Maternal Diabetes Induced Hydrocephaly in Newborn Rats

M. Tehrani-pour, J. Khayyat-zade and Z. Ghorbani
Department of Biology, Faculty of Science, Islamic Azad University, Mashhad Branch, Mashhad, Iran

Abstract: In the present study, we examined the effects of maternal hyperglycemia on the volume of brain and lateral ventricle in newborn Wistar rats. At 7th day of pregnancy hyperglycemia was induced by a single injection (i.p.) of streptozotocin (55 mg kg\(^{-1}\)). Control animals were given an equal volume of citrate buffer. After parturition 1 pups were randomly selected from each litter, their brain dissected, fixed in 10% formalin, sectioned in 7 μm thickness and stained by H.E. By applying stereological techniques and systematic random sampling scheme the volume of the brain and lateral ventricles were estimated. In comparison with controls, statistical analysis showed significant increases (p<0.05) in the volume of the brain and lateral ventricles. In conclusion it seems that, maternal diabetes effect on blood brain barrier permeability in newborn rats that could cause large amount of CSF generation. These effects could lead to brain disorders such as hydrocephalus.

Key words: Maternal diabetes, hydrocephaly, lateral ventricle, CSF

INTRODUCTION

Maternal diabetes is associated with an increased risk of several complications in the offspring, such as growth disturbances and congenital malformations (Aberg et al., 2001). Diabetes Mellitus is a chronic progressive disease that often results in vascular complications, including the development of microangiopathy, which is characterized by basement membrane thickening (Hill and Williams, 2004) cytoskeleton rearrangement and increased paracellular leakage (Idris et al., 2004). Extensive research has been conducted on endothelial cell dysfunction in a number of tissues, including kidney, peripheral nerve, retina, heart and skeletal muscle (Jason et al., 2006). Also, diabetes increases blood brain barrier permeability (Malcolm, 2000) and is a risk factor for normal pressure hydrocephalus. Normal pressure hydrocephalus occurs when the volume of CSF increases, but it is pressure remains normal or just slightly elevated (Flanagan, 2002). Hydrocephalus is a net accumulation of CSF. Hydrocephalus results from an alteration of a normal physiological process and has multiple causes.

With the rare exception of CSF overproduction from a choroids plexus papilloma, all types of hydrocephalus are either communicating or non-communicating (Perez-Figares et al., 2001). In non-communicating hydrocephalus, impairment of CSF flow is within the ventricular system whereas in communicating hydrocephalus, the impairment is distal to the ventricles, mostly in the subarachnoid space (Vio et al., 2000). Non-communicating hydrocephalus results from lesions such as aqueductal occlusion, obstruction of the outlets of the fourth ventricles, tumors adjacent to the ventricular wall, hemorrhage and infection within the ventricular system (Perez-Figares et al., 2001). Most of infant from diabetic mother suffer from hydrocephaly and problems related to that. This study investigated the reasons induced hydrocephaly in newborn rats from diabetic mothers.

MATERIALS AND METHODS

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2008). In this study, Wistar rats (300-350 g) prepared from Razi Institute. Diabetes was induced in female rats at the 7 days of gestation (dg) via an intraperitoneal injection of (55 mg kg\(^{-1}\)) STZ (streptozotocin) in sterile phosphate buffered saline (Tehrani-pour et al., 2007). Control animals were injected intraperitoneally with phosphate buffered saline. The animals were hosed under standard (12 h) light-dark conditions and in a temperature controlled room (20°). The food and water was available to the animals ad libitum. Induction of diabetes was assessed by blood glucose levels. Animals have had above (300 mg dL\(^{-1}\)) glucose levels were confirmed as diabetic animals.

CSF extraction: CSF was extracted from 1 day old rat newborns. Only one neonate was selected from each

Corresponding Author: Maryam Tehrani-pour, Department of Biology, Faculty of Science, Islamic Azad University, Mashhad Branch, Mashhad, Iran Tel: +98511835050 Fax: +985118424020
diabetic mother randomly. Selected neonates were without obvious anomaly. Then they were anesthetized and CSF was collected into a glass micropipette from cisterna magna. Samples which were pink colored and contaminated by blood discarded. Clear samples were centrifuged and were kept in -70°C. Then total protein concentrations in neonates were determined in both control and experimental groups. All processes in CSF extraction were performed at freeze temperature, due to preventing CSF evaporation.

**Sampling:** After CSF extraction, of each diabetic and normal mother’s one neonate was selected. All infants were anesthetized and decapitated. The brain were rapidly removed and fixed in 10% Para formaldehyde. NaCl was added to the fixative to make the tissue float in order to ever come deformities during the fixation period, paraffin embedded tissue blocks were sectioned at 7 μm thickness coronally and stained with haematoxylin-eosin. In addition, tissue blocks containing samples (brains) were serially cut throughout. Form several hundred sections per block; of each 20 section 3 serial sections were obtained. For example for the first series: 5th, 6th, 7th section and for the second series: 27th, 28th, 29th section and so on. Therefore, we mounted every 3 section on a slide. At a practical level, Stereological methods are precise tools for obtaining quantitative information about three-dimensional structures based mainly on observations made on sections. The volume of the lateral ventricle were measured with cavaliers method (Gundersen et al., 1988). All experiments were performed a minimum of two times.

Student’s t test was used for comparison when only 2 groups were analyzed. Statistical significance was chosen as p<0.05. All results are reported as Mean±SEM.

**RESULTS**

Diabetes was assessed in this study by monitoring blood glucose levels of both PBS and STZ-injected rats. There was a significant increase (p<0.01) in blood glucose levels, from 80±15 mg dL⁻¹ in control to 200±18 mg dL⁻¹ in diabetic rats. After CSF extraction, levels of total protein were analyzed. Protein concentration in the CSF was as follows:

In neonates from diabetic mother: 159±7 mg dL⁻¹ and in neonates from normal mother: 233±7 mg dL⁻¹. Statistical analysis was shown a significant increase (p<0.01) (Fig. 1).

**Brain volume in neonate (1 day old rat):** In neonates from diabetic mother the brains become large (Fig. 2a, b). The mean of brain volume in newborn of diabetic mother was 5.68±0.5 in compare with control that was 2.96±0.5 (p<0.05).
important factors, including prolonged hyperglycemia, hypertension increases oxidant stress, dyslipidemia and insulin resistance (Barnes-Powell, 2007) have been shown to play a role in diabetes induced endothelial cell dysfunction. Few studies have investigated the effects of diabetes on the vasculature of the central nervous system (Ristow, 2004). However, recent clinical evidence suggests diabetes leads to increased incidences of vascular dementia, ventricular hypertrophy, lacunar infarcts and hemorrhage and may be a predisposing factor for Alzheimer's disease (Arvanitakis et al., 2004). Many studies measure BBB disruption by increased permeability of the microvasculature to albumin (Jason et al., 2006). We argue that by the time there is significant increase in total proteins in newborns CSF from diabetic mothers to camper with control (p<0.01). Recent studies demonstrated that small openings in the BBB can have a significant impact on BBB function and structure (Banks, 2006). Additionally, a recent study showed that STZ-induced diabetes in rats altered the molecular structure of BBB tight junctions by decreasing the expression of occluding, with no change in the accessory protein zonula occludens. Because of the progressive nature of diabetes and the unique phenotype of the BBB the effects of diabetes on the cerebrovascularity are different from other microvascular beds and barider systems, such as seen at the retina and peripheral nerves. We hypothesize that diabetes has a long term progressive effect on BBB endothelial cells resulting at first in small transient breaches that over time, grow larger and more pronounced (Jason et al., 2006). The main findings of this study were that STZ-induced diabetes produced a progressive increase in BBB permeability. Further studies will be needed to evaluate the role of BBB endothelial cell tight junction regulation and basement membrane alterations in increased microvascular permeability and focus on how alterations in neurovascular unit function in the identified brain regions related to the etiology of adverse CNS effects.

In other site, it seems likely that if the drainage systems at the base of the brain are inadequate by design or become obstructed from aging or injury, that it could eventually lead to hydrodynamic failure and chronic NPH (Flanagan, 2002). In this type of hydrocephalus, the brain would fill from the bottom up. The structure that would fill first would be the subarachnoid spaces and basilar cisterns. The location and size of the cisterns and subarachnoid space gives them a greater capacity to absorb excess CSF (Miyamoto et al., 2003). After the cisterns become overfilled, the ventricles start to fill up. When they become overfilled they begin to stretch eventually they become enlarged. Similar it was shown in (Fig. 3).
Many reports have dealt with the probable functions of the SCO-RF complex (Perez-Figares et al., 2001). One of the working hypotheses relates the SCO to the circulation of CSF. During the fetal life, the material secreted by the SCO into the ventricular CSF prevents the closure of the Sylvian aqueduct, thus allowing the CSF to circulate freely between the third and fourth ventricle. A maldevelopment of the SCO might lead to the aqueductal stenosis and a congenital hydrocephalus. Hyperglycemia alters physiological condition to pathological. We are still in the process of understanding the pathophysiological mechanisms underlying hydrocephalus in infants from diabetic mothers.

In total, it is concluded that maternal diabetes effect on blood brain barrier permeability in newborn rats that could cause large amount of CSF generation. These effects could lead to brain disorders such as hydrocephalus or hyperglycemia, stenosed sylvain aqueduct then lateral ventricles, the third and the fourth ventricles become large.

REFERENCES


