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Research article

Trans-resveratrol enriched maternal diet protects the immature hippocampus from perinatal asphyxia in rats

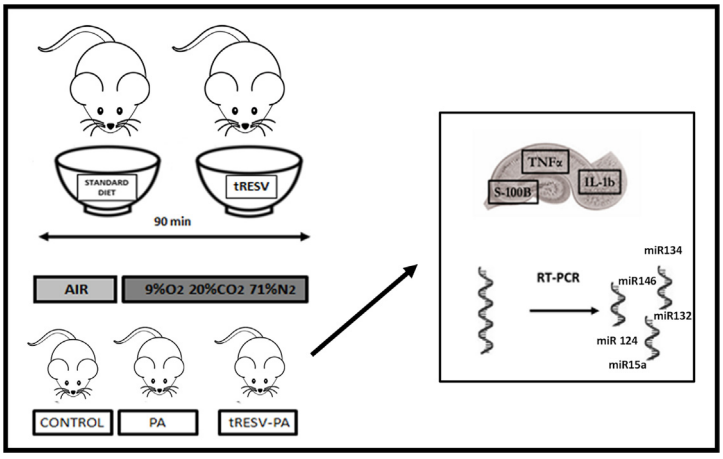
Sebastian Isac[a], Anca Maria Panaitescu[a,b], Ana Spataru[a], Mara Iesanu[a], Alexandra Totan[c], Amalia Udriste[d,e], Natalia Cucu[d], Gheorghe Peltecu[b], Leon Zagrean[a], Ana-Maria Zagrean[a,*]

[a] Division of Physiology and Neuroscience, Carol Davila University of Medicine and Pharmacy, 050474 Bucharest, Romania
[b] Filantropia Clinical Hospital, 011171 Bucharest, Romania
[c] Division of Biochemistry, Carol Davila University of Medicine and Pharmacy, 050474 Bucharest, Romania
[d] Association for Epigenetics and Metabolomics, Bucharest, Romania
[e] Research Center for Studies of Food Quality and Agricultural Products, Bucharest, Romania

HIGHLIGHTS

- Neuronal modulation after perinatal asphyxia has an epigenetic component.
- Maternal trans-resveratrol reduces asphyxia-related hippocampus vulnerability.
- Trans-resveratrol modifies at epigenetic level the hippocampal damage.

GRAPHICAL ABSTRACT



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ABSTRACT

Trans-resveratrol (tRESV), a polyphenol with antioxidant properties, is common in many food sources, hence easily accessible for study as a maternal dietary supplement in perinatal asphyxia (PA). Hypoxic-ischemic encephalopathy secondary to PA affects especially vulnerable brain areas such as hippocampus and is a leading cause of neonatal morbidity. The purpose of this study is to identify new epigenetic mechanisms of brain inflammation and injury related to PA and to explore the benefit of tRESV enriched maternal diet. The hippocampal interleukin 1 beta (IL-1b), tumour necrosis factor alpha (TNFα) and S-100B protein, at 24–48 h after 90 min of asphyxia were assessed in postnatal day 6 rats whose mothers received either standard or tRESV enriched diet. The expression of non-coding microRNAs miR124, miR132, miR134, miR146 and miR15a as epigenetic markers of hippocampus response to PA was determined 24 h post-asphyxia. Our results indicate that neural response to PA could be epigenetically

Abbreviations: IL-1b, interleukin 1 beta; miRNAs, microRNAs; PA, perinatal asphyxia; RT-PCR, reverse transcription PCR; S-100B, S-100 beta protein; TNFα, tumour necrosis factor alpha; tRESV, trans-resveratrol.

* Correspondent author at: Division of Physiology and Neuroscience, Carol Davila University of Medicine and Pharmacy, 8 Eroii Sanitari Blvd., 050474 Bucharest, Romania.
E-mail address: ana-maria.zagrean@umfcd.ro (A.-M. Zagrean).

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controlled and that tRESV reduces asphyxia-related neuroinflammation and neural injury. Moreover, tRESV could increase, through epigenetic mechanisms, the tolerance to asphyxia, with possible impact on the neuronal maturation. Our data support the neuroprotective quality of tRESV when used as a supplement in the maternal diet on the offspring's outcome in PA.

1. Introduction

The potential influence of maternal diets on neonatal neuroplasticity is becoming a topic of intense research [1]. The interest is driven by the prospect of maternal nutrition modulating the severity of perinatal pathologies like hypoxic-ischemic encephalopathy [2–4], secondary to perinatal asphyxia (PA).

One dietary compound that recently proved to be effective in nervous system disease models, trans-resveratrol (tRESV) is a thoroughly studied polyphenol with antioxidant properties. This compound is present in a variety of common dietary sources, such as grape skin, blueberries, peanuts and pistachios [5] and has reported positive effects in seizures [6], Parkinson's disease [7], or high-fat diet induced neuroinflammation [8]. Resveratrol was shown to reduce oxidative stress and improve metabolic profiles of low-protein diet female rats and their offspring [9]. tRESV maternal effects on offspring's immature hippocampus in PA is yet to be elucidated.

PA incidence ranges between 1/1000 and 5–10/1000 [10]. Using targeted intervention during the gestation period, the outcome of the offspring can be improved. Therefore, effective prevention and neuroprotective strategies could be developed through maternal diet.

The range of clinical conditions caused by PA is determined by two factors: the severity of the asphyxia episode and the impact it has on selective vulnerable areas of the immature brain structures, especially on the hippocampus [11]. Asphyxia-related neonatal encephalopathy causes variable brain damage, ranging from attention deficit hyperactivity disorder to further neurobehavioral impairments and cerebral palsy [12,13].

This study aimed to evaluate hippocampal epigenetic programming induced by PA and to investigate the role of maternal tRESV enriched diet in attenuating the hippocampal damage. Using ELISA, the hippocampal tumour necrosis factor alpha (TNFα), interleukin 1 beta (IL-1b) and S-100B protein (S-100B) from tissue homogenate were assessed as neuroinflammation and neural injury markers, respectively. Using reverse transcription PCR (RT-PCR), the epigenetic profile of the hippocampus post-asphyxia was investigated, by measuring the expression of some small non-coding RNA (miRNAs) species, miR124, miR132, miR134, miR15a and miR146, involved in the epigenetic control of neuroinflammation, tolerance to asphyxia, apoptosis, angiogenesis and neuronal maturation.

2. Materials and methods

2.1. Animals and diets

Experiments were carried out on Wistar rats, which had access to standard diet and water *ad* libitum. All animal procedures were carried out with the approval of the local ethics committee for animal research in accordance with the European Communities Council Directive 86/609/EEC on the protection of animals used for scientific purposes.

Female Wistar rats (n = 16) received starting with postnatal day 30 (after weaning) either standard diet (n = 10) or tRESV (USP, FCC Grade – NutriVita®, Irvine, California, US) supplemented diet (n = 6). tRESV was dissolved in 0.5% dimethyl sulfoxide and offered in the drinking water (50 mg/kg body weight/day). All the animals were kept under standard conditions in 12 h light–dark cycles. The rats were mated at maturity (postnatal day 90–100) and the diets were administered until their offspring attained postnatal day 7. At mating, the rats among the groups showed no significant differences in body weight. Also, there was no difference in the time interval from mating to giving birth.

2.2. Neonatal exposure to asphyxia

On postnatal day 6, a total of 24 pups were randomly selected from the two dietary groups which were investigated. Sixteen pups were selected from mothers who received a standard diet and eight pups from mothers that received a tRESV supplemented diet. Eight pups from each group were separated from their mothers and exposed to a 90-min asphyxia (9% O_2, 20% CO_2 and 71% N_2) using a birth asphyxia model described by Helmy [14] and modified by us [15]. The asphyxia gas mixture was provided at a constant flow of 2 l/min using an open non-rebreathing system. The remaining eight pups from the standard diet group were held for 90-min in atmospheric air, separated from their mothers. The temperature was maintained at 37 °C during exposure using a heating pad (FHC Inc., USA). All the pups were returned to their mothers immediately after exposure.

2.3. Experimental groups

The experimental groups were as follows: Control group (n = 8) (pups exposed to atmospheric air whose mothers received a standard diet), PA group (n = 8) (pups exposed to PA whose mothers received a standard diet) and tRESV-PA group (n = 8) (pups exposed to PA whose mothers received a tRESV supplemented diet). The experimental design is illustrated in Fig. 1.

2.4. Tissue collection

At 24 and 48 h post exposure, the pups from all experimental groups were sacrificed and the hippocampi were dissected and isolated from meningeal structures on ice and rinsed in ice-cold PBS (0.02 mol/L, pH = 7.0–7.2). The tissue was then minced, homogenized with a glass homogenizer, sonicated for 10 min and centrifuged for 5 min at 5000*g* and 4 °C. The supernatant was removed and stored at −20 °C until all samples were collected and analysed.

2.5. ELISA assessment of neuroinflammation and neural injury markers

Using ELISA technique performed on a Stat Fax-303 Plus Strip Reader (Awareness Technology Inc., USA), and following manufacturer's recommendations, we measured the levels of TNFα (EIAbScience Co. Ltd., China), IL-1b (RayBiotech, USA), and the total protein content (Biosystems, Spain) from hippocampal supernatant collected 24–48 h post exposure. S-100B (Cusabio Biotech Co. Ltd., China) was determined 24 h post exposure. All results for IL-1b,