Progress in Neuro-Psychopharmacology & Biological Psychiatry 71 (2016) 76–82

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Maternal gut and fetal brain connection: Increased anxiety and reduced social interactions in Wistar rat offspring following peri-conceptional antibiotic exposure

Stéphanie Degroote [a], Darel J. Hunting [a], Andrea A. Baccarelli [b], Larissa Takser [a,*]

[a] Faculté de Médecine et Sciences de la Santé, Université de Sherbrooke, 3001 12e Avenue Nord, Sherbrooke, QC J1H 5H3, Canada
[b] Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115, United States

ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form 19 June 2016
Accepted 19 June 2016
Available online 21 June 2016

Keywords:
Antibiotic
Behavior
Rat
Microbiota
SuccinylSulfaThiazole

ABSTRACT

Background: A growing body of evidence indicates that gut microbiota characteristics may be closely related to mental dysfunctions. However, no studies have investigated fetal brain development in relation to the maternal gut microbiota, despite the extensive use of antibiotics in obstetric practice.
Objective: To determine how periconceptional exposure to SuccinylSulfaThiazole (SST), a non-absorbable antibiotic, can affect behavior in rat offspring. This antibiotic drug has previously been shown to substantially perturb the gut microbiota in rats following a 28-day exposure.
Methods: Female Wistar rats were divided in two groups: control, or exposed during one month before breeding until gestational day 15 to a diet containing 1% SST. We administered behavioral tests to offspring, i.e., open field (post-natal day 20), social interactions (P25), marble burying (P30), elevated plus maze (P35), and prepulse inhibition of the acoustic startle reflex (sensory gating) (P45).
Results: Both male and female offspring exposed peri-conceptionally to SST showed reduced social interactions, with a decrease of about half in time spent in social interactions compared to controls, reduced exploration of the open arm by 20% in the elevated plus maze test indicating increased anxiety and altered sensorimotor gating, with a 1.5–2-fold decrease in startle inhibition.
Conclusion: Maternal periconceptional exposure to SST provokes alterations in offspring behavior in the absence of maternal infection. Because we administered SST, a non-absorbable antibiotic, only to the dam, we conclude that these neurobehavioral alterations in the offspring are related to maternal gut microbiota alterations.

1. Introduction

A growing body of evidence indicates that the gut microbiota affects mental functions. Several studies in germ-free rats have shown that the absence of gut microbiota leads to stress and anxiety (Crumeyrolle-Arias et al., 2014; O'Mahony et al., 2014). However, experimental studies on the influence of the maternal gut microbiota on fetal brain development are almost non-existent. One experimental study in mice showed that exposure during late pregnancy to a mixture of non-absorbable antibiotics in the drinking water, altered maternal microbiota composition, and provoked decreased locomotor activity in offspring (Tochitani et al., 2016). Moreover, alterations in vaginal microbiota by maternal stress, in a mouse model, have been linked to metabolic reprogramming of the gut and brain in the offspring (Jašarević et al., 2015). The question of whether maternal gut microbiota affect brain development is crucial, because >40% of pregnant women are administered antibiotics (Broe et al., 2014), often to treat urinary tract infections or for prophylaxis for preterm membrane rupture. Exposure to antibiotics has been associated with an increased risk of cerebral palsy in preterm babies (Kenyon et al., 2008). A1.2 to 2-fold increased risk of autism was reported in a Danish cohort following the use of antibiotics during pregnancy, including sulfonamides (Atladóttir et al., 2012). The use of the sulfa antibiotic trimethoprim, which inhibits a key step in the folate pathway, during the 12 weeks before conception was associated with increased risk of congenital malformations including neural tube defects (Andersen et al., 2013). However, epidemiological studies cannot distinguish the effects of the infections from those of antibiotics used to treat them. Therefore, experimental models are required to test the hypothesis that the maternal microbiome affects fetal brain development and to separate the effect on the developing fetus of gut microbiota alterations caused by an antibiotic drug from

Abbreviations: SST, SuccinylSulfaThiazole; Gx, Gestational day x; Py, Post-natal day y; PPI, Prepulse inhibition.

* Corresponding author at: Département de Pédiatrie, Faculté de Médecine, Université de Sherbrooke, 3001, 12e Avenue Nord, Sherbrooke, Québec J1H 5N4, Canada.
E-mail addresses: stephanie.degroote@usherbrooke.ca (S. Degroote), darel.hunting@usherbrooke.ca (D.J. Hunting), abaccare@hsph.harvard.edu (A.A. Baccarelli), larissa.takser@usherbrooke.ca (L. Takser).

http://dx.doi.org/10.1016/j.pnpbp.2016.06.010
0278-5846/

the effects due to the infection itself. In the present study, we used SuccinylSulfaThiazole (SST), a long-acting non-absorbable sulfonamide antibiotic drug. SST is used in clinical practice mainly to treat intestinal tract infections, since it remains in the gut much longer than absorbable antibiotics and has no systemic toxicity (Patrick, 1995). Once SST, a prodrug, reaches the slightly alkaline large intestine, it is slowly hydrolyzed by bacterial esterases to sulfathiazole, the active form. <4% of SST is absorbed into the bloodstream and >95% is retained in the gut (Patrick, 1995; Welch et al., 1942).

The term "gut-brain axis" refers to the bidirectional relationship between the gut and the brain, as the microbiota activity can directly modify the availability of metabolites and/or precursors used for the synthesis of neurotransmitters (Holzer and Farzi, 2014). In humans, 95% of serotonin, a neuropeptide which influences mood and behavior, is produced in the gut from tryptophan (Camilleri, 2009), and tryptophan availability can be altered by either diet modification (Zhang et al., 2006), or altered microbiota composition as in germ-free mice (Wikoff et al., 2009). Serotonergic neurons are among the earliest neurons to be differentiated and modulate a number of developmental events in the developing brain. Alterations in serotonin homeostasis cause permanent changes to adult behavior and modify the wiring of brain connections (Gaspar et al., 2003).

The objective of this study was to determine if periconceptional perturbation of the maternal gut microbiome by exposure to SST can affect behavior in rat offspring. Given that SST is also used in animal studies in combination with folate deficient diets when modeling folate deficiency, we measured homocysteine in the blood from dams after SST treatment to control for folate status, which is an important determinant of (i) brain development via its role in DNA synthesis and methylation (Bailey, 2009) and (ii) tetrahydrobiopterin (BH4) synthesis, an essential cofactor in the biosynthesis of monoamine neurotransmitters such as serotonin (Miller, 2008). Under conditions of folate/one-carbon deficiency, high homocysteine levels indicate a deficit of one-carbon donors needed for the transformation of homocysteine to methionine.

Our hypothesis is that periconceptional exposure to SST, a non-absorbable antibiotic, will provoke neurobehavioral alterations in the offspring due to alterations in the gut microbiome in dams. Based on previous findings in germ-free rats (Crumeyrolle-Arias et al., 2014; O'Mahony et al., 2014) and antibiotic exposure in mice (Tochitani et al., 2016), we expect to observe an anxiogenic phenotype and decreased locomotor activity.

2. Methods

2.1. Animals and treatment

We obtained 12 female Wistar rats (250–290 g) and 8 male Wistar rats (310–340 g) from Charles River Laboratories (St. Constant, Québec, Canada). They were housed 6 per for females and 4 per for males in plastic cages with bedding and regulated temperature (21 ± 2 °C) and humidity (50 ± 10%), and a 12 h light/dark cycle (6 h–18 h). Food and water were provided *ad libitum*. All animals received care in compliance with the Guide to the Care and Use of Experimental Animals from the Canadian Council of Animal Care and the protocol was approved by our institutional animal research ethics committee. Male rats used for breeding were fed with Rodent Chow 5075; Charles River Laboratories. As soon as we received the females, we randomly assigned them to two groups of six dams each: control (Basal Diet 5755, from TestDiet ®), or exposed to the diet with 1% SuccinylSulfaThiazole (SST) (Basal Diet 5755 with SST 1% added, from TestDiet ®). This diet was administered for one month before breeding and was maintained until gestational day 15 (G15), when all dams were fed with standard Rodent Chow 5075, Charles River Laboratories.

After one month on a given diet, a blood sample was taken from the saphenous vein of the female rats which were then placed in a new cage with a male. Blood was immediately centrifuged at 5000 Rotation Per Minute (RPM) for 20 min and the supernatant was aliquoted and stored at −80 °C until analysis. We checked every morning for the presence of a vaginal plug, a sign of breeding during the night. When a plug was found, the female was placed in an individual cage and the day was considered as G1. Two females from the control group and one from the SST group were not pregnant.

Dams were allowed to raise all the litters, and dams and pups were kept in the same cage from birth until weaning at post-natal day 21 (P21). After weaning, males and females were separated. For all behavioral tests, we used 2 males (housed together after weaning) and 2 females (housed together after weaning) from each litter, which results in 16 pups for the control group and 20 pups for the SST-exposed group. Dams and all untested siblings were euthanized at P21. Adult offspring were euthanized after behavioral testing at P50.

2.2. Analysis of homocysteine and tryptophan levels

For homocysteine dosage, we used the Axis® Homocysteine Enzyme Immunoassay (EIA) kit for quantitative determination of total L-homocysteine in plasma from pre-conception blood in dams. Tryptophan dosages were performed by PhenoSwitch Bioscience Inc. (Sherbrooke, Canada) using liquid chromatography-mass spectrometry (LC-MS). Blood samples from dams were aliquots from pre-conception and euthanasia (P21), and for offspring they were aliquots from euthanasia of the untested siblings (P21). All samples were prepared as follows: for each plasma sample, 20 μl were mixed with acidified methanol, doped with internal standards, to precipitate the proteins. The samples were clarified by centrifugation at 13,000 RPM for 5 min. The supernatant was dried and resuspended in 20 μl of acidified water (0.2% formic acid). A standard curve ranging from 0.09 μM to 500 μM for tryptophan was prepared. Samples are analysed undiluted and 100-fold diluted. Acquisition was performed with a ABSciex TripleTOF 5600 (Sciex, Foster City, CA, USA) equipped with an electrospray interface with a 25 μm iD capillary and coupled to an Eksigent μUHPLC (Eksigent, Redwood City, CA, USA). Analyst TF 1.6 software was used to control the instrument and for data processing and acquisition. The source voltage was set to 5.5 kV and maintained at 400oC, curtain gas was set at 27 psi, gas one at 12 psi and gas two at 15 psi. Acquisition was performed in MRM mode. Separation was performed on a reversed phase Halo PFP column 0.5 mm i.d., 2.7 μm particles, 50 mm long (Advance Materials Technology, Wilmington, DE). Quantification was done using the area under the curve with the MuliQuant software (Sciex).

2.3. Behavioral testing

All behavioral tests were performed between 9 a.m. and 4 p.m. by the same person.

2.3.1. Open field ($n = 16$ controls and $n = 20$ SST exposed)

This test measures observable spontaneous motor activities (Bignami, 1996). At P20, the rat was placed in the apparatus, a box with a 40 cm^2 base with a 40 lx light intensity and a video camera placed above connected to a computer running ANY-Maze® software (Stoelting CO, USA). Each rat was placed in the same orientation and all trajectories, including time of mobility and distance travelled, were analysed during a 5 min session.

2.3.2. Social interactions ($n = 8$ pairs of controls and $n = 8$ pairs of SST exposed, 4 pairs of males and 4 pairs of females in both groups)

At P25, two rats from the same group, of the same sex, and approximately the same weight, but who had never met before, were placed in the same cage (26 cm × 17 cm × 14.5 cm) with a 40 lx light intensity. The day before the test, all animals had a 5 min session alone in the apparatus to become familiarized with the cage. The 10 min session of testing was recorded with a camera placed above the apparatus and