Behavioural Brain Research 291 (2015) 289–298

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Broccoli sprout supplementation during pregnancy prevents brain injury in the newborn rat following placental insufficiency

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HIGHLIGHTS

- Placental insufficiency causes fetal growth restriction (FGR).
- FGR is associated with developmental disability and increased cerebral palsy (CP).
- Broccoli sprouts is a natural health product and a potent phase II enzyme inducer.
- Broccoli sprouts prevents FGR induced behavioral and pathologic alterations.

ARTICLE INFO

Article history:
Received 23 October 2014
Received in revised form 13 May 2015
Accepted 16 May 2015
Available online 23 May 2015

Keywords:
Intrauterine growth restriction
Cerebral palsy
Broccoli sprout
Developmental delay
Preventative therapy

ABSTRACT

Chronic placental insufficiency and subsequent intrauterine growth restriction (IUGR) increase the risk of hypoxic-ischemic encephalopathy in the newborn by 40 fold. The latter, in turn, increases the risk of cerebral palsy and developmental disabilities. This study seeks to determine the effectiveness of broccoli sprouts (BrSp), a rich source of the isothiocyanate sulforaphane, as a neuroprotectant in a rat model of chronic placental insufficiency and IUGR. Placental insufficiency and IUGR was induced by bilateral uterine artery ligation (BUAL) on day E20 of gestation. Dams were fed standard chow or chow supplemented with 200 mg of dried BrSp from E15 – postnatal day 14 (PD14). Controls received Sham surgery and the same dietary regime. Pups underwent neurologic reflex testing and open field testing, following which they were euthanized and their brains frozen for neuropathologic assessment. Compared to Sham, IUGR pups were delayed in attaining early reflexes and performed worse in the open field, both of which were significantly improved by maternal supplementation of BrSp ($p < 0.05$). Neuropathology revealed diminished white matter, ventricular dilation, astrogliosis and reduction in hippocampal neurons in IUGR animals compared to Sham, whereas broccoli sprout supplementation improved outcome in all histological assessments ($p < 0.05$). Maternal dietary supplementation with BrSp prevented the detrimental neurocognitive and neuropathologic effects of chronic intrauterine ischemia.

These findings suggest a novel approach for prevention of cerebral palsy and/or developmental disabilities associated with placental insufficiency.

1. Introduction

Neonatal hypoxic-ischemic encephalopathy is a leading cause of neurodevelopmental disabilities, inclusive of cerebral palsy and mental retardation [1]. Placental insufficiency resulting in growth restriction of the fetus is a significant predisposing ante-partum risk factor, shown to increase the probability of hypoxic-ischemic encephalopathy 40-fold [2].

Evidence indicates that up to 90% of cases of cerebral palsy and developmental disability occur prior to birth [2–4]. This, combined with the toxicity of conventional medications to the immature brain [5,6], highlights the need for neuroprotective strategies that are safe, efficacious and preventive in nature.

Broccoli sprouts are a rich source of sulforaphane, a potent isothiocyanate shown to be protective in models of oxidative stress and inflammation [7–9]. Our study tested the effectiveness of this natural product in a rodent model of placental insufficiency.

Abbreviations: BrSp, broccoli sprout; BUAL, bilateral uterine artery ligation; IUGR, intra-uterine growth restriction; GFAP, glial fibrillary acidic protein; MBP, myelin basic protein; DTC, dithiocarbamate; ITC, isothiocyanate.

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http://dx.doi.org/10.1016/j.bbr.2015.05.033
0166-4328/

We asked whether maternal supplementation with BrSp during gestation and the early newborn period reduces adverse neurodevelopmental effects associated with placental insufficiency, and whether improvement in neuropathologic outcome would coincide with functional improvement.

2. Materials and methods

2.1. Animals

Pregnant female Long–Evans rats (Charles River Laboratories) were used in this study ($n = 17$). Animals were housed in the Health Sciences Laboratory Animal facility at the University of Alberta. Following a 5-day acclimatization period, animals were timed-pregnant whereby a vaginal lavage containing sperm denoted day one of gestation (E1). Pregnant dams were then assigned to one of the following four groups: (1) *Sham-operated (Sham)*; (2) *Sham-operated with broccoli sprout supplement (Sham + B)*; (3) *Intra Uterine Growth Restriction (IUGR)*; (4) *IUGR with broccoli sprout supplement (IUGR + B).*

Rat pups were born spontaneously and reared with their dams. Litters were culled to 8 where more than eight pups were born. Only litters of at least 5 pups were included in the study. Three to six pups from each litter were used to represent each of the four experimental groups throughout the study ($n = 88$ pups). All animals were maintained on a 12 h light/dark schedule and received food and water ad libitum throughout the study. The Health Sciences Animal Care and Use Committee at the University of Alberta approved all procedures.

2.2. Surgical procedure

Bilateral uterine artery ligation (BUAL), adapted from Wigglesworth's original model of fetal growth restriction, was performed on E20, of a 23 day gestation, to induce chronic placental insufficiency [10]. This stage of brain development for the fetal rat is comparable to human brain development at approximately 22–26 weeks gestational age [11,12].

Pregnant rats were anesthetized with 4% isoflurane (approximately 2% maintenance) in 21% oxygen and received a vertical, low midline abdominal incision, 2–3 cm long. Both uterine arteries, proximal to the uterine bifurcation, were permanently ligated with 4–0 Vicryl coated suture (Ethicon Inc.,Somerville, NJ, USA). After suturing the muscular layer with Vicryl, 0.05 ml of bupivicaine (Sensorcaine by AstraZeneca Can Inc., Ontario, Canada) was administered in a drop wise fashion for analgesia and the skin layer was closed with 5–0 silk suture (Angiotech Surgical Specialties Corp., Reading, PA, USA).

Following surgery, the animals were monitored over a 4–6 h to ensure full recovery. Sham-operated rats underwent identical anesthetic and surgical procedures with the exception of BUAL.

2.3. Intrauterine growth restriction and cephalization index assessment

We used the IUGR definition previously described by Olivier et al., namely – birth weight ≤ 2 SD below the mean [13]. Birth weights were collected from four naïve litters ($n = 56$) to ascertain the mean birth weight for our breeding colony and define the criteria for IUGR. The mean birth weight of the naïve animals was 6.28 ± 0.38 g, therefore animals ≤ 5.52 g, at birth, were considered growth restricted. In this study, IUGR rats were rats born to BUAL operated females, with a birth weight ≤ 5.52 g. Sham rats were rats born to sham operated females, with a birth weight > 5.52 g.

On the day of delivery (PD1), newborn rat pups were counted, weighed, sexed and returned to their dam for 48 h to ensure attachment.

The cephalization index (CI = head circumference/birth weight) was calculated for individual litters of Sham ($n = 10$, 5 female and 5 male) and IUGR ($n = 14$, 7 female and 7 male) as described by Bassan et al. [14], to ensure the neurological significance of the BUAL model. Animals were chosen randomly from all BUAL and Sham litters in the study.

2.4. Broccoli sprout supplementation

Broccoli sprouts were prepared as previously described [15]. The appropriate groups received 200 mg/day dried broccoli sprouts as a supplement to their regular chow, in a separate dish, from E15 (beginning of the third trimester) to PD14. Animals were then observed to be sure that they consumed all of the BrSps. Any animal that did not eat BrSp for 2 days was excluded from the study.

2.5. Analysis of isothiocyanates/dithiocarbamates (ITC/DTC) in fetus

In order to determine the bioavailability of the sulforaphane to the fetus, additional pregnant dams in their third trimester ($n = 6$) were intraperitoneally injected with either 50 or 500 μg of sulforaphane in 500 μl normal saline, or were fed 200 mg of dried broccoli sprouts for two days. Rats were euthanized 1 h after the sulforaphane injection or consumption of broccoli sprouts.

Samples of whole fetuses were collected rapidly from uterine horns, frozen on liquid nitrogen, and stored at −80 °C prior to analysis. Assessment of sulforaphane activity is performed via measurement of its collective metabolites, dithiocarbamates (DTC), by a cyclocondensation reaction that has been previously described [16,17].

The cyclocondensation product (1,3-benzodithiole-2-thione) was detected by a Waters Model 996 photodiode array detector at 365 nm. The DTC level of the tissue was adjusted to tissue weight.

2.6. Evaluation of neurobehavioral development and maturation

Testing began on PD3 and continued daily through to PD21 to assess the emergence of reflexes, maturation and other sensorimotor behaviors. Rats were allowed to acclimatize to the testing environment for approximately 1 h. To avoid temperature effect, newborn rats were tested in an incubator maintained at 34.5 °C where possible, or under a warm lamp (31 °C) for tests that could not be performed inside the incubator.

The following is a brief description of the reflex tests used, which were adapted from Fox and Lubics et al. [18,19].

1. *Righting reflex:* An animal positioned on its back will turn over to rest in a normal prone position.
2. *Grasp reflex:* Palms are stroked with a blunt instrument and the limb flexes to grasp the instrument. The day each pup could grasp the instrument with both fore or hind limbs was the postnatal day of attainment for that reflex.
3. *Hind limb placing response:* While suspending the pup, the dorsal side of one hind paw is touched to the edge of a flat surface causing the foot to be raised and be placed on the surface. The day each pup performed the placing task with both hind limbs was the day of attainment.
4. *Cliff aversion:* When placed at the edge of a flat surface with forepaws and head over the edge, the rat it will turn and crawl away from the edge. We scored this test 0 (pup completely disregards cliff), 1 (hesitation or struggle) or 2 (pup immediately