Available from: https://doi.org/10.1182/blood-2003-11-3909

10. Braidy N, Guillemin GJ, Grant R. Effects of kynurenine pathway inhibition on NAD metabolism and cell viability in human primary astrocytes and neurons. Int J Tryptophan Res. 2011;4:29-37. Available from: https://doi.org/10.4137/ijtr.s7052

11. Braidy N, Grant R, Adams S, Brew BJ, Guillemin GJ. Mechanism for quinolinic acid cytotoxicity in human astrocytes and neurons. Neurotoxicol Teratol. 2009;16(1):77-86. Available from: https://doi.org/10.1007/s12640-009-9051-z

12. Bordelon Y, Chesselet M-F. Early effects of intrastriatal injections of quinolinic acid on microtubule-associated protein-2 and neuropeptides in rat basal ganglia. Neuroscience. 1999;93(3):843-53. Available from: https://doi.org/10.1016/s0306-4522(99)00239-0

13. Andiné P, Lehmann A, Ellrén K, Wennberg E, Kjellmer I, Nielsen T, et al. The excitatory amino acid antagonist kynurenic acid administered after hypoxic-ischemia in neonatal rats offers neuroprotection. Neurosci Lett. 1988;90(1-2):208-12. Available from: https://doi.org/10.1016/0304-3940(88)90813-0

14. Schwarcz R, Bruno JP, Muchowski PJ, Wu H-Q. Kynurenines in the mammalian brain: when physiology meets pathology. Nat Rev Neurosci. 2012;13(7):465-77. Available from: https://doi.org/10.1038/nrn3257

15. Pedraz-Petrozzi B, Elyamany O, Rummel C, Mulert C. Effects of inflammation on the kynurenine pathway in schizophrenia—a systematic review. J Neuroinflammation. 2020;17(1):56. Available from: https://doi.org/10.1186/s12974-020-1721-z

16. Won E, Kim Y-K. Stress, the autonomic nervous system, and the immune-kynurenine pathway in the etiology of depression. Curr Neuropharmacol. 2016;14(7):665-73. Available from: https://doi.org/10.2174/1570159x14666151208113006

17. Vancassel S, Capuron L, Castanon N. Brain kynurenine and BH4 pathways: relevance to the pathophysiology and treatment of inflammation-driven depressive symptoms. Front Neurosci. 2018;12:499. Available from: https://doi.org/10.3389/fnins.2018.00499

18. Hunt C, Macedo e Cordeiro T, Suchting R, de Dios C, Cuellar Leal VA, Soares JC, et al. Effect of immune activation on the kynurenine pathway and depression symptoms—a systematic review and meta-analysis. Neurosci Biobehav Rev. 2020;118:514-23. Available from: https://doi.org/10.1016/j.neubiorev.2020.08.010

19. van Eijk M, van Roomen CP, Renkema GH, Bussink AP, Andrews L, Blommaart EF, et al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol. 2005;17(11):1505-12. Available from: https://doi.org/10.1093/intimm/dxh328

20. Fujigaki H, Mouri A, Yamamoto Y, Nabeshima T, Saito K. Linking phencyclidine intoxication to the tryptophan-kynurenine pathway: therapeutic implications for schizophrenia. Neurochem Int. 2019;125:1-6. Available from: https://doi.org/10.1016/j.neuint.2019.02.001

21. Guillemin GJ, Brew BJ. Microglia, macrophages, perivascular macrophages, and pericytes: a review of function and identification. J Leukoc Biol. 2004;75(3):388-97. Available from: https://doi.org/10.1189/jlb.0303114

22. McDonough A, Weinstein JR. Neuroimmune response in ischemic preconditioning. Neurotherapeutics. 2016;13(4):748-61. Available from: https://doi.org/10.1007/s13311-016-0465-z

23. Guðmundsdóttir S. Effect of chitin derivatives on macrophages. The role of chitinases and chitinase-like proteins. 2014. Available from: https://skemman.is/bitstream/1946/18199/1/MSritger%C3%B0_SteinunnGudmundsdottir.pdf

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213

24. Barone R, Simporé J, Malaguarnera L, Pignatelli S, Musumeci S. Plasma chitotriosidase activity in acute Plasmodium falciparum malaria. Clin Chim Acta. 2003;331(1-2):79-85. Available from: https://doi.org/10.1016/s0009-8981(03)00089-5

25. Sotgiu S, Barone R, Arru G, Fois M, Pugliatti M, Sanna A, et al. Intrathecal chitotriosidase and the outcome of multiple sclerosis. Mult Scler J. 2006;12(5):551-7. Available from: https://doi.org/10.1177/1352458506070614

26. Sotgiu S, Barone R, Zanda B, Arru G, Fois ML, Arru A, et al. Chitotriosidase in patients with acute ischemic stroke. Eur Neurol. 2005;54(3):149-53. Available from: https://doi.org/10.1159/000089935

27. Verbeek M, Lefeber D, Jongen P. Chitotriosidase activity in controls and multiple sclerosis. Acta Neurol Scand. 2010;121(5):356. Available from: https://doi.org/10.1111/j.1600-0404.2010.01339.x

28. Hollak C, van Weely S, Van Oers M, Aerts J. Marked elevation of plasma chitotriosidase activity: A novel hallmark of Gaucher disease. J Clin Invest. 1994;93(3):1288-92. Available from: https://doi.org/10.1172/jci117084

29. Smythe G, Braga O, Brew BJ, Grant R, Guillemin GJ, Kerr S, et al. Concurrent quantification of quinolinic, picolinic, and nicotinic acids using electron-capture negative-ion gas chromatography–mass spectrometry. Anal Biochem. 2002;301(1):21-6. Available from: https://doi.org/10.1006/abio.2001.5490

30. Güemes M, Rahman SA, Hussain K. What is a normal blood glucose? Arch Dis Child. 2016;101(6):569-74. Available from: https://doi.org/10.1136/archdischild-2015-308336

31. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol. 2006;147(S1). Available from: https://doi.org/10.1038/sj.bjp.0706400

32. Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T. Inflammatory response in acute traumatic brain injury: a double-edged sword. Curr Opin Crit Care. 2002;8(2):101-5. Available from: https://doi.org/10.1097/00075198-200204000-00002

33. Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. FEBS J. 2009;276(1):13-26. Available from: https://doi.org/10.1111/j.1742-4658.2008.06766.x

34. Remarque E, Bollen E, Weverling-Rijnsburger A, Laterveer J, Blauw G, Westendorp R. Patients with Alzheimer's disease display a pro-inflammatory phenotype. Exp Gerontol. 2001;36(1):171-6. Available from: https://doi.org/10.1016/s0531-5565(00)00176-5

35. Bettcher BM, Kramer JH. Inflammation and clinical presentation in neurodegenerative disease: a volatile relationship. Neurocase. 2013;19(2):182-200. Available from: https://doi.org/10.1080/13554794.2011.654227

36. Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RM, et al. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. Biol Psychiatry. 2009;65(4):304-12. Available from: https://doi.org/10.1016/j.biopsych.2008.07.024

37. La Cognata V, Golini E, Iemmolo R, Balletta S, Morello G, De Rosa C, et al. CXCR2 increases in ALS cortical neurons and its inhibition prevents motor neuron degeneration in vitro and improves neuromuscular function in SOD1G93A mice. Neurobiol Dis. 2021:105538. Available from: https://doi.org/10.1016/j.nbd.2021.105538

38. McDonald SJ, Sharkey JM, Sun M, Kaukas LM, Shultz SR, Turner RJ, et al. Beyond the brain: peripheral interactions after traumatic brain injury. J Neurotrauma. 2020;37(5):770-81. Available from: https://doi.org/10.1089/neu.2019.6885

39. Zareen Z, Strickland T, Mc Eneaney V, Kelly LA, McDonald D, Sweetman D, et al. Cytokine dysregulation persists in childhood post neonatal encephalopathy. BMC Neurol. 2020;20(1):1-9. Available from: http://dx.doi.org/10.21203/rs.2.16375/v3

40. Stone TW, Behan WM. Interleukin-1β but not tumor necrosis factor-α potentiates neuronal damage by quinolinic acid: Protection by an adenosine A2A receptor antagonist. J Neurosci Res. 2007;85(5):1077-85. Available from: https://doi.org/10.1002/jnr.21212

41. Ceresoli G, Guidetti P, Schwarcz R. Metabolism of [5-3H] kynurenine in the developing rat brain in vivo: effect of intrastriatal ibotenate injections. Dev Brain Res. 1997;100(1):73-81. Available from: https://doi.org/10.1016/s0165-3806(97)00029-1

42. Lipner E, Murphy SK, Ellman LM. Prenatal maternal stress and the cascade of risk to schizophrenia spectrum disorders in offspring. Curr Psychiatry Rep. 2019;21(10):99. Available from: https://doi.org/10.1007/s11920-019-1085-1

43. Sävman K, Heyes MP, Svedin P, Karlsson A. Microglia/macrophage-derived inflammatory mediators galectin-3 and quinolinic acid are elevated in cerebrospinal fluid from newborn infants after birth asphyxia. Transl Stroke Res. 2013;4(2):228-35. Available from: https://doi.org/10.1007/s12975-012-0216-3

44. Neuro-inflammatory Sequelae of Minimal Trauma in the Non-traumatized Human Brain: A Microdialysis Study. J Neurotrauma. 2021;38(8):1137-50. Available from: https://doi.org/10.1089/neu.2011.1790

45. Martinez G. The role of immune response in traumatic injury: Boston University; 2020.

46. Woodcock T, Morganti-Kossmann C. The role of markers of inflammation in traumatic brain injury. Front Neurol. 2013;4:18. Available from: https://doi.org/10.3389/fneur.2013.00018

47. Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, et al. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr Dis Treat. 2015;11:97. Available from: https://doi.org/10.2147/ndt.s65815

48. Adamczak S, Dale G, de Rivero Vaccari JP, Bullock MR, Dietrich WD, Keane RW. Inflammasome proteins in cerebrospinal fluid of brain-injured patients as biomarkers of functional outcome. J Neurosurg. 2012;117(6):1119-25. Available from: https://doi.org/10.3171/2012.9.jns12815

49. Zeiler FA, Thelin EP, Czosnyka M, Hutchinson PJ, Menon DK, Helmy A. Cerebrospinal fluid and microdialysis cytokines in severe traumatic brain injury: a scoping systematic review. Front Neurol. 2017;8:331. Available from: https://doi.org/10.3389/fneur.2017.00331

Citation: Galipo O, Scucces L, Morganti-Kossmann MC, Musumeci S. Tryptophan Metabolism and Birth Asphyxia: What Implications for Neurodevelopment? Arch Community Med Public Health. 2024;10(4):028-034. Available from: https://dx.doi.org/10.17352/2455-5479.000213