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Neonatal isoflavone exposure interferes with the reproductive system of female Wistar rats

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HIGHLIGHTS

- Rats were fed with a soy based isoflavone extract during neonatal period.
- Neonatal direct isoflavone exposure resulted in estrogenic effect in rats.
- Neonatal isoflavone exposure resulted in more irregular estrus cycles later in life.

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ABSTRACT

There is increasing concern about possible adverse effects of soy based infant formulas (SBIF) due to their high amount of isoflavones (ISO). The aim of the present study was to investigate effects of neonatal exposure to ISO on reproductive system of female Wistar rats.

Animals were exposed to an ISO depleted diet or a diet enriched with an ISO extract (IRD; 508 mg ISO/kg) during embryogenesis and adolescence. Pups of each group were fed daily by pipette with ISO-suspension (ISO+; 32 mg ISO/kg bw) or placebo from postnatal day (PND) 1 until PND23 resulting in plasma concentrations similar to levels reported in infants fed SBIF.

The visceral fat mass was reduced by long-term IRD. Vaginal epithelial height was increased at PND23 and vaginal opening was precocious in ISO+ groups. Later in life, more often irregular estrus cycles were observed in rats of ISO+ groups. In addition, FSH levels and uterine epithelial heights were increased at PND80 in ISO+ groups.

In summary, the results indicate that neonatal ISO intake, resulting in plasma concentrations achievable through SBIF, has an estrogenic effect on prepubertal rats and influences female reproductive tract later in life.

1. Introduction

Soy based infant formulas (SBIF) have been used approximately for one century in different compositions as an alternative to cow milk or as an supplement or replacement for breast milk (Merritt and Jenks, 2004). The reasons for its use vary and include intolerance to cow milk, vegetarianism but also lifestyle factors. The usage of SBIF differs between European countries (2–7% of infant formula sales) and the United States where it decreased from 1999 to 2009 from 22.5% to 12.7% (McCarver et al., 2011). SBIF include the soy derived isoflavones (ISO) genistein (GEN), daidzein

Abbreviatons: bw, body weight; DAI, daidzein; FSH, follicle-stimulating hormone; GEN, genistein; GLY, glycitein; GnRH, gonadotropin-releasing hormone; HPG, hypothalamic-pituitary-gonadal; IDD, isoflavone depleted diet; IRD, isoflavone rich diet; ISO, isoflavones; LH, luteinizing hormone; LOD, limit of detection; LOQ, limit of quantification; NTP, National Toxicology Program; PND, postnatal day; SBIF, soy based infant formula; Sult, sulfotransferases; T4, thyroxin; UWW, uterine wet weight; VO, vaginal opening.

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(DAI) and glycitein (GLY) which belong to the group of phytoestrogens because of their binding affinity to the estrogen receptors (Mortensen et al., 2009). The beneficial or adverse impact of ISO on reproductive function, lipid metabolism but also hormone related cancer for instance is controversially discussed (Orgaard and Jensen 2008; Jefferson et al., 2012; Spagnuolo et al., 2015). SBIF are of special interest in this context. On the one hand exposure to SBIF occurs during a critical period of development and on the other hand ISO plasma levels might exceed those in adults. ISO intake by SBIF varies between 3 and 11 mg/kg bw/day (Mortensen et al., 2009) and Setchell et al. reported a mean ISO plasma level of 980 ng/ml after an intake of 6–9 mg/kg bw/day (Setchell et al., 1997). Even though ISO have a lower estrogenic activity compared to estradiol (Kuiper et al., 1998), in this young age the concentration of ISO is approximately 13 000–22 000 times higher than the plasma concentration of endogenous estradiol (Setchell et al., 1997). It also exceeds the ISO concentration observed in Japanese adults (Morton et al., 2002) or in Western postmenopausal women consuming soy diet (Van der Velpen et al., 2014).

Therefore, the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction reviewed animal experiments and epidemiological studies regarding SBIF in 2011 and classified it as “minimal concern for adverse developmental effects in infants fed soy infant formula” (McCarver et al., 2011). Reported adverse effects in rodents mainly include a disruption of the female reproductive tract such as an influence on estrus cycle (Talsness et al., 2015), a premature pubertal onset (Dinsdale et al., 2011) or an alteration of ovarian follicle (Jefferson et al., 2002). Nevertheless, the NTP concluded that there are still critical data gaps which have to be filled for the risk assessment; such as exposure to ISO mixtures instead of the single compounds or direct ISO exposure during lactational period (resembling SBIF) instead of exposure through breast milk, where ISO content seems to be very limited (Doerge et al., 2006).

As an attempt to fill above mentioned gaps, a breeding experiment with female Wistar rats was conducted and neonatal pups were orally exposed to a well characterized soy ISO extract in doses relevant for human exposure or placebo from postnatal day (PND) 1–23. Acute effects, but also effects on reproductive system later in life were investigated.

2. Materials and methods

2.1. Animal study

Experimental design is depicted in Fig. 1. For the two-generation intervention study parental Wistar rats (Janvier Laboratories, Le Genest St. Isle, France) received either an ISO-depleted diet (IDD) (Ssniff Sm R/M-H, 10 mm, phytoestrogenfree, Ssniff, Soest, Germany) or an ISO-rich diet (IRD; 508 mg ISO/kg diet, sum of aglycone equivalents of DAI, GEN and GLY) based on IDD enriched with an ISO extract (NovaSoy650®, ADM, Decatur, Illinois, USA) with beginning of trio breeding. 13 pregnant dams of each diet group were included in this study and individually housed. On PND1 (day of birth), pups with the same day of birth were cross-fostered within diet groups and randomized into two subgroups, respectively. The subgroups were named IDD-, IDD+, IRD-, IRD+ (− = placebo feeding during lactational period; + = ISO feeding during lactational period). The number of pups per dam after cross-fostering was as follows: IDD- (n = 6–11), IDD+ (n = 7–13), IRD- (n = 8–19), IRD+ (n = 7–18). Cross-fostering was used to diminish the influence of the genetic background of the dams and to avoid contamination and mistake during the feeding as described below. Pups of subgroups were orally treated via pipette either with a NovaSoy650® enriched suspension (7.62 mg Novasoy650®/ml, 10% glucose, 5% cornoil, water) or placebo (without Novasoy650®). All pups in the same cage were treated with the same suspension. Pups were fed daily with 1 μl/g bw (32 mg ISO/kg bw/day) until ablactation on PND23. ISO levels were exemplarily measured in all groups at PND5, 10 and 15 (n = 1 − 3) at different time points after feeding to control the success of the feeding and to compare ISO plasma levels with other studies. At PND23 female rats were separated from males and randomized within their diet groups (n = 7 − 8). Rats received the same diet as their dams until dissection at PND23 and 80. Rats were sacrificed at the next metestrus cycle phase from PND80 on, when leucocytes were visible in the vaginal smear (which was not possible for all rats due to cycle irregularities; see discussion).

Body weight and food intake were measured twice a week. All rats were kept under controlled conditions of temperature (21 ± 1 °C), humidity (50–70%) and illumination (12 h light/12 h dark). Three to seven animals were housed per cage with free

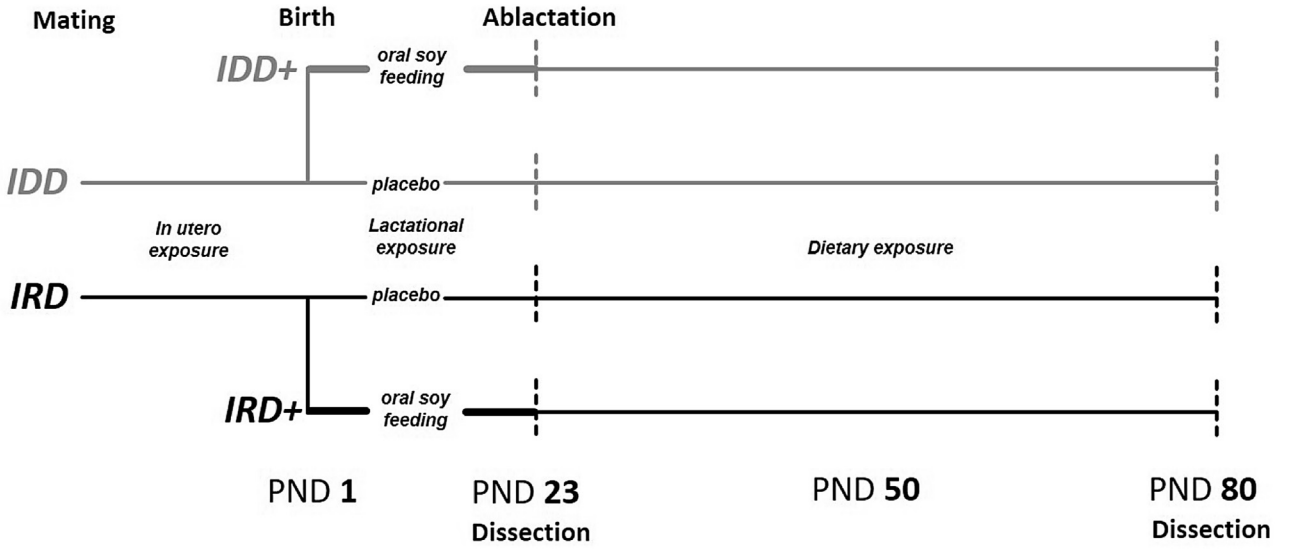

Fig. 1. Timeline and study design. Exposure to isoflavones [by isoflavone-rich diet (IRD)] started in utero, followed during lactational period and maintained by diet until dissection at PND23 and 80. At day of birth, rats within diet groups were separated into two subgroups and were fed orally either with soy suspension (+) or placebo once a day. IDD = isoflavone-depleted diet.