Effect of Dehydration in Late Pregnancy on Fetal Rat Growth and Fetal Brain RAS Receptor Expression

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ABSTRACT

To study the effect of maternal dehydration on fetal growth and fetal brain RAS development in late pregnancy. The dehydration model of the maternal mice during the late pregnancy was established. The experiment was divided into two groups: use blood-gas analyzer to detect the blood gas and electrolyte parameters of the mother and fetus, and use the osmometer to detect the plasma osmotic pressure; use LaserDoppler to detect the fetal brain surface blood flow; detect the levels of angiotensin II receptor protein and angiotensinogen in fetal brain. The dehydration of maternal mice during the late pregnancy can significantly reduce the dry and wet weights of fetus and fetal brain. The serum sodium, hematocrit (HCT) and plasma osmotic pressure of fetal rats increase, and there is a statistical difference compared with the control group; the fetal cerebral blood flow (CBF) of dehydration group is significantly lower than that of the control group. Dehydration in late pregnancy can significantly affect fetal rat growth and fetal brain RAS receptor expression.

Key Words: Late Pregnancy, Dehydration, Fetal Development, Fetal Brain RAS, Cerebral Blood Flow (CBF)

Introduction

Basic research on perinatal medicine has progressed rapidly in recent years and has gradually become a more independent theory and technology system. "Perinatology" has been known and approved by experts and scholars at home and abroad as a discipline term. Human fetal development accounts for approximately 80% of the entire gestation period (from fertilization to childbirth) and multiple factors (genetic or environmental factors, etc.) can affect fetal development. According to the latest statistics from the Ministry of Health, China has about 30 million babies born each year, and the birth defect rate is about 4-6% (i.e., nearly one million children are deficient each year) (Barker et al., 1986). This data refers only to birth defects that are visible malformations. The fetus may be abnormally stimulated in the mother's uterus. Some of them may not be teratogenic or lead to severe "visible" congenital defects, but may cause potential problems for the health of individuals after birth, and the resulted fetal origin problems are not included in the discussion. A large number of studies have reported that environmental factors lead to "blot" effects of fetal diseases (aka: DOHaD), such as changes in the nutritional status of the fetus can lead to cardiovascular, cerebrovascular and endocrine metabolic diseases in postnatal adults. This conclusion has been confirmed by a large number of epidemiological studies and related basic experimental studies; the "blot" effect caused by maternal smoking during pregnancy (active or passive smoking) and drug abuse (such as cocaine addiction) is also a hot topic in this field (Paul et al., 2006), it is confirmed that the fetus’s exposure to nicotine and cocaine during pregnancy can...
lead to a significant increase in the susceptibility of their offspring to hypertension, coronary heart disease, type 2 diabetes, cardiovascular and cerebrovascular diseases, endocrine and metabolic diseases, etc.

It is well-known that, during gestation period, both vomiting, diarrhea, fever and other pathological conditions, and heat, sweating, eating habits and other non-pathological conditions, can cause maternal dehydration and lead to water and salt metabolism imbalance. Although the fetus has a placental barrier and amniotic fluid as a buffer, in the mother's body, long term water and salt metabolism disorder would inevitably affect the fetus through multiple ways, such as: the change of maternal blood volume and (or) ion levels can directly affect the fetal fluid environment and amniotic fluid ingredients, as well as its system functions such as endocrine metabolism, etc.; changes in hormones caused by water and electrolyte imbalances in the mother's body can also directly affect fetal endocrine homeostasis through the placental barrier, thereby altering fetal water salt metabolism and its deglutition, thus indirectly influencing the humoral and amniotic fluid environment of the fetus; changes in fetal humoral and hormone levels can further affect the balance of many systems (e.g., cardiovascular system, nervous system, blood oxygen metabolism, hematopoietic system, etc.) (Haustetter et al., 1998). In short, maternal dehydration can regulate multiple fetal systems through multiple pathways. If these conditions occur in the "window period" of intrauterine fetal development, the "blot" effect may occur. However, whether maternal dehydration during pregnancy affects the health of fetuses and adult offspring, and whether it has a "programmed" effect on adult offspring, in particular, studies on the effects of dehydration on brain renin-angiotensin system (RAS) have not been reported at home and abroad yet. Clinically, water and salt imbalances in pregnant women can occur during any period of pregnancy. However, a large number of studies have shown that organogenesis occurs in the early pregnancy, while further maturation of the organs occurs in the middle and late pregnancy (Huxley et al., 2000). Considering that in the late pregnancy, the fetus may be more sensitive to related stimuli. Therefore, this study focuses on maternal dehydration during late pregnancy and the resulting "blot" effect, it plans to use mother rates in late pregnancy for three-days dehydration (this dehydration method has been used in many research institutes) to study these issues in detail.

Methods
Many causes of pregnancy such as excessive activity, fever, dehydration. Dehydration affects the balance of water and electrolytes in body fluids. Bleeding, vomiting, and diarrhea can cause mixed dehydration to the body of the pregnant woman (i.e., dehydration inside and outside the cell), thereby affecting the balance of water and electrolyte in body fluids (Langley et al., 1994). The regulation of humoral balance involves three major factors: nervous system, endocrine system and behavior. In this experiment, the dehydration model of the maternal mice during the late pregnancy was established to investigate the fetal growth and fetal brain RAS development. The results of the study will provide experimental and theoretical basis for perinatal care and prenatal and postnatal care for pregnant women in perinatal period.

The quasi-SD clean grade rats were 280-300 g males and 220-250 g females. The male and female rats were mated every night at 6:00 pm at the proportion of 1:1, if the vaginal plug was observed in the bottom plate the next morning, it was considered pregnant, and marked as the first day of pregnancy, the pregnant rats were randomly divided into dehydration group and control group, 10 rats per group. The dehydration group was dehydrated for 72 hours in late pregnancy (day 18 to day 20). The control group received normal drinking water, and both groups received normal food. Both groups were given normal food and water at other times during pregnancy. On the 21st day of gestation, mother rats were anesthetized with 4% chloral hydrate. Open their abdominal cavity and uterus and take out the fetal rats, weigh the wet weight of the placenta, fetal rat and fetal brain. And then, the rats were anesthetized with 4% chloral hydrate. Open their abdominal cavity and uterus and take out the fetal rats, weigh the wet weight of the placenta, fetal rat and fetal brain. And then, the fetal rat, fetal brain, and placenta were placed in an oven, dry for 8h at 70°C and weigh their dry weights respectively; and measure the body length and tail length of the fetuses. On day 21 of pregnancy, the fetuses were removed and placed on a warm cotton pad. The PF5010 LDPM blood flow monitoring probe was placed on the surface of the measurement site to measure changes in blood flow on the skin of the arm and fetal brain surface. The probe was fixed for one or two minutes under stable conditions, PeriFlux5000 system automatically records the graphics, and its software converts the graphics into data. Take the
average value which represents the average blood flow value of the body part. After maternal abdominal cavity was exposed on the 21st day of gestation, the mother rat's blood was collected from the abdominal aorta. The blood of fetal rats is collected from the heart according to the method used by the laboratory, that is to open the fetal rat's chest with ophthalmic scissors, expose the heart, carefully insert the heparinized 1 ml syringe (with a 23G needle) into the fetal heart chamber to take blood from the cardiac apex. Normally, for each fetal rat, it can collect 0.1-0.15ml of blood, the blood of each litter is mixed and tested. RNA quantification: use water without RNA enzyme to dissolve the RNA precipitate to 40μl, take 10μl of the solution and dilute 100 times, measure OD260 and OD280 with 752 UV Spectrophotometer. The integrity of the RNA was checked by 1% formaldehyde denaturing gel electrophoresis, the purity of the RNA was determined by the ratio of OD260/D280, the amount of RNA was determined by the OD260 value and diluted to a final concentration of 1 μg/μl (Barker, 2002).

Results and discussion
After 3 days of maternal dehydration in the late pregnancy, the wet weight of the fetal rat brain and the fetal rat fetus can be significantly reduced. After drying, the weight of the fetus is significantly lower than that of the control group. In addition, the dehydration of the mother mouse during late pregnancy can significantly shorten the length of fetal rat body and tail, but the dry and wet weight of the placenta was not statistically different from the control group (Table 1, 2).

| Table 1. Effects of maternal dehydration on fetal morphological parameters in late pregnancy |
|------------------------|------------------------|------------------------|------------------------|------------------------|
| Groups                | Body weight | Pure weight | Tail length | Body length |
| Control group         | 3.923 ±0.14 | 0.635 ±0.06 | 1.437 ±0.018 | 4.181 ±0.113 |
| Dehydration group     | 2.847 ±0.14 | 0.412 ±0.02 | 1.246 ±0.015 | 3.805 ±0.062 |

Table 2. Effects of maternal dehydration on fetal morphological parameters in late pregnancy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Brain weight</th>
<th>Pure weight</th>
<th>Placental weight</th>
<th>Placental net weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.160 ±0.06</td>
<td>0.0203 ±0.001</td>
<td>0.473 ±0.03</td>
<td>0.082 ±0.005</td>
</tr>
<tr>
<td>Dehydration group</td>
<td>0.143 ±0.04</td>
<td>0.0178 ±0.001</td>
<td>0.442 ±0.03</td>
<td>0.081 ±0.005</td>
</tr>
</tbody>
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In late pregnancy, maternal dehydration significantly increased Na+ concentration, Hct and Osm levels in the blood of the mother and fetus, which was significantly different from that of the control group, but for the blood of the mother and the fetus, pH, pO2, pCO2, S02%, Hb, Glu, Lac and other indicators were not statistically significant compared with the control group (Table 3).

3-day dehydration of the maternal mice during the late pregnancy can significantly increase AT1R, AT2R mRNA levels in the fetal rat
forebrain (P < 0.01), but AT\textsubscript{B}R mRNA levels were not statistically different from the control group (Figure 3, 4).

**Figure 3.** AT\textsubscript{1}R, AT\textsubscript{2}R mRNA levels of control group and Dehydration group

**Figure 4.** Hypothalamic nucleus illustration

**Conclusions and prospects**

The results of this study showed that although maternal dehydration during the late pregnancy decreased fetal body weight and brain weight at day 21 of pregnancy, there was no significant difference in individual body weight and brain weight in adults compared to the control group. This indicates that the adult offspring of the dehydrated mothers during the late pregnancy had a catch-up growth in body weight and brain weight, the microscopic changes still existed from the fetal period to the adulthood. In addition, the expression level of angiotensinogen in the brain of the offspring rats in the basal state was significantly higher than that in the control group. This indicated that the dehydration of mother rats during the late pregnancy increased the expression of angiotensinogen in the adult offspring, which may affect body fluid balance and neuroendocrine function (Fitzsimons, 1998).

Dehydration of the maternal mice during the late pregnancy can significantly reduce the weight and brain weight of the fetus, and significantly increase serum sodium, hematocrit, plasma osmotic pressure, cerebral angiotensinogen mRNA levels, significantly increase the expression level of AT\textsubscript{1}R and AT\textsubscript{2}R in the forebrain and hindbrain of the fetal rats, and significantly reduce the blood flow to the fetal brain surface. The results showed that the dehydration of the maternal mice during the late pregnancy can cause the imbalance of the humoral balance in the intrauterine fetus, resulting in fetal growth retardation, affecting fetal brain RAS development and the regulation of fetal body fluid metabolism.

For dehydrated mother rat in the late pregnancy, its adult offspring rats (female, male) have no significant differences in body weight, brain weight, blood gas and electrolyte levels compared with the control group, but in the dehydrated group, the adult offspring rats have significant difference in the expression levels of AT\textsubscript{2}R, AT\textsubscript{2}R and AT\textsubscript{G} mRNA compared with the control group. The results showed that, the dehydration of mother rats during the late pregnancy had a long-term "blot" effect on the expression of RAS-related molecules in the adult offspring rats.

After intracerebroventricular injection of AngII, the water intake of male offspring of the dehydrated mother in late pregnancy increased significantly, accompanied by increased expression of c. Fos in the brain-associated nuclei (such as AV3V), and increased cellular activity (Godfrey et al., 2001). The results showed that the dehydration in late pregnancy enhanced the response of adult offspring to central Ang II, and may cause certain changes in thirst behavior of offspring.
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