Brain damage and congenital cataract due to autogenously fecal peritonitis in pregnant Wistar rats[1]

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ABSTRACT

PURPOSE: To investigate the morphological aspects of brain and eyes in newborn rats whose mother underwent autogenously fecal peritonitis.

METHODS: Four pregnant rats that underwent fecal peritonitis, with a 10% fecal suspension in dose of 4 ml per kilogram received two antimicrobial treatments: 1. intraperitoneal moxifloxacin and dexamethazone; and 2. Intravenous meropenem. After head inspection, the brain consistencies and the eyes belonging to all offspring were analyzed.

RESULTS: The brains of newborn from rats that received 4 ml/kg of 10% suspension of feces showed, significantly smaller and less than the firm consistency of those in the control group. Congenital cataract was observed in 9 (34.6%). No cataract was observed in the 20 newborn rats from the mothers that received the combination of moxifloxacin and dexamethasone. Cataract could be observed in three (13.6%) offspring from mothers that received meropenem.

CONCLUSIONS: Peritonitis can produce brain damage and congenital cataract in rats. The translation to humans is that intra abdominal infection in pregnant women may be associated with damage in brain and eye structures of their concepts. This can be averting using the adequate early therapeutically approach.

Key words: Peritonitis. Quinolones. Carbapenems. Cataract. Rats.

Introduction

The most important treatable cause of childhood remains untreated or poorly treated cataract, which is responsible for 5–20% of all cases, although corneal scarring can present similar or even higher prevalence in some countries[1-3].It can be diagnosing at birth or shortly thereafter, or even it develops during the first two years of life[4-6].

Among the causes of human congenital cataract are intrauterine infections (toxoplasmosis, rubella, cytomegalovirus, herpes, varicella and syphilis).Worldwide, it is estimated that more than 100 000 infants are born with congenital rubella syndrome (CRS) each year, and the burden in developing countries is very high. Congenital cataract is one of the corn stone of this syndrome[7,8]. However, the majority of cataracts in newborns are genetically caused[9-11].The mutations in structurally and functionally important genes such as EPHA2 in the lens may contribute to a significant proportion of the congenital cataract burden[12]. There have been other suggestions of a possible association between pregnancy events and congenital cataracts, including: very low birth weight; maternal substance abuse; urinary tract infection; and aspirin ingestion[12].

As regard to experimental model of this disease, it is well recognized that congenital cataract is present in over 45% of the offspring of diabetic rats[13]. Pregnant rats with hypercholesterolemia can also have pups with congenital cataract[14]. Additionally, excessive maternal caffeine exposure during pregnancy can be cataractogenic for neonatal crystalline lenses in these animals[15].

Several mouse models of congenital cataract have genetic basis as exemplified by the following: (i) the most frequent mutations in congenital cataracts affect genes coding for γ-crystallins (gene symbol: Cryg); (ii) some postnatal, progressive cataracts have been characterized by mutations in the ß-crystallin encoding genes (Cryb); (iii) mutations in genes coding for membrane proteins like MIP or connexins lead to congenital cataracts; (iv) mutations in genes coding for transcription factors such as FoxE3, Maf, Sox1,andSix5 cause cataracts; (v) mouse models suffering from hereditary age-related cataracts have not yet been characterized genetically[16]. It has also been described a mouse mutantAca47 that carries a missense mutation in the Lim2 gene (c.T151C; p.C51R) that leads to decreased sizes of eye axis and lens. Since homozygous mutants further develop congenital lens opacities, Aca47 might be used as a model for congenital cataract formation in humans[17].

Gram-negative urinary tract infections can complicate human pregnancies. However, the effects of maternal endotoxemia from these microorganisms have rarely been evaluated by animal experiments or by human investigations. The main affected area from this aggression is the central nervous system[18]. Neuroinflammation can sensitize the brain to injurious insults in the embryonic development and produces several anomalies in this system, however not specifically in the eye embryology this injury has been described[19]. On the other hand, autoimmune optic neuritis is a common early manifestation of multiple sclerosis, yet early therapeutic interventions for this disease often have high ocular toxicity associated with increased risks for glaucoma, cataract, or retinopathy. This phenomenon can be studied in rat model[20].

To the best of our knowledge there is no experimental rat model associating bacterial peritonitis and congenital cataract. Thus, the purpose of this study is to report the occurrence of this congenital anomaly in newborn rats from mothers that underwent autogenously fecal peritonitis early in pregnancy; and also to show two antimicrobial treatments for sepsis and their effects in the prevention of this condition.

Methods

This was a prospective and interventional study and all experiments were conducted in accordance with the national laws for the use of animals in research and approved by the local Ethical Committee at Medical School from Campina Grande – Paraiba, Brazil.

The study design was threefold: 1st: To develop a model of autogenously fecal peritonitis in pregnant rats in a way to produce significant brain damage in the offspring. With this aim it was injected into the abdominal cavity 3ml/kg and 4ml/kg of autogenously filtered 10% fecal suspensions in two groups of 10 animals each at the 9th day of proved pregnancy. After pup birth the female adult rats underwent abdominal and thorax inventory for assessing the findings associate with the peritonitis. The weights of the offspring and their brain were recorded. The morphological aspects of the newborn animals were also described, with special attention to the head structures and the central neural system. Ten animals without any intervention were used as a control group.

2nd: After concluding that the most damaging model for the central nervous system was the injection into the peritoneal cavity of 4ml/kg of filtered 10% fecal suspension at the 9th day of proved pregnancy, two rats with autogenously peritonitis were treated with the combination of moxifloxacin and dexamethasone at the single intra-peritoneal doses of 40mg/kg and 0.2mg/kg, respectively. Both drugs were given at 48 h and 72 h after peritonitis induction.

3rd: Using the same model two pregnant rats as the above mentioned protocol received a single doses of meropenen 60 mg/kg/d i.v at 48 h and 72 h after peritonitis induction.

Since after birth the offspring were separated from their mothers and inspected for any congenital malformation and weighed in a precise scale. They were euthanized by halothane and decapitated. After close morphological inspection, the brains were carefully removed and equally weighed.

The quantitative parameters were expressed by their mean and standard deviation. As the parameters did not pass the normality test, the following statistical analyses were used: the Mann-Witney test for unpaired mean differences, the Kruskal-Wallis test and the post-test of Dunn for multi comparisons among means. $p \leq 0.05$ was used for rejecting the null hypothesis.

Results

After delivery the two groups of rats were euthanized and the abdomen and thorax were widely open for inspection, and pictures were taken for recording the lesions. The multiple infected lesions, inside both cavities, were more frequent and evident in their aggressiveness appearance into the pregnant rats that received 4ml/kg of autogenously filtered 10% fecal suspensions when compared with those that received 3ml/kg (Figures 1-4).

FIGURE 1 -Residual retroperitoneal abscess in the left lateral abdominal wall close by the left kidney could be observed. The pregnant rat had received 4ml/kg of autogenously filtered 10% fecal suspension.

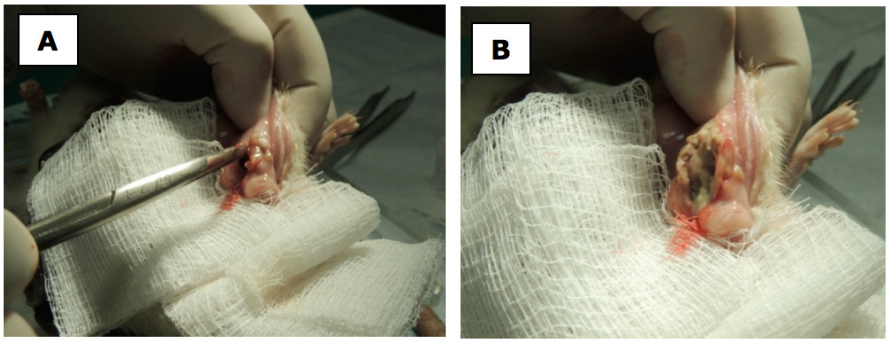

FIGURE 2 – **A.** Residual abscess opened showing a yellowish mass with no odor. Microbiological study revealed no bacteria. **B.** Closer view of the same abscess.

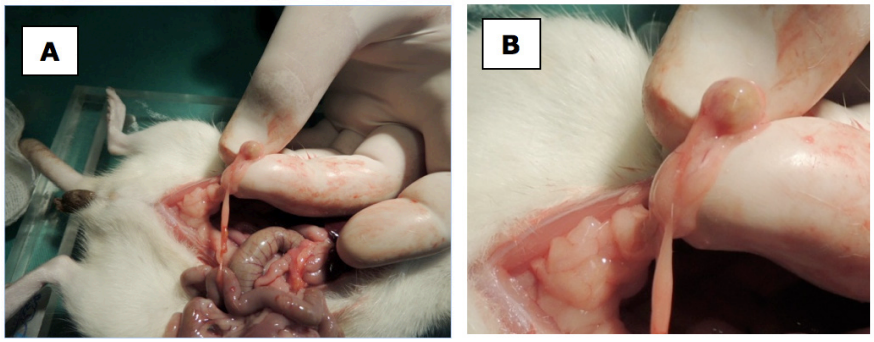

FIGURE 3 – A. Residual abscess close by the uterus could be observed. The pregnant rat had received 4ml/kg of autogenously filtered 10% fecal suspension. B. Closer view of the same abscess.

FIGURE 4 - Residual abscesses or scars within the left lung. The pregnant rat had received 4ml/kg of autogenously filtered 10% fecal suspension.

The mean weight of newborn rats from the animals that received 4 ml/kg of 10% autogenously fecal suspension was significantly smaller than those from animals that received 3 ml/ kg of 10% autogenously fecal suspension: {(4 ml/ Kg) - X_2 5.56 g ± 0.5g versus X_1 (3ml/ Kg): 5.80 g ± 0.97g – p = 0.0194}; however, the mean weight of the encephalic organs showed no significantly statistical difference: {(4 ml/ Kg) - X_2 1.18g ± 0,29g versus X_1 (3ml/ Kg) -1.19 g ± 0,03g – p = 0.3770}; but either means were significantly smaller than the mean weight of the control ones ($X_{control}$ 1.21g ± 0.08g – p=0.0004).

The most striking observations in the newborn rats from mothers that received 4ml/kg (10% fecal suspension) were in encephalic and eye organs. The brains from these offspring were jelly like organs that could not maintain intact the morphology (shape) and in 9 (34.6%) from 26 puppies congenital cataracts could be observed; seven had bilateral and two unilateral (Figures 5-14).

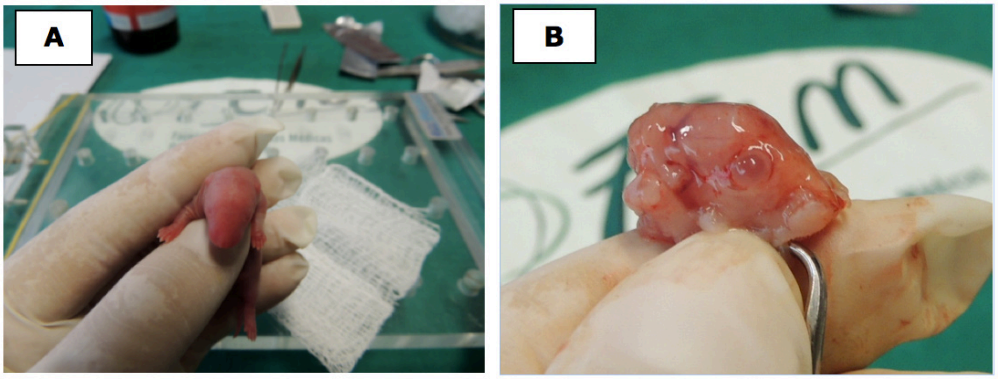

FIGURE 5 – A. Normal offspring from control group. Thin membrane (blepharophimosis) over the normal eyes can be observed. **B.** After removal of the blepharofimosis and the skin of the skull, the lenses are transparent

FIGURE 6 - Skin removed from skull of an offspring from a mother that eceived 4ml/kg of fecal suspension. Newborn with bilateral congenital cataract.

FIGURE 7 - Offspring from a mother that received 4ml/kg of fecal suspension with congenital cataract. Close view of the eyes with lenses opacification.

FIGURE 8 - Offspring from a mother that received 4ml/kg of fecal suspension with congenital cataract. Both eyes with lens opacification.

FIGURE 9 - Offspring from a mother that received 4ml/kg of fecal suspension with congenital cataract. Both eyes with lens opacification. Closer view.

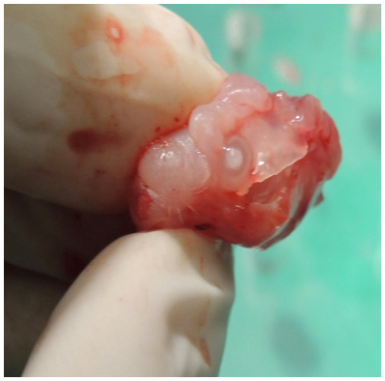

FIGURE 10 - Offspring from a mother that received 4ml/kg of fecal suspension with congenital cataract. Close view of the left eye with lens opacification.

The normal offspring rat encephalic area measured at an average of 1.2 cm for the largest extension (from head to tail direction). The normal consistency was parenchyma one (Figures 11 and 12).

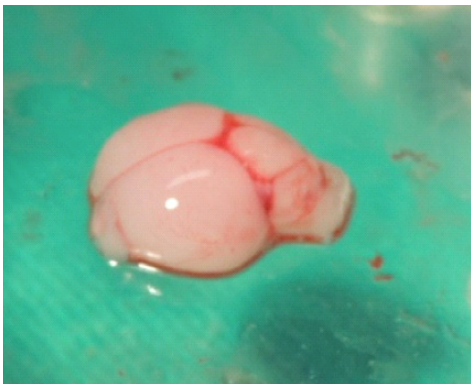

FIGURE 11 - Offspring from a normal control mother. Normal brain.

FIGURE 12 - Offspring from a normal control mother. Normal brain.

The brains from the offspring of rats that received 4/ml/kg of 10% autogenously fecal suspension were jelly like consistency (brain melting) and could not be maintained in their normal position or appearance (Figures 13 and 14).

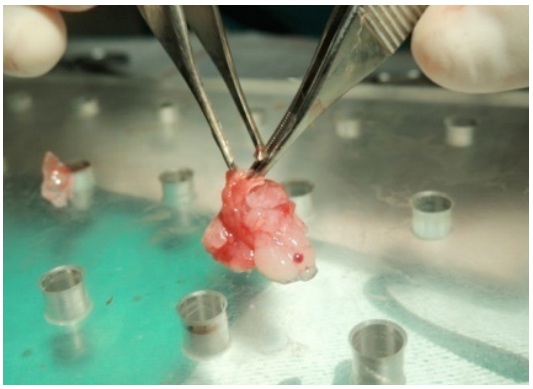

FIGURE 13 - Like brain from an offspring from a rat that received 4 ml/kg of 10% fecal suspension inside the abdomen. View during careful dissection.

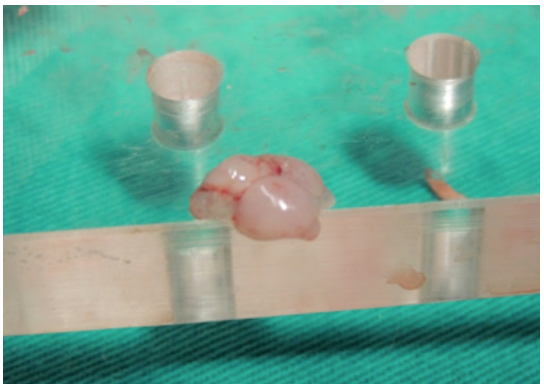

FIGURE 14 - Jelly like brain from an offspring from a rat that received 4 ml/kg of 10% fecal suspension inside the abdomen. Isolated brain, after dissection.

The brains from the newborn rats, which mothers had received the combination of moxifloxacin and dexamethasone at the single intraperitoneally doses of 40 mg/kg and 0.2 mg/kg, respectively were morphologically normal, the brains were also of gross normal morphology and consistency, and no single cataract was observed in all 20 offspring.

The brain morphology of the newborn rats, which mother have received intravenous meropenem were close to normal as regard to the consistency and shape; however in three (13.6%) out of 22 of these offspring, opacification of the normally transparent crystalline lens (cataract) could be observed; two were bilateral and one unilateral.

Discussion

There is no doubt that the enforcement of the VISION 2020 principles may eliminate congenital cataract as the main cause of childhood blindness[1], however the lack of resources in the developing countries may impair the fundamental human right of child, which is to have the right to sight. Taking into account that rubella is one of viral infection that frequently can

produce cataract in newborns in which the pregnant mothers had this infection early in the organogenesis phase, and less-than-optimal postoperative visual outcome for surgical treatment of this condition frequently occurs, one can suggests the need to look at primary prevention of rubella, especially in developing countries. Thus, rubella prevention is an urgent task in order to improve maternal and child health avoiding the rubella syndrome that includes congenital cataract. Thus, avert this disease based on genetics alteration[7,8]. To achieve this important goal is necessary that a high coverage immunization for this viral infection should be implemented before childbearing age.

As regard to offspring from experimental diabetic rats, the treatment with *Morus alba* extract can be ameliorated effect of mulberry leaves on retinal neurotransmitters, retinal neuronal cells and anti-cataract activity, attributed to its flavonoids content, which shows potential anti-oxidative activity and has potential hypoglycaemic and anti-hypercholesterolemic effects[14].

The literature regarding bacterial infection in pregnant human beings as a source of brain damage is scarce and no reference was found linking peritonitis and congenital cataract.

Maternal endotoxemia, in rat late gestation, seems not to induce fetal rat endotoxemia from maternal endotoxemia, and consequently could not affect the central nervous system. Although neuronal necrosis can be identified in offspring from rats at appropriate gestational ages with *E. coli* endotoxin[18], the effect of the same insult early in the organogenesis phase has not yet been studied. Thus, the present investigation is original and provides a newborn rat model for brain damage and congenital cataract from severe infected mothers.

It is relevant to stress the difference of the amount of enterobacteria and consequently the endotexemia injected into the abdominal cavity of pregnant rats regarding the effect on newborn brain and eye. Using 3ml/kg of 10% filtered fecal suspension no important features were observed inside the abdomen of rats after delivery and almost no gross damage was observed in the brain and eyes of the offspring. Thus, it was chosen to produce severe abdominal infection in pregnant rats with the dosage of 4ml/kg of 10% filtered fecal suspension for obtaining a reproducible model for severe brain damage and congenital cataract in the newborns.

As comparing the two therapeutically interventions it was observed that the use of moxifloxacin and dexamethasone at the single intra-peritoneal doses of 40mg/kg and 0.2mg/kg, respectively was more effective in protecting the brain and eye damage of the offspring from rats with severe peritonitis. Since the approach of using antibiotics via intra peritoneal cavity is not frequently done for peritonitis, in general, in pregnant woman with severe abdominal infection the use of moxifloxacin and dexamethasone could provide a new alternative treatment, since in rats with severe intra-abdominal infection it proved to be very effective[21].

Conclusions

The newborns of rats whose mothers underwent autogenously fecal peritonitis, with a dose of 4 ml/kg showed: brains consistency more liquefied than the control group; and the development of congenital cataract. These findings allow translation to humans, in the sense that intra abdominal infection in pregnant women may be associated with changes in brain and eye structures of their concepts. This can be averting using the adequate early therapeutically approach.

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