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Short communication

Vasodilatory Prostaglandins in Perinatal Hypoxic Brain Damage

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A B S T R A C T

Prostaglandin (PGE_2 and PGI_2) synthesis was determined in the cerebrospinal fluid (CSF) and serum of 19 hypoxic neonates at the age of 5–96 hours by using Enzyme Linked Immunosorbent Assay (ELISA) method. Control group consisted of 8 children of the same age whose samples were taken due to initial suspicion of neonatal meningitis. The prostaglandin concentrations in CSF were correlated with initial hypoxic-ischemic encephalopathy (HIE) stage and neurological findings of patients at the age of 12 months. The values of PGE_2 and PGI_2 in the CSF of children with perinatal hypoxia (PNH) were significantly higher than in the children from the control group. The values of PGI_2 in serum were significantly higher than in »CSF« of patients with PNH. Although average values of PGE_2 and PGI_2 in the liquor were higher in children with advanced stage of HIE, the differences between different stages were not statistically significant. We did not find any significant correlation between average concentrations of prostaglandins and neurological findings of the 12-month-old children.

***Key words**: prostaglandins (PG), perinatal hypoxia (PNH), hypoxic-ischemic encephalopathy (HIE), cerebrospinal fluid (CSF), neurological outcome*

Introduction

Perinatal hypoxia (PNH) is, still, nowadays, when perinatal care is improved, very important clinical problem because of possible, early and late, consequences for child[1,2]. Clinical signs of central nervous system (CNS) in the form of hypoxic-ischemic encephalopathy (HIE) are very important, early symptoms in neonatal clinical practice[3]. Late signs of perinatal hypoxia are manifested very clearly at the age of 12 months in the form of more or less obvious motoric or mental disorders[4,5].

Pathophysiological base of damage in PNH means exhaustion of mechanism of circulatory and metabolic adaptation of fetus or neonate to hypoxic-ischemic stress[6]. Signs of CNS dysfunction in form of HIE which are graded, according to Sarnat/Sarnat method, in three grades, can be important predictor of later damages[7,8]. Prostaglandins (PG) are derivatives of arachidonic acid which rises in the process of phospholipids tissue membranous breakdown. PGI_2, PGE_2 and PGF_2[9–11] have especially important biological effect. There are a number of unidentified processes in which some representatives of this group are included. Vasodilatory effect of some PGs is an important mechanism in compensatory phase of hypoxic-ischaemic brain damage. However, their dynamics and possible consequences on early and late neurological outcome are still insufficiently defined and they are still subject to researches[12–14].

Patients and Methods

In the period from 2003 to 2005, a prospective cohort analysis of vasodilatory mediators of hypoxia (PGE_2 and PGI_2) has been carried out on neonates, hospitalized at

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Neonatal Intensive Care Unit of Children's department, University Hospital Mostar, due to perinatal hypoxia. Only neonates who fulfilled at least three of the following criteria for perinatal hypoxia/asphyxia (American Pediatric Academy – APA) were included in the study[13]: 1. Birth distress evaluated on cardiotocography (more than an hour of serious disorders, deceleration or bradycardia longer than half an hour); 2. Early passage of thick meconium; 3. pH in fetal scalp blood lower than 7.2 or in capillary blood in the first four hours lower than 7.25. 4. Need for neonatal resuscitation with positive pressure ventilation for more than one minute; 5. Apgar score ≤7 in the fifth minute of life; 6. Abnormal neurological signs in the first 72 hours of life (abnormal tonus, reflexes and consciousness); 7. Multi-system perinatal failure. Neonates with infection confirmed, and those with congenital malformations or metabolic disorders, as well as cases with artificial hemorrhage (traumatic hemorrhage at lumbar puncture) were excluded.

Control group was formed from non-asphyxic/non-hypoxic neonates who underwent spinal tap and were treated hematologically, due to clinical suspicion on perinatal infection. Cerebrospinal fluid (CSF) and blood microbial cultures were negative. PGE_2 was determined in CSF samples of 19 patients at the age of 5–96 hours, and of 8 examinees of the control group. PGI_2 was determined in CSF samples of 19 patients and in serum of 9 patients. It was not determined in the serum of control examinees because of insufficient sample quantity. Patients and participants of control group were not significantly different in birth weight, sex, gestational age, proteins in CSF and cells in CSF. There was a significant difference between patients with PNH and control group for Apgar score in the first and the fifth minute of life, for blood pH, pCO_2 and base excess (–EB). pO_2 was not significantly different between groups as artificial ventilation with oxygen was applied immediately after birth (Table 1). CSF samples were taken from patients at the age of 5–96 hours and they were stored at –20 °C in Chemical-Biological laboratory of The School of Medicine in Mostar, and at –70 °C at Department of Physiology of the School of Medicine, University of Zagreb, prior to analysis. Prostaglandin PGE_2 was determined according to Enzime Linked Immunosorbent Assey (ELISA) method using original R&D kit (Catalog no. RD-DE2100). PGI_2 was determined according to ELISA method using the kit of the same producer (Catalog no. RD-DE1700), and values are expressed in pg/mL. Optical reader for microplates of Dynatech Company (M 5000) was used for result reading.

Patients were clinically observed and their neurological status was evaluated according to Sarnat/Sarnat method[7] as: 1-mild HIE (1st grade of HIE) (excitation, emphatic reflexes, frequent convulsions); 2-moderate HIE (2nd grade of HIE) (lethargy, diminished reflexes, convulsions +/–); 3-severe HIE (3rd grade of HIE) (stupor/coma, absence of reflexion, convulsions +).

Neurological status of the 12-month-old was estimated by neuropediatricians and physiotherapists and the patients were classified, according to Amiel-Tison method[5], into three groups: 1-normal result; 2-mild motoric damage (abnormality in muscular tonus); 3-abnormal result (cerebral palsy or death). Ultrasound examination of brain, (at mobile Ultrasound Toshiba instrument, with probe of 3.75 mHz, 5 and 6 mHz) made during the first week, was used in total estimation of neurological state of a neonate.

Statistical analysis was made by using Statistica for Windows programme, Version 7. StatSoft, Inc. (2004). Non-parametric statistic tests (Kruskal-Wallis, Mann-Whitney U test) were used, and results were considered statistically significant if $p \leq 0{,}05$[15].

The research was approved by Ethical Committee of the School of Medicine, University of Mostar, with the parents' written consent.

Results

Group data on the asphyxia group (n=19) and the control group (n=8) are given in Table 1. The infants in

TABLE 1
MAIN PERINATAL DATA ON THE STUDIED NEONATES (MEDIANS AND INTERQUARTILE RANGE- MANN-WHITNEY U TEST, p<0.05)

Observed variable (medians, IQR)	Children with PNH (N=19)	Control group (N=8)	P value
Gestational age (weeks)	39.00 (2.75)	39.50	0.716
Birth weight (g)	3270.00 (550.00)	3000.00	0.386
Apgar score (1st minute)	6.00 (2.00)	8.00 (2.00)	<0.001
Apgar score (5th minute)	7 (1.00)	10 (2.00)	<0.001
CRP (mg/L)	1.9 (5.50)	4.25 (4.20)	0.591
Proteins in CSF (mg/mL)	1082.0 (1284.0)	721.0 (962.75)	0.911
Blood pH (capillary)	7.23 (0.08)	7.34 (0.19)	0.016
pCO_2 (mmHg)	58.40 (15.70)	41.10 (18.88)	0.016
pO_2 (mmHg)	48.00 (10.40)	56.05 (23.13)	0.689
–EB	7.30 (2.30)	2.25 (1.58)	0.002

IQR – interquartile range, PNH – perinatal hypoxia, CRP – C reactive protein, CSF – cerebrospinal fluid, EB – excess base

the asphyxia group were classified as having mild (n=9), moderate (n=7) and severe HIE (n=3). Two infants in the group with severe HIE died in the neonatal period due to multiorgan postasphyctic failure. Other infants have been followed during 12 months of age. Six infants have normal neuromotor outcome, eight infants at the age of 12 months have mild motor impairment. Five infants, one from severe HIE group, other from moderate HIE group, have adverse neuromotor outcome (cerebral palsy).

Significantly higher values of PGE_2 were found in CSF samples of children with PNH than those in the control group (p=0.0476, Mann-Whitney U test) (Table 2).

Patients also had significantly higher PGI_2 values in CSF than examinees in the control group (p=0.0215, Mann-Whitney U test) (Table 3). Average values of PGI_2 are significantly higher in the serum of patients than in CSF (Table 3), while, there are no comparable results in the control group because of little sample quantity.

Children with 3rd grade of HIE had higher average values of PGE_2 and PGI_2 in CSF than children with 1st and 2nd grade, but differences were not statistically significant (Table 4). Average concentrations of PGE_2 were not significantly different comparing to neurological result at the age of 12 months. Average values of PGI_2 were obviously higher at children with good neurological result, but difference between groups was not statistically significant (Table 5). In serum also, there was not statistically significant difference in average concentrations of PGI_2 between groups with different neurological results (Table 5).

TABLE 2
PGE_2 LEVELS (pg/mL) IN CSF OF PATIENTS WITH PNH AND CONTROL GROUP (MANN-WHITNEY U TEST, p<0.05)

Participants	PGE_2 (pg/mL)	
	Number	Levels (medians)
Patients	19	30.78
Control	8	18.30
Statistical significance	p= 0.0476	

PGE_2 – Prostaglandin E_2, CSF – cerebrospinal fluid, PNH – perinatal hypoxia

TABLE 3
PGI_2 LEVELS IN CSF AND SERUM (pg/mL) OF PATIENTS AND CONTROL GROUP (MANN-WHITNEY U TEST, p<0.05)

Participants	CSF		Serum	
	Number	Levels (medians; pg/mL)	Number	Levels (medians; pg/mL)
Patients	19	86.02	9	497.64
Controls	7	22.21	7	n.d.
Statistical significance	p=0.0215			

PGI_2 – Prostaglandin I_2, CSF – cerebrospinal fluid, n.d. – not determined

TABLE 4
VALUES OF VASODILATORY PROSTAGLANDINS (MEDIANS) IN RELATION TO GRADE OF HIE (KRUSKAL-WALLIS TEST, p<0.05)

Prostaglandin (pg/mL)	Ist grade of HIE (N=9)	IInd grade of HIE (N=7)	IIIrd grade of HIE (N=3)	p value
PGE_2 (CSF)	29.21	31.24	115.39	0.4090
PGI_2 (CSF)	177.39	37.20	1209.74	0.1563
PGI_2 (serum)	675.82	256.46	934.51	0.1027

HIE – hypoxic-ischemic encephalopathy, PGE_2 – Prostaglandin E_2, PGI_2 – Prostaglandin I_2, CSF – cerebrospinal fluid

TABLE 5
VALUES OF VASODILATORY PROSTAGLANDINS (MEDIANS) IN RELATION TO NEUROLOGICAL OUTCOME AT THE AGE OF 12 MONTHS (KRUSKAL-WALLIS TEST, p<0.05)

Prostaglandin (pg/mL)	Normal outcome (N=6)	Moderate neuromotor damage (N=8)	Adverse outcome (N=5)	p value
PGE_2 (CSF)	31.74	29.19	38.65	0.811
PGI_2 (CSF)	392.46	63.76	86.46	0.190
PGI_2 (serum)	675.82	120.80	497.64	0.099

PGE_2 – Prostaglandin E_2, PGI_2 – Prostaglandin I_2, CSF – cerebrospinal fluid

Discussion

There are numbers of studies which confirm that prostaglandins are significantly produced in entire perinatal period[16–21]. They are important autacoids with many physiological and pathological influences. Results of their secretion are conditioned by their dynamics, and also by stress stimuli in which asphyxia/hypoxia have special place[19]. They play an important role in the adaptation of blood vessels to the perfusion pressure changes, to the carbonic dioxide (pCO_2) and oxygen (pO_2) partial pressure changes, as well as many other functions[17].

In our research, significantly higher PGE_2 values were found in CSF samples of children with PNH than in the control group ($p=0.047$) (Table 2). Similar results, with significantly higher values of PGE_2 in CSF of children with HIE, are stated by Vilanova et al.[21], while Reznichenko et al.[22] find increase of PGE_2 in the serum of neonates with acute and chronic perinatal hypoxia. Correlation of higher PGE_2 values and grade of HIE, in our analysis, was not statistically confirmed, but higher values were noticed at children with 3rd grade of HIE than in children with 1st grade (115.387 pg/mL *versus* 29.214 pg/mL). Also the concentration of PGE_2 in CSF and neurological result were not significantly correlated ($p=0.811$). Researches on animal models show different dynamics of certain prostaglandins secretions depending on hypoxia duration (acute/chronic), different intensity of secretion and different duration of high values of some prostaglandins (that is PGE_2 and PGI_2)[23,24]. Recently, it was shown that PGE_2 is responsible for apnea and irregular breathing in hypoxic mice[25].

In this research, significantly higher values of PGI_2 were also found in CSF of children with PNH than in the control group ($p=0.0215$), which is similar to results of the above mentioned researches[21,22]. Although PGI_2 values are obviously higher in children with 3rd grade of HIE than in children with 1st grade (6,6 times higher), difference between three groups of children with HIE is not statistically significant. Liu et al.[26] find higher values of PGI_2 in CSF of children with moderate and severe HIE and in children with adverse neurological outcomes. They conclude that PGI_2 (with TXA_2 and cAMP) is a sensitive marker of serious brain damage in early phase and it can be a good predictor of future neurological development. Our analysis did not show statistically significant difference between PGI_2 value and neurological result at the age of 12 months (small sample), although PGI_2 values in group of children with good result were obviously higher than those in children with moderate motoric damage or with severe results (Table 5). These, at first sight, controversial data could be possibly explained by researches on neurological tissue *in vitro* where neuroprotective role of PGI_2 was found[27,28]. Cytoprotective effect of PGI_2 observed in those researches, out of its vascular effect, could be a possible basis for explanation of its positive long-lasting effect after hypoxic-ischemic brain damage[27,28].

PGI_2 in the serum of patients with PNH has similar pattern as it has in CSF, that is, children with 3rd grade of HIE had the highest values of PGI_2 (Table 4). However, difference between groups was not statistically significant. Raznichenko et al.[22] also find similar values of PGI_2 in serum and CSF. Our result, which shows slightly higher values of PGI_2 in serum than in CSF, can be explained by vascular net changes in hypoxia, which are more dominant than complete cerebral production of prostaglandins which is reflexed in CSF[23].

Contrary to results of the above mentioned authors, in our study the increased values of PGE_2 and PGI_2 in CSF of children with PNH were not in correlation with grade of HIE and neurological outcome. This may be due to the methodological differences in our study and in the studies of other authors (e.g. time of sampling, grading of HIE or neurological scoring) or to the well known protective effect of PGE_2 and PGI_2 in various diseases. Thus, the increased concentration of these parameters can be some kind of balance between their increased production in patients with more severe disease and compensatory effects of these agents to ameliorate the disease. These antagonist influences can obviously abolish their correlation with clinical parameters. The letter possibility merrits further investigations, perhaps in animal model.

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VAZODILATACIJSKI PROSTAGLANDINI U PERINATALNOJ HIPOKSIČNOJ OZLJEDI MOZGA

S A Ž E T A K

Proizvodnja prostaglandina (PGE_2 i PGI_2) je istraživana u likvorima i serumima 19 novorođenčadi u dobi od 5–96 sati u kojih je postojala perinatalna hipoksija (PNH). Analiza je vršena enzimskim imunotestom (ELIZA). Kontrolnu skupinu je sačinjavalo osmero djece iste dobi koja su zbog sumnje na novorođenački meningitis bila punktirana. Koncentracije prostaglandina u likvoru su uspoređivane sa stupnjem hipoksično-ishemične encefalopatije (HIE) i s neurološkim ishodom istih pacijenata u dobi od 12 mjeseci. Koncentracije PGE_2 i PGI_2 u likvoru su bile statistički značajno više u djece s PNH nego u djece iz kontrolne skupine. Koncentracije PGI_2 u serumu su bile značajno više od onih u likvoru pacijenata s PNH. Iako su prosječne koncentracije PGE_2 i PGI_2 u likvoru bile više u djece s težim stupnjem HIE, razlike nisu bile statistički značajne. Nismo našli niti statistički značajnije korelacije između prosječnih koncentracija prostaglandina i neurološkog ishoda u dobi od 12 mjeseci života.