THE EFFECT OF VERAPAMIL AND MAGNESIUM SULFATE ON REGIONAL CEREBRAL BLOOD FLOW IN A PUP MODEL OF PERINATAL SPHYXIA

UGUR DILMEN*
EMIN METE*
ERHAN VAROGLU*
MEMDUF AKYÜZ*
YASEMIN AKIN*
RAHMI ÖRS*

SUMMARY: The purpose of this study was to observe the effects of three minutes asphyxia on regional cerebral blood flow (rCBF) in newborn puppies. Another aim was to see the influence of verapamil and magnesium sulfate on rCBF. Blood pH, pO_2, pCO_2 were measured before and after asphyxia. Meanwhile, rCBF was measured by using single photon emission computerized tomography (SPECT) before and after asphyxia with 1 mCi Tc-99 m HMPAO and 2.5 mCi Tc-99 m HMPAO respectively. Then, the puppies were divided into three groups (verapamil, magnesium sulfate and serum physiologic). After administering the drugs intravenously, rCBFs of the puppies were measured by SPECT. The differences between basal and post-asphyxial pO_2, pCO_2 and pH values were significant. However, basal post-asphyxial and postdrug measurements for rCBF revealed no statistically significant differences. We therefore conclude that, three minutes asphyxia in puppies seem to be a safe period. Additionally verapamil and magnesium sulfate have not altered rCBF.

Key Words: Asphyxia neonatorum, verapamil, magnesium sulfate, regional cerebral blood flow.

INTRODUCTION

Prenatal asphyxia and its neuro-developmental consequences remain a major clinical challenge despite recent advances in obstetrical and neonatal care. Brain injury is not associated with the degree of hypoxia or acidosis (1-4). However, the fetus or the neonate redistributes the blood flow to the heart, brain and adrenals to ensure the adequate oxygen and substrate delivery to these vital organs (5). Also, regional cerebral blood flow (rCBF) was reported to be decreased in the term babies with hypoxic ischemic encephalopathy after the forth day of the life (6). Additionally, hypoxic ischemic injury may increase the intracellular calcium and lead to successive neuronal damage (7). We in the experiments reported here employed verapamil, a calcium channel blocking agent, and magnesium sulfate to test if they would improve cerebral blood flow (CBF) and thus prevent the metabolic alterations that accompany the insult in the newborn pup model of perinatal asphyxia.

* From Fatih University, Medical Faculty, Department of Neonatology, Emek, Ankara, Türkiye.

MATERIALS AND METHODS

Newborn pups (24-72 hours) were anesthetized with 20 mg/kg intraperitoneal pentobarbital. Then, femoral vein and artery were catheterized the baseline rCBF was measured by using single photon emission computed tomography (SPECT). For baseline SPECT study 1 mCi Tc-99 m HMPAO was administered via femoral vein catheter. In twenty minutes SPECT

study was performed using a rotating gamma camera interfaced to a dedicated computer system (General Electric 4000 XCT). Total 64 matrix 64 frames of 20 sec in a circular orbit were collected. Transaxial, coronal and sagittal slices were generated using back projection method after a high frequency cutoff. Slice thickness was 6.4 mm. The pups were fixed under the camera with special apparatus to avoid motion artifacts. The pups randomly divided into three groups: Verapamil group, magnesium sulfate group and control group. Then, mouth and noses of the pups were obstructed by a plastic bag for 3 minutes. After this procedure, SPECT study was performed again. For second SPECT study 2.5 mCi Tc-99 m HMPAO was administered intravenously. Some of the pups needed resuscitation. However, none of them were delayed more than 15 minutes, and bicarbonate was not used during resuscitation. After measuring CBF, verapamil 0.5 mg/kg or magnesium sulfate 0.075 mg/kg were administered intravenously to the pups in the verapamil group and magnesium sulfate group and physiologic saline was administered to the control group. Then, the CBF was measured by using the same method, but saline dose Tc-99m HMPAO was 5 mCi.

SPECT studies were interpreted quantitatively. For quantitative evaluation three region of interests (ROIS) were drawn on transaxial slices. First ROI was drawn on whole, second ROI in anterior portion and third ROI in posterior portion of brain as visible as brain maturity was concerned. The mean counts of all ROIS were calculated for three separate SPECT studies in order to measure rCBF. The ratios of posterior cere-

Table 1: Birth weight, blood gases and pH values in the verapamil group before and after asphyxia.

No	Birthweight	PO_2 before asphyxia	PCO_2 before asphyxia	pH before asphyxia	PO_2 after asphyxia	PCO_2 after asphyxia	pH after asphyxia
1	700	54.4	33.6	7.30	34.8	64.8	7.10
2	660	80.3	26.0	7.40	43.5	63.0	6.90
3	700	90.5	16.0	7.20	44.6	65.6	6.90
4	700	73.3	36.0	7.30	27.2	66.0	7.00
5	760	78.8	52.0	7.20	12.2	79.0	6.60
6	650	59.6	39.2	7.30	30.9	43.8	7.10
7	640	44.0	46.0	7.40	21.0	57.0	7.10
8	760	47.0	33.0	7.40	27.0	42.0	7.00
9	660	82.0	31.0	7.40	30.0	75.0	6.50
10	700	51.1	25.4	7.10	30.2	45.9	6.80
11	550	70.8	31.3	7.30	32.9	51.1	7.00
Mean S.D.	6.80±58.82	66.53±15.34	33.59±9.88	7.3±0.1	30.39±9.17	59.38±12.47	6.91±0.20

Table 2: Birth weight, blood gases and pH values in the magnesium sulfate group before and after asphyxia.

No	Birthweight	PO_2 before asphyxia	PCO_2 before asphyxia	pH before asphyxia	PO_2 after asphyxia	PCO_2 after asphyxia	pH after asphyxia
1	740	110.5	13.0	7.46	36.4	53.1	7.28
2	700	60.8	24.0	7.35	35.1	60.1	7.23
3	720	72.2	33.0	7.38	48.9	37.0	7.28
4	780	45.6	31.8	7.31	40.8	60.7	7.26
5	600	43.6	45.2	7.32	22.0	50.6	7.20
6	620	50.1	32.6	7.40	30.1	60.2	7.10
7	640	36.3	44.9	7.20	26.8	42.1	6.80
8	700	62.1	32.1	7.32	30.3	60.8	6.70
9	600	63.4	40.3	7.30	38.8	70.1	7.10
Mean S.D.	677.8±65.1	60.51±21.94	32.99±10.17	73.38±0.73	34.36±8.07	54.97±10.37	71.06±0.214

Table 3: Birth weight, blood gases and pH values in the control group before and after asphyxia.

No	Birthweight	PO_2 before asphyxia	PCO_2 before asphyxia	pH before asphyxia	PO_2 after asphyxia	PCO_2 after asphyxia	pH after asphyxia
1	570	64.0	37.5	7.43	32.8	62.1	7.10
2	600	51.8	39.5	7.51	33.5	50.8	7.28
3	560	80.1	38.8	7.35	28.5	65.8	6.90
4	660	73.0	37.7	7.37	22.0	52.4	6.00
5	700	61.6	39.6	7.32	40.1	65.1	7.10
6	700	90.3	30.1	7.22	45.5	68.7	6.80
7	550	85.5	42.0	7.44	40.1	75.8	6.50
Mean S.D.	620.0±65.60	72.33±13.92	37.89±3.74	7.377±0.094	34.64±7.96	62.96±8.86	6.811±0.437

bral cortex/whole brain were used to statistical analysis, since posterior cerebral cortex was more mature than the anterior portion due to normal mode of development.

Consequently, the effects of verapamil and magnesium sulfate on rCBF were calculated.

Blood gases and pH analysis:

Blood gases and pH analysis were made using the blood samples obtained from the femoral artery catheter before and after asphyxia. Blood pH, pO_2 , and pCO_2 measurements were made by using NOVA Blood Gases Analysis Instruments.

Statistical methods

Wilcoxon signed ranks analysis method was used for comparing the initial and post-asphyxial results of SPECT analysis, pH, pO_2, pCO_2 in verapamil, magnesium sulfate and control groups. Mann-Whitney U test was used for comparing the basal SPECTs between control/verapamil, control/magnesium sulfate and verapamil/magnesium sulfate groups. Kruskal-Wallis one-way analysis of variance was used to evaluate the difference between the three groups with respect to basal SPECT, postdrug SPECT, basal pH, final pH and post-asphyxial SPECT.

For all tests values of $p<0.05$ were considered significant.

RESULTS

Physiologic variables: Values for PaO_2, $PaCO_2$ and pH before and after asphyxia are given in Table 1 (Verapamil group), in Table 2 (magnesium sulfate group), and those of the control group in Table 3.

It is important to note that the pO_2, pCO_2 and pH before and after asphyxia were found significantly different as expected ($p<0.005$).

Table 4: SPECT studies in the verapamil group.

No	Basal (P.C.C/W.B.)	After asphyxia (P.C.C/W.B.)	Verapamil (P.C.C/W.B.)
1	0.9995	1.0253	1.0035
2	1.0003	1.0635	1.0283
3	1.0258	1.0884	1.0351
4	1.0421	1.0678	1.0428
5	1.0685	1.0474	1.0562
6	1.1170	1.1045	1.0149
7	1.0154	0.9948	1.0579
8	1.0267	1.0652	1.1061
9	1.0505	1.0021	1.0203
10	1.1097	0.9762	1.0583
11	1.0838	1.1109	1.0978

P.C.C.: posterior cerebral cortex.
W.B.: Whole brain.

rCBF studies with SPECT:

Basal, post-asphyxial, postdrug SPECT values are seen in verapamil, magnesium sulfate and control groups (Tables 4, 5, 6) respectively. Basal, post-asphyxial and postdrug SPECT values were similar in three groups (p for all>0.05).

DISCUSSION

In experimental neonatal asphyxia, it is possible to produce acute and total interruption of gas exchange to the newly delivered animal. Such animals, after resuscitation and extended survival, exhibit damage to structures in their brainstems (8). However, the effects of

Table 5: Table 4 SPECT studies in the magnesium sulfate group.

No	Basal (P.C.C/W.B.)	After asphyxia (P.C.C/W.B.)	Magnesium Sulfate (P.C.C/W.B.)
1	1.0183	0.9815	0.9882
2	1.0000	0.9692	1.0176
3	0.9926	1.0332	1.0217
4	0.9900	0.9751	0.9657
5	1.0341	1.0382	1.3135
6	1.0768	1.0973	1.0549
7	1.0655	1.0539	1.0143
8	1.0819	1.0619	0.9138
9	1.0829	1.0339	1.0521

P.C.C.: posterior cerebral cortex.
W.B.: Whole brain.

Table 6: SPECT studies in control group.

No0	Basal (P.C.C/W.B.)	After asphyxia (P.C.C/W.B.)	Physiologyic Saline (P.C.C/W.B.)
1	1.0000	1.3672	1.0000
2	1.0000	1.0575	1.0519
3	1.0822	1.0716	1.0706
4	1.1837	1.0649	1.0978
5	0.9052	1.0061	0.9775
6	0.9922	1.0346	1.0617
7	0.9922	1.0346	1.0617

P.C.C.: posterior cerebral cortex.
W.B.: Whole brain.

hypoxia on cerebral hemispheres are controversial. The damage on the hemispheres may show the degree of hypoxic insult (9). Fetal hypoxemia and acidosis produce a striking redistribution of fetal blood flow from non-vital organs to the vital organs (10).

The study with newborn piglets showed increased cerebral blood flow remained above baseline values in different grades of hypoxia (11). It has been suggested that, following a hypoxic ischemic episode, fetal CBF increases markedly with preferential shunting to some parts of the fetal brain (12).

Since relatively uniform perfusion rates throughout the brain at birth there gradually emerged a marked heterogeneity, in parallel with the structural and functional maturation and differentiation known to occur in the brain during this period of life, we studied with the brain of neonatal puppies in order to measure the rCBF (13).

Previous studies in different pathological conditions in the fetal lamb revealed different rCBF findings. When partial oxygen tension or content decreased, fetal CBF was increased linearly with a different hierarchy of responsivity occurred (brainstem>sub-cortex and cortex), fetal CBF increased as carbon dioxide tension increased with a different hierarchy of responsivity (brainstem>sub-cortex>cortex), auto regulation of fetal CBF over a wide range of blood pressure or cardiac output was maintained for both total CBF and the various brain regions (14). However, in newborn lambs and in newborn puppies acute hypocarbia created by using hyperventilation with decreased cerebral oxygen consumption, did not make any difference on the cerebral circulation (15,16).

We used pentobarbital in order to stabilize the puppies for rCBF studies. Although, some studies showed controversial results about the effect of Phenobarbital on CBF (11,17), the effect of pentobarbital was not studied. Being a hypnotic agent, it caused active sleep in the neonatal dogs. Peripheral and central chemo receptors are less sensitive during active sleep. In term newborns slow cyclical variations in cerebral blood flow velocity were observed (18). However, the equivalent situation was not studied in the dogs and other animals.

The advantage of Tc-99 m HMPAO is the clearance from the blood. Uptake in the brain reaches a maximum of 3.5-7% of the injected dose within 1 minute. Up to 15% of the cerebral activity was washed away from the brain by 2 minutes post injection after which there occurred loss of minor activity for the following 24 hours (19).

This experiment revealed that three minutes asphyxia in puppies does not change rCBF. Consequently, we can say 3 minutes may be a safe time for the puppies for resuscitation.

In hypoxic ischemic encephalopathy, besides changes in cerebral homodynamic, acute neurochemi-

cal and biophysical alterations play a significant role on the degree of the damage (7,8,20-22). Cerebral energy failure, calcium activated phospholipid degradation, post-ischemic injury and increased release of neurotransmitters (excitatory amino acids [EAA]) are interrelated and consequently neuronal damage increases (7,23,24).

After understanding some new mechanisms of hypoxic ischemic encephalopathy, some researchers tried some new drugs in order to block the cascading reactions. Calcium channel blockers, flunarizine and lidoflazine have been tested in animal experiments (25,26). The administration of combination of lidoflazine and free radical scavengers during resuscitation after severe asphyxia was shown to improve cerebral blood flow (26). In addition to the blockade of calcium flow to the cytosol, some of these agents may act as a free radical scavenger (27). This study has shown that verapamil was not effective on rCBF. Other calcium channel blocking agents should be tested in either prophylactic or resuscitative treatment experiments.

EEAs (particularly L-glutamate and L-aspartate) release was suggested as effective as calcium channel blockers on the neuronal damage (28). Being a neurotransmitter EAAs exert trophic influences and contributes to the development of neuronal signaling and participates in the neuronal architecture (7). EAAs has special receptors in central nervous system (CNS): N-methyl D-aspartate (NMDA), kainate and quisqualate (29). After hypoxic ischemic excessive amounts of glutamate and aspartate was measured in CNS. By stimulating NMDA receptors, glutamate may cause neuronal injury by massive influx of calcium into the neurons (7,30).

Understanding the importance of EAAs and receptors some NMDA blocking agents, (MK-801) were used for experimental and therapeutic purposes (30,31). However, the deleterious effect associated with calcium influx is blocked by magnesium on rCBF in this study. The results showed that it was not changed. The effect of magnesium should be studied on the molecular basis.

We believe, in the near future new drugs or some ancient drugs are going to be used for the treatment of hypoxic ischemic encephalopathy. Further studies with calcium channel blocking agents and EAA blockers will enlighten the exact mechanism of these agents in hypoxic ischemic encephalopathy.

ACKNOWLEDGEMENT

The authors are greatly indebted to Dr Mete F. Toppare for his help during statistics.

REFERENCES

1. Dennis J, Johnson A, Mutch L, Yudkin P and Johnson P : Acid base status at birth and neuro-developmental outcome at four and one half years. Am J Obstet Gynecol, 161:213-220, 1989.

2. Dijxhoorn MJ, Visser GH, Fidler VJ, Touwen BC and Huisjes HJ : Apgar score, meconium and acidemia at birth in relation to neonatal neurological morbidity. Br J Obstet Gynecol, 93:217-222, 1986.

3. Gunn AJ, Parer JT, Mallard EC, Williams CE and Gluckman PD : Cerebral hystologic and electrophysiological changes after asphyxia in fetal sheep. Pediatr Res, 31:486-491, 1992.

4. Marrin M and Paes B : Does the Apgar score have diagnostic value. Obstet Gynecol, 72:120-123, 1988.

5. Reid DL, Parer JT, Williams K, Darr D, Phernetton TM and Rankin JH : Effects of severe reduction in maternal placental blood flow on blood flow distribution in sheep fetus. J Dev Physiol, 15:183-188, 1991.

6. Shankaran S, Peters K and Finer N : Estimated cerebral blood flow in term infants with hypoxic ischemic encephalopathy. Rediatr Res, 92:365-372, 1981.

7. Giacoia GP : Asphyxial brain damage in the newborn. South Med J, 66:676-682, 1993.

8. Myers RE : Two patterns of prenatal brain damage and their conditions of occurrence. Am J Obstet Gynecol, 112:246-276, 1972.

9. Pasternak JF : Hypoxic-ischemic brain damage in the term infant. Pediatr Clin North Am, 40:1061-1072, 1993.

10. Tweed WA, Cote J and Wade JG : Preservation of fetal brain blood flow relative to other organs during hypovolemic hypotension. Pediatr Res, 16:137-141, 1982.

11. Cortey A, Monin P, Hascoet JM, Hamon I and Vert P : Effects of Phenobarbital on cerebral blood flow during hypoxia. Biol Neonate, 65:396-405, 1994.

12. Ashwal S, Majcher JS and Longo LD : Patterns of fetal lamb regional cerebral blood flow during and after prolonged

hypoxia: Studies during the post-hypoxic recovery period. Am J Obstet Gynecol, 139:365-372, 1981.

13. Kennedy C, Grave GD, Jehle JW and Sokoloff L : Changes in blood flow in the component structures of the dog brain during postnatal maturation. J Neurochem, 19:2423-2433, 1972.

14. Ashwal S, Dale PS and Longo LD : Regional cerebral blood flow: Studies in the fetal lamb during hypoxia, hypercapnia, acidosis and hypotension. Pediatr Res, 18:1309-1316, 1984.

15. Rosenberg AA : Response of the cerebral circulation to hypocarbia in post-asphyxia newborn lambs. Pediatr Res, 32:537-541, 1992.

16. Reuter JH and Disney TA : Regional cerebral blood flow and cerebral metabolic rate of oxygen during hyperventilation in the newborn dog. Pediatr Res, 20:1102-1106, 1986.

17. Andersen K, Jensen KA and Ebbesen F : The effect of Phenobarbital on cerebral blood flow in newborn infants with fetal distress. Eur J Pediatr, 153:584-587, 1994.

18. Ferrari F, Kelsall AWR, Rennie JM and Evans DH : The relationship between cerebral blood flow velocity fluctuations and sleep state in normal newborns. Pediatr Res, 35:50-54, 1994.

19. Verbruggen L : Nuclear Medicine in neurological and psychiatric disorders. In Nuclear Medicine in Clinical Diagnosis and Treatment. Ed by IPC Murray, DJ EII, Livingston, London, 1:445-629, 1994.

20. Johnston MV : Cellular alterations associated with prenatal asphyxia. Clin Invest Med, 16:122-132, 1993.

21. Blennow M, Hagberg H, Ingvar M, Zeman J, Wang YS and Lagercrantz H : Neurochemical and biophysical assessment of neonatal hypoxic ischemic encephalopathy. Semin Prenatal, 18:30-35, 1994.

22. Oriot D, Betremieux P, Baumann N and Le Marec B : CSF ascorbic acid and lactate levels after neonatal asphyxia: preliminary results. Acta Pediatr, 81:845-846, 1992.

23. Lorek A, Takei Y, Cady EB, Wyatt EB, Penrice J, Edwards AD, Peebles D, Wylezinska M, Reece HO, Kirkbiride V, Cooper CE, Aldrige RF, Roth SC, Brown G, Delpy DT and Reynolds OR : Delayed ('secondary') cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: Continuous 48 hour studies by phosphorus magnetic resonance spectroscopy. Pediatr Res, 36:699-706, 1994.

24. Van Bel F, Dorrepal CA, Benders MJNL, Zeeuwe PEM, Van de Bor M and Berger HM : Changes in cerebral hemodynamics and oxygenation in the first 24 hours after birth asphyxia. Pediatrics, 92:365-372, 1993.

25. Cunn AJ, Mydlar T, Bennet L, et al : The neuroprotective actions of a calcium channel antagonist, flunarizine in the infant rat. Pediatr Res, 25:573-576, 1989.

26. Thiringer K, Hrber A, Karlsson K, et al : Post-asphyxial cerebral survival in newborn sheep after treatment with oxygen free radical scavengers and a calcium antagonist. Pediatr Res, 22:62-66, 1987.

27. Goncalves T, Carvalho AP and Oliveira Cr : Antioxidant effect of calcium antagonists on microsomal membranes isolated from different brain areas. Eur J Pharmacol, 204:315-322, 1991.

28. Rothman SM and Olney JM : Glutamate and the pathophysiology of hypoxic ischemic brain damage. Ann Neurol, 19:105-111, 1986.

29. Warkins JC and Olverman JH : Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci, 10:265-272, 1987.

30. Hill A : Current concepts of hypoxic-ischemic cerebral injury in the term newborn. Pediatr Neurol, 7:317-325, 1991.

31. Meldrum B : Possible therapeutic applications of antagonists of excitatory amino acid neurotransmitters. Clin Sci, 68:113-122, 1985.

32. Gill R, Foster AC and Woodruff GN : MK 801 is neuroprotective in gerbils when administered during the post ischemic period. Neuroscience, 25:847-855, 1988.

33. Ascher P and Nowark L : Electrophysiological studies of NMDA receptors. Trends Neurosci, 10:284-288, 1987.

Correspondence:
Ugur Dilmen
Çiftlik Cad. No: 57
06510 Emek,
Ankara, TÜRKIYE.