2.4. Effects of diesel exhaust (DE) and diesel exhaust particles (DEPs) on inflammatory response and immunological systems in offspring

Several studies are available on the effects of pre- and postnatal exposure to DE and DEPs on development of the immunological system of offspring (Table 4).

F344/DuCrj rats (5–7 rats/group) inhaled total DE containing 1.73 mg/m^3 DEPs and 0.79 ppm NO_2, HEPA-filtered DE, or clean air (control) daily for 6 h on GD 7 to 3 days after delivery, PNDs 4–22, or PNDs 23–41 [41]. Pups were immunized against Japanese cedar pollen allergens 3 or 4 times at 2-week intervals from PND 49 and the titers of immunoglobulin E (IgE) antibody were determined. It was described that in HEPA-filtered DE, 99.9998% of particles measuring 50 nm or more were eliminated. The total DE, F-DE, or clean air was also inhaled by F344/DuCrj rats (6 rats/group) on GD 3 to 3 days after delivery and serum testosterone and estradiol levels of pups were determined on PNDs 3 and 23. In this study, only male offspring were examined. After exposure on GD 7 to 3 days after delivery, decreases were observed in thymus weight on PNDs 4 and 49 and spleen weight on PND 4 in both DE groups and thymus weight on PND 82 in the total DE group. After exposure on PNDs 4–22, decreases were noted in thymus weight on PNDs 23 and 96 in the filtered DE group and increases were noted in serum tesosterone levels on PND 23 in both DE groups. The production of IgE against pollen was increased in offspring of both DE-exposed groups. No effects were found in offspring exposed on PNDs 23–41. The authors of this study concluded that exposure to DE during the period for differentiation of the immune system accelerated the production of IgE against pollen and F-DE that included the gaseous phase and UFPs measuring less than 50 nm might be responsible.

Pregnant Slc:ICR mice (26–32/group) inhaled DE or clean air (control) on GDs 2–13 daily for 12 h and placentas and fetuses were examined on GD 14 [42]. The DE was mixed with clean air that was first passed through a HEPA filter and charcoal filter. The concentrations of DEPs were similar to in the study described above [20]. There were no effects of DE on the implantation rate, number of fetuses, or sex ratio of fetuses. The number of resorbed placentae was increased at 3.0 mg/m^3. The mRNA levels of CYP1A1 were decreased and those of interleukin (IL)-2, -4, and -5 increased in the placentae in DE-exposed groups. Although the authors of this study noted the possibility that maternal stress caused fetal and placental resorption and the immune system might play some role in the induction of placental resorption, no dose-dependent changes in the incidence of resorbed placentae or gene expression were observed.

Pregnant BALB/c mice on GD 14 or nonpregnant control mice were administered respirable-sized DEPs, that is less than 10 μm in particle size [43], suspended in phosphate-buffered saline (PBS) at 0.05 mg/50 μL/mouse or PBS (control) by a single intranasal insufflation [44]. Pups received a single intraperitoneal injection of ovalbumin (OVA) with alum on PND 4. These pups were exposed to aerosolized OVA on PNDs 12–14, and subjected to an examination of pulmonary function and a pathological analysis. Airway responsiveness to increasing concentrations of aerosolized methacholine was measured using whole body plethysmography. Bronchoalveolar lavage (BAL) differential cell counts and histopathological examinations of the lung were also performed. Lung inflammatory responses were determined 48 h post-administration in nonpregnant and pregnant mice. Pregnant mice exposed to DEPs had higher serum levels of cytokines, including IL-1β, IL-6, tumor necrosis factor-α (TNF-α), and chemokine, at 48 h after exposure compared with nonpregnant mice. Offspring of dams exposed to DEPs showed increased airway hyperesponsiveness (AHR), an increased percentage of eosinophils, and pulmonary inflammation. These findings indicate that DEPs caused acute cellular inflammation in pregnant mice and increased allergic susceptibility in their pups.

In the study of Hougaard et al. [37], inflammatory effects of DEPs were also evaluated by analysis of gene expression of cytokines in the liver of mouse pups on PND 2. Although there was a tendency for increased inflammation in the liver of pups, they concluded that the levels of inflammatory were generally similar in exposed and control offspring.

DE was mixed with HEPA-filtered air and diluted to approximately 0.8 and 3.1 mg/m^{3}of particulate matter [45]. Low DE contained 0.782 mg/m^3 of particulate matter, 5.7×10^7 particles/cm^3. It had a number median diameter for size distribution (NMDSD) of 66 nm, a volume median diameter for size distribution (VMDSD) of 162 nm, 5.7 ppm of CO, 6.3 ppm of NO, 0.43 ppm of NO_2, and <1 ppm of SO_2. High DE contained 3.09 mg/m^3 of particulate matter, 1.8×10^8 particles/cm^3. It had a NMDSD of 74 nm, a VMDSD of 199 nm, 17.7 ppm of CO, 21.2 ppm of NO, 1.22 ppm of NO_2, and <1 ppm of SO_2. BALB/c mice (20 mice/group) inhaled high or low DE or HEPA-filtered air (control) for 4 h daily for 10 consecutive days on GDs 9–18. The gestation index was lower in both DE groups (50%) than the control (75%) and the high DE group had the smallest litter size, but these changes were not statistically significant. No effect of DE was observed on the body weight of offspring. Further details of reproductive effects of DE were not available. Immune and inflammation status were assessed in offspring on PNW 6 or later. DE did not affect standard immunotoxicity endpoints in offspring immunized with experimental antigens or markers of allergic lung disease following mucosal allergen sensitization and challenge. Gender-specific alterations in lung protein and inflammatory cells, and splenic T cell subsets were observed in both DE groups.

BALB/c mice were intranasally administered *Aspergillus fumigatus* 5 times, 4 days apart, beginning 20 days prior to mating, inhaled DE during the 2nd and 3rd weeks of gestation for 5 h/day, 5 days/week, and were treated again with *A. fumigatus* on GDs 7 and 14 [46]. Offspring were treated with 5 or 6 doses of *A. fumigatus* 4 days apart, serum IgG levels were determined during PNWs 9–10, and BAL differential cell counts, AHR, and histopathology of the lungs were examined at PNWs 14–15. DE was diluted to a desirable level (1.09 mg/m^3 of particulate matter). The DEP atmosphere had a count median aerodynamic diameter (CMAD) of 80 nm and a MMAD of 152 nm. Offspring of dams that inhaled DE alone, or allergen and DE, developed lower total IgE levels, and higher levels of IgG_1. Eosinophil counts in BAL were lower in offspring of dams that inhaled allergen and DE than those of dams that inhaled allergen alone. The authors of this study noted that the findings were contradictory to several studies that showed prenatal sensitization to be associated with greater allergic immune responses in the offspring.

C57BL/6 mice inhaled DE containing 0.5 or 2.0 mg/m^3 of particulate matter on GDs 9–17 for 4 h daily, or received twice-weekly oropharyngeal aspirations of the collected DEPs at 0.05 mg for 3 weeks from GD 3[47]. In the online data supplement, it is described that mice inhaled DE 4 h/day for 11 consecutive days on GDs 7–17. Low DE had a NMDSD of 73 nm and VMDSD of 119 nm, and contained 0.5 mg/m^3 of particulate matter, 2.1×10^6 particles/cm^3, 21% O_2, 1.1 ppm CO, 14 ppm NO, <1 ppm NO_2, and <1 ppm SO_2. High DE had a NMDSD of 74 nm and VMDSD of 159 nm, and contained 2.0 mg/m^3 of particulate matter, 3.4×10^6 particles/cm^3, 22% O_2, 37 ppm CO, 44 ppm NO, 1.2 ppm NO_2, and <1 ppm SO_2. A cytokine analysis was performed on fetal lungs and placentae on GD 18. Dams and pups that prenatally inhaled DE or filtered air were exposed to filtered air or 1 ppm of ozone for 4 weeks (3 h/day, 3 days/week) beginning on PND 3. No effect of DE or DEPs was noted on litter size. Levels of cytokines, including IL-1β, IL-6, TNF-α, regulated upon activation, normal T cell expressed and secreted

Table 4
Effects of diesel exhaust (DE) and diesel exhaust particles (DEPs) on inflammatory response and immunological system in offspring.

Animals	Test materials/concentration	Exposure		Developmental effects	References
		Method (no. pregnant animals)	Duration (time)		
F344 rats	Total DE (1.73 mg/m^3 particles, 0.79 ppm NO_2) HEPA-filtered DE (99.9998% of particles measuring 50 nm or more were eliminated)	Inhalation (5–7/group)	GD 7-PND 3 (6 h/day, 7 days/week) PNDs 4–22 (6 h/day, 7 days/week) PNDs 23–41 (6 h/day, 7 days/week)	↓ Spleen and thymus weight in both DE groups ↑ IgE in both DE groups ↓ Thymus weight in filtered DE group ↑ Serum testosterone in both DE groups ↑ IgE in both DE groups No effect	Watanabe and Ohsawa [41]
ICR mice	DE (0.3, 1.0, or 3.0 mg/m^3 DEPs with 0.4 μm MMAD)	Inhalation (26–32/group)	GDs 2–13 (12 h/day, 7 days/week)	↓ Fetal weight of both sexes at 3.0 mg/m^3 ↑ No. of resorbed placentae at 3.0 mg/m^3 ↓ Expression of CYP1A1 in males of DE groups ↑ Expression of IL-2, -4, and -5 in placenta of DE groups	Fujimoto et al. [42]
BALB/c mice	Respirable-sized DEP (0.05 mg/50 μL/mouse)	Intranasal insufflation	GD 14	↑Inflammation in lungs of dams and offspring ↑Susceptibility to allergy in pups	Fedulov et al. [44]
C57BL/6BomTac mice	DEPs (SRM2975, 19.1 mg/m^3 particles, ~1 × 10^6 particles/cm^3, median diameter of 240 nm, surface area of 90 mg^2/g)	Inhalation (20/group)	GDs 7–19 (1 h/day)	Tendency for increased inflammation in liver of pups	Hougaard et al. [37]
BALB/c mice	Low DE (0.782 mg/m^3 particles, 5.7 × 10^7 particles/cm^3, 66 nm NMDSD, 162 nm VMDSD, 5.7 ppm CO, 6.3 ppm NO, 0.43 ppm NO_2, <1 ppm SO_2 High DE (3.09 mg/m^3 particles, 1.8 × 10^8 particles/cm^3, 74 nm NMDSD, 199 nm VMDSD, 17.7 ppm CO, 21.2 ppm NO, 1.22 ppm NO_2, <1 ppm SO_2	Inhalation (20/group)	GDs 9–18 (4 h/day, 7 days/week)	↓ Gestation index in both DE groups ↓ Litter size in high DE group Gender-specific alterations in lung protein, lung inflammatory cells, and splenic T cell subset in both DE groups	Sharkhuu et al. [45]
BALB/c mice	DE (1.09 mg/m^3 particles with 80 nm CMAD, 152 nm MMAD)	Inhalation	2nd and 3rd weeks of gestation (5 h/day, 5 days/week)	↓ IgE ↑ IgG_1 ↓ BAL eosinophils in DE + allergen group	Corson et al. [46]
C57BL/6 mice	Low DE (0.5 mg/m^3 particles, 2.1 × 10^6 particles/cm^3, 21% O_2, 11 ppm CO, 14 ppm NO, <1 ppm NO_2, <1 ppm SO_2, 73 nm NMDSD, 119 nm VMDSD) High DE (2.0 mg/m^3 particles, 3.4 × 10^6 particles/cm^3, 20% O_2, 37 ppm CO, 44 ppm NO, 1.2 ppm NO_2, <1 ppm SO_2, 74 nm NMDSD, 159 nm VMDSD) DEPs (0.05 mg/mice, 18–200 μm size distribution)	Inhalation Oropharyngeal aspiration	GDs 9–17 (4 h/day, 7 days/week) Twice weekly from GD 3 for 3 weeks	↑ IL-1β, IL-6, TNF-α, RANTES, KC, and eotaxin in placentae and fetal lungs after exposure to DE or DEP ↑ AHR in 2.0 mg-DE group	Auten et al. [47]

(RANTES), keratinocyte-derived chemokine (KC), and eotaxin, were increased in fetal lungs and placentae of dams exposed to DE or DEPs. The ozone-induced AHR was more robust in offspring of dams exposed to DE at 2.0 mg/m^3 and persisted in the 4-week recovery period.

In summary, pre- and postnatal exposure to DE and DEPs decreased the weight of the thymus and spleen, accelerated the production of IgE against pollen, modified the expression of immune-related genes, increased allergic susceptibility, altered inflammatory indices in the lung, and increased AHR. The results of one study were contradictory to several studies that showed prenatal sensitization to be associated with greater allergic immune responses in offspring.

2.5. Effects of diesel exhaust (DE) and diesel exhaust particles (DEPs) on genotoxicity in offspring

Studies of the effects of pre-natal exposure to DE and DEPs on genotoxicity in offspring are summarized in Table 5.

Golden Syrian hamsters inhaled DE diluted with air to 12 mg/m^3 of particulate matter on GDs 5–13 for 8 h daily [48]. The frequency of sister chromatid exchange (SCE) in fetal liver was determined on GD 13. Prenatal exposure to DE did not alter the frequency of SCE or mitotic activity. No detailed information on experimental conditions was available.

C57BL/6Jp^{un}/P^{un} mice (4–6 dams/group) were administered DEPs at 31.25, 62.50, 125, 250, or 500 mg/kg/day on GDs 10–15 by oral gavage, and DNA deletion frequency in the retina pigment epithelium was determined in 20-day-old offspring. Oxidative DNA damage and DNA adducts were measured in fetuses on GDs 15 and 17 [49]. The DEPs contained elemental carbon at 9%, organic carbon at 50%, and extractable organic material at 26.3% [50]. Prenatal exposure to DEPs increased the frequency of DNA deletions. DEPs did not increase the levels of oxidative damage or DNA adducts in fetuses.

Hougaard et al. [37] also examined offspring for genotoxic biomarkers. DNA strand breaks in the liver were determined by comet assay in 2-day-old pups of dams exposed to SRM2975 on GDs 7–19. They concluded that there was no indication of DNA damage in the liver of offspring of dams prenatally exposed to DEPs.

The DEPs used were NIST 2975 (standard reference material from the exhaust of an industrial forklift) [51]. Animals were bred and exposed as described in the previous study [37]. Briefly, C57BL/6 mice were exposed to DEPs at 19 mg/m^3 (approximately 1×10^6 particles/cm^3, MMD of 240 nm) or air on GDs 7–19 for 1 h/day and allowed to give birth to F1 offspring. Matured F1 offspring were cross-mated with unexposed CBA/J mice, producing F2 offspring. Three days after weaning, lung inflammation was evaluated by BAL differential cell counts analysis in maternal mice. Tissue was obtained from the F2 offspring shortly after birth or weaning and from the F1 parents. To determine the germline mutation rate, an expanded simple tandem repeat mutation analysis was conducted using isolated DNA of the spleen of the F1 parents and F2 offspring. Lowered body weight during lactation was found in F1 offspring of the DEP-exposed group. No effect of DEPs was observed on clinical signs or cellular profile in BAL in F0 dams, litter size or sex ratio of F1 or F2 offspring, or body weight of F2 offspring during lactation. The germline mutation rate was increased in the males exposed prenatally to DEPs, but not in the females. The authors of this study concluded that maternal exposure to DEPs increased mutations in sperm.

To summarize, the findings of studies on genotoxicity in offspring prenatally exposed to DE and DEPs have been contradictory. DEPs increased the male germline mutation rate and frequency of DNA deletions. Meanwhile, reports that DE and DEPs did not have genotoxic effects in offspring are also available.

Table 5
Effects of diesel exhaust (DE) and diesel exhaust particles (DEPs) on genotoxicity in offspring.

Animals	Test materials/concentration	Exposure		Developmental effects	References
		Method (no. pregnant animals)	Duration (time)		
Golden Syrian hamsters	DE (12 mg/m^3 particles)	Inhalation	GDs 5–13 (8 h/day, 7 days/week)	No effect on frequency of SCE or mitotic activity in fetal liver	Pereira et al. [48]
C57BL/6Jp^{un}/P^{un} mice	DEPs (31.25, 62.50, 125, 250, or 500 mg/kg/day)	Oral gavage (4–6/group)	GDs 10–15	↑ Frequency of DNA deletions in retina pigment epithelium	Reliene et al. [49]
C57BL/6BomTac mice	DEPs (SRM2975, 19.1 mg/m^3 particles, ~1 × 10^6 particles/cm^3, median diameter of 240 nm, surface area of 90 mg^2/g)	Inhalation (20/group)	GDs 7–19 (1 h/day)	No change in genotoxic response in liver of offspring	Hougaard et al. [37]
C57BL/6 mice	DEPs (NIST 2975, 19 mg/m^3, ~1 × 10^6 particles/cm^3, 240 nm MMD)	Inhalation	GDs 7–19 (1 h/day)	↓ Body weight of F1 offspring ↑ Male germline mutation rate	Ritz et al. [51]

2.6. Developmental toxicity of carbon black (CB)

Soot from combustion sources, such as DE soot, consists of a carbonaceous core and inorganic and organic compounds [15,52]. CB is essentially pure elemental carbon particles devoid of hydrocarbons [14], and Printex 90 (14 nm in diameter, 300 m^2/g in surface area, Evonic Inds. AG, Essen, Germany), a commercially available CB, is a well characterized carbonaceous core particle that has been used extensively as a benchmark and as a model for DE particles [15]. Both reports referred to in our previous paper [53] and those that newly appeared after publication of our review are summarized in this section (Table 6).

In the study of Reliene et al. [49], the effect of a prenatal oral administration of CB at 500 mg/day on DNA deletions in the retina pigment epithelium in fetuses was determined. CB did not increase the rate of DNA deletion.

Pregnant BALB/c mice were administered CB at 250 μg/50 μL/mouse or PBS by a single intranasal insufflation on GD 14 [44]. The treatment of animals was similar to that in the DEP-experiment described above [44]. Pups of dams exposed to CB showed an increased susceptibility to allergies.

Pregnant ICR mice (20 mice/group) were intratracheally instilled with 14-nm CB nanoparticles at 1.2 mg/mouse/day or with 0.1 mL of saline containing 0.05% Tween 80 (control) on GDs 7 and 14, and male offspring were examined at PNWs 5, 10, and 15 [54]. No marked effects were found on body weight, testicular or epididymal weights, or serum testosterone levels in male offspring. However, histopathological changes, such as vacuolation of the seminiferous tubules and low cellular adhesion of seminiferous epithelia, in the testes and decreased DSP were observed in male offspring after prenatal exposure to CB.

Expression of *Collagen, type VIIIal (Col8al)* in the tubular cells in the kidney was examined at PNWs 3 and 12 and increased in 12-week-old male offspring of ICR mouse dams (12-13 mice/group) intratrachelly instilled with Printex 90 at 0.05 mg/mouse/day or with saline containing 0.05% Tween 80 (control) on GDs 5 and 9 [55]. In male offspring, the serum levels of creatinine and blood urea nitrogen were not affected by prenatal instillation of CB. The authors of this study noted that prenatal exposure to CB caused renal abnormalities similar to the tubulointerstitial fibrosis in diabetic nephropathy.

Pregnant C57BL/6BomTac mice inhaled Printex 90 at 42 mg/m^3 or HEPA-filtered air (control) for 1 h/day on GDs 8–18 (44 mice) or were intratracheally instilled with Printex 90 at a total dose of 0.011, 0.054, or 0.268 mg/mouse or with saline containing 10% BAL (control) on GDs 7, 10, 15, and 18 (80 mice) [15,56]. Detailed data on physic-chemical characteristics of Pritex 90 were reported [15]. In brief, Printex 90 had a geometric mean size of 65 nm, surface area of 292–338 m^2/g, and pynometric particle density of 2.1 g/cm^3. The aggregates ranged in size from <100 nm to 20–30 μm; the typical size was approximately 200 nm. The highest instillation dose was comparable to the expected inhaled pulmonary dose. Maternal lung inflammation was found 3–5 and 24–27 days after exposure in the CB-inhaled and highest CB-instilled groups, and the inflammation was stronger after the instillation than inhalation of CB. No adverse effects of CB on reproductive and developmental parameters were observed after the inhalation or intratracheal instillation. Levels of DNA strand breaks were increased in maternal liver cells 3–5 days after the inhalation of CB and offspring liver cells 22–23 and 50 days after the inhalation of CB, but not after the instillation of CB. These findings indicate that prenatal inhalation of CB caused DNA damage in the dams and their offspring.

Using the offspring from the previous intratracheal instillation study [15], the effect of prenatal exposure to CB on the sexual development (all dose groups) and behavior (control and 0.268 mg/mouse group) of offspring was determined [57]. Prenatal CB did not have an effect on weight gain after weaning, AGD, vaginal opening, preputial separation, or acoustic startle of offspring. Although no differences were observed in open field activity, the female offspring in the 0.268 mg/mouse group displayed a different pattern of habituation. The authors of this study noted that the change in neurobehavior was likely an indirect effect of maternal inflammation.

The hypothesis that pulmonary exposure to CB and CB-induced responses resulted in secondary effects on offspring was examined [58] using the tissues of the dams and offspring from the intratracheal instillation study [15]. Histopathology, DNA microarrays, pathway-specific RT-PCR arrays, focused RT-PCR, and tissue protein analysis were applied to characterize pulmonary responses in dams exposed to CB. Global hepatic gene expression profiling was also performed to characterize the response of the offspring to prenatal CB exposure. In the lungs of dams on 26–27 days after instillation, retention of CB was found at 0.054 and 0.268 mg/mouse and neutrophil-marked inflammation and altered expression of several cytokines and chemokines were observed at the highest dose. A hepatic response at the mRNA level was caused in male and female offspring on PND 2 at the highest dose and a higher response was observed in females. The majority of the altered genes in offspring belonged to metabolic pathways. Analysis of newborn livers by DNA microarrays showed that female offspring were more sensitive to maternal exposure than males. The authors of this study speculated that prenatal exposure to CB caused metabolism-mediated disorders that manifested later in life.

In summary, pre- and postnatal exposure to CB altered susceptibility to allergies, inflammatory responses, the histopathology of the testes, spermatogenesis, genotoxic responses, and postnatal behavior in offspring.

3. Discussion and conclusions

Papers on the developmental toxicity of DE were summarized in this review. Various adverse effects of maternal exposure to DE, DEPs, and CB on development in the offspring of experimental animals have been reported. It is difficult to evaluate the dose-response effect and clarify the most sensitive adverse effect of prenatal exposure to DE, because DE contains DEPs and gaseous phase and there are many differences in mass and number concentrations and size distribution of DEPs and in components and concentrations of gaseous phase among studies. However, the adverse effects of DEPs and CB may be possible to evaluate dose-dependent effect. The adverse effects on male reproductive system seem to be the most sensitive endpoint after prenatal exposure to DE or DEPs. These effects were observed after prenatal exposure to CB.

There is a lack of detailed information on experimental conditions including numbers of animals and exposure conditions, and the characterization of DE and DEPs used in some studies cited in this review. A relatively small number of animals and only one dose level were used. The number of animals per group should be sufficient to allow meaningful interpretation of the data for developmental toxicity studies, and a dose–response analysis is also needed to allow more realistic comparisons with actual human exposure.

The propensity for fetuses of a given litter to exhibit similar responses to toxic insult, thereby artificially inflating the apparent group response, has been designated the "litter effect" [59]. The proper experimental unit in the analysis should be the litter because the litter is the unit that is randomized, and individual fetuses or offspring within litters do not respond completely independently [59]. To reduce the rate of Type I errors (false positives) and increase the validity of experiments in developmental toxicity

Table 6
Developmental toxicity of carbon black (CB).

Animals	Test materials/concentration	Exposure		Developmental effects	References
		Method (no. pregnant animals)	Duration (time)		
C57BL/6Jp^{un}/P^{un} mice	CB (500 mg/kg/day)	Oral gavage (4–6/group)	GDs 10–15	No effect on DNA deletion frequency	Reliene et al. [49]
BALB/c mice	CB (0.25 mg/50 μL/mouse)	Intranasal insufflation	GD 14	↑ Susceptibility to allergy in pups	Fedulov et al. [44]
ICR mice	14 nm-Carbon black (0.2 mg/mouse/day)	Intratracheal instillation (20/group)	GDs 7 and 14	↑ Vacuolation of seminiferous tubules ↓ Cellular adhesion of seminiferous epithelia ↓ DSP	Yoshida et al. [54]
ICR mice	Printex 90 (0.05 mg/mouse/day, 14 nm in particle size, 300 m^2/g in surface area)	Intranasal instillation (12–13/group)	GDs 5 and 9	↑ Expression of *Collagen, type VIIIal* (tubulointerstitial fibrosis) in male offspring	Umezawa et al. [55]
C57BL/6BomTac mice	Printex 90 (42 mg/m^3, 65 nm in geometric mean size, 295–338 m^2/g in surface area, 2.1 g/cm^2 in pynometric density, 99% C, 0.8% N, 0.01% H_2)	Inhalation (44 for filtered air or Pritex 90)	GDs 8–18 (1 h/day)	↑ Total cell count, neutrophils, and lymphocytes after inhalation and instillation of highest dose	Jackson et al. [15]
	Printex 90 (total dose of 0.011, 0.054, or 0.268 mg/mouse)	Intratracheal instillation (80 for vehicle or Pritex 90)	GDs 7, 10, 15, and 18	↑ DNA strand breaks in maternal and offspring liver cells after inhalation	
C57BL/6BomTac mice	Printex 90 (total dose of 0.011, 0.054, or 0.268 mg/mouse)	Intratracheal instillation (17–24/group)	GDs 7, 10, 15, and 18	Different pattern of habituation in open field in female offspring at 0.268 mg/mouse	Jackson et al. [57]
C57BL/6BomTac mice	Printex 90 (total dose of 0.011, 0.054, or 0.268 mg/mouse)	Intratracheal instillation (17–24/group)	GDs 7, 10, 15, and 18	Retention of CB in lungs of dams at 0.054 and 0.268 mg/mouse. Neutrophil-marked inflammation and altered expression of several cytokines and chemokines in lungs of dams at 0.268 mg/mouse. Hepatic response at the mRNA level in male and female offspring on PND 2 at 0.268 mg/mouse. Higher response in females	Jackson et al. [58]

studies, a statistical analysis of the offspring before weaning should be carried out using the litter as an experimental unit. Only three of the reports [15,37,57] cited in this review provided a description regarding the experimental unit for statistical analysis. The statistical approach using the litter as an experimental unit might be also adequate for postnatal data evaluation because influences persist beyond weaning [60].

In some studies, inhalation was performed for 5 days a week [23,24,33,34,38,40,46] and GDs 2–13, except for GDs 4, 5, 11, and 12 [21,22]. There is a possibility that the exposure period may vary and researchers may underestimate the outcome and pass over specific effects on critical developmental events that occur during non-exposure days because most critical developmental events occur during a very narrow time period in the gestation of rodents [61]. The exposure period for test materials should cover the period for critical developmental events.

Not enough information was provided on maternal toxicity in some reports. The investigation of maternal toxicity is essential for developmental toxicity studies, because the toxicity to offspring may be modified or influenced by toxicity to the mother and toxicity to offspring often occurs in conjunction with maternal toxicity in animal studies. In several studies, changes in maternal hormone levels [19,25] and maternal behavior [20], maternal inflammation [15,44,57,58], and DNA strand breaks in the maternal liver [15] were reported after exposure to DE, DEPs, or CB. Effects in offspring occurred at concentrations which were toxic to the mother. The inhalation of high concentrations of poorly soluble particles causes an overload of particles, which is generally characterized by a pulmonary burden that is greater than the steady-state burden predicted from the deposition rates and clearance kinetics of particles inhaled during exposure [62], in rodent lungs. Rats may be more susceptible to pulmonary overload than mice and hamsters, and pulmonary inflammation is more severe in rats [63]. Maternal inflammation due to prolonged exposure resulting from the deposition of particulate matter in the maternal lungs have the potential to adversely affect offspring. Jackson et al. [58] suggested that potential implications of prenatal exposure to CB might include metabolism-mediated disorders in later life. They also speculated that the neurobehavioural changes in offspring were more likely indirect effects of maternal inflammation [57]. These findings suggest that the developmental toxicity of DE in offspring of exposed dams occurred in conjunction with maternal toxicity.

Although polycyclic aromatic hydrocarbons were reported to pass into maternal blood and transfer into fetuses and into breast milk after maternal exposure to DE in rats [64], translocation of DEPs from the maternal lungs into fetal organs was not demonstrated in the reports cited in this review paper. However, carbon nanoparticles were translocated from the lungs to the systemic circulation and extrapulmonary organs [65,66] and to the olfactory bulb, cerebrum, and cerebellum [67]. Whole-body inhalation results in significant dosing of the gastrointestinal tract with particles via direct ingestion, movement from the respiratory tract due to mucociliary clearance and swallowing, and fur grooming [65]. Titanium dioxide (TiO_2) particles given by oral gavage were transported to other organs after uptake by the gastrointensinal tract in mice and rats [68,69]. As for the placental transfer of particles, it has been reported that aggregates of TiO_2 nanoparticles were detected in Leydig cells, Sertoli cells, and spermatids in the testes and brain cells of PNW 6-pups after the subcutaneous maternal injection of TiO_2 nanoparticles during gestation [70]. After the intravenous injection of $[^{14}C]C_{60}$ fullerene during pregnancy and lactation, $[^{14}C]C_{60}$ was distributed to the placentae and fetuses of exposed pregnant rats and to the milk and pups of exposed lactating dams, respectively [71]. Furthermore, an *in vitro* study showed the uptake of DEPs and CB into Leydig cells, and inhibition of cell viability and increased StARP mRNA expression [72] and suggested that DEPs and CB have direct effects on the production of steroid hormone in Leydig cells resulting in a defect of spermatogenesis. A recent review of the transplacental transfer of particles showed that most UFPs tested so far were detected in the fetal circulation or tissues and suggested that the placenta did not provide a tight barrier to the transfer of UFPs to fetuses [73]. Taken together, these findings may suggest that nanosized DEPs are transported to pre- and postnatal offspring and have a direct effect on the offspring of dams exposed to DE or DEPs. On the other hand, reports suggesting that air pollution and particulate matter have a secondary effect on the development of offspring are available. Maternal exposure, before and/or during pregnancy, to air pollution including particulate matter emitted by traffic adversely affected growth and survival of mouse offspring, and these phenomena were indicated to be mediated by morphological changes in the placenta and umbilical cord, imbalances in the endogenous regulators of vascular tone, and oxidative stress [74–76]. It is necessary to clarify whether or not the translocation of DE and/or its components including DEPs from the maternal lungs into fetal organs and into pups occurs through maternal milk.

As for the causative agent for the developmental toxicity of DE, conflicting findings have been reported. In the studies using NR-DE and filtered-DE [25], it is suggested that nanoparticles exert no significant toxic effects and toxic chemicals in filtered-DE may be responsible for toxic effects because there was no difference in toxicity between the NR-DE and filtered-DE groups. Certain studies revealed that developmental effects caused by exposure to total DE and HEPA-filtered DE had almost the same affect [19,26,41]. A HEPA filter has a minimum particle removal efficiency of 99.97% for all particles of 300 nm diameter, with high efficiency for both larger and smaller particles [77].These findings may support the above suggestions. The gaseous phase of DE contains more than 40 toxic air contaminants [78]. Gaseous components of DE include carbon dioxide, oxygen, nitrogen, water vapor, carbon monoxide, nitrogen compounds, sulfur compounds, and numerous low-molecular-weight hydrocarbons, and the DEPs are composed of a center core of elemental carbon and absorbed organic compounds, as well as small amounts of sulfate, nitrate, metals, and other trace elements [3]. It is necessary to clarify the causative agent for the developmental toxicity of components of the gaseous phase of DE because some chemical components in the gaseous phase possess developmental toxicity [79,80] and endocrine disrupting activity [81]. However, a possibility that DEPs were a responsible toxicant cannot be ruled out because filtered-DE still retained DEPs.

Meanwhile some studies cited in this review were performed using DE containing various concentrations of DEPs and filtered-DE. These studies showed that exposure to DE containing higher concentrations of DEPs produced more severe developmental toxicity and the severity of the toxicity depended on the concentration of DEPs [20,28,35,42]. Furthermore, exposure to DEPs [31,37,49,51] and CB including nanosized CB [15,44,54,55,57,58] was reported to cause developmental toxicity and the toxicity was similar to that induced by exposure to DE [44,54]. These observations suggest the causative agent for the developmental toxicity of DE to be DEPs.

Determination of the mass concentration, number concentration, and size-distribution of the particles in total DE and filtered-DE is needed to identify the toxicant responsible for the developmental toxicity of DE. Regarding measurement of particles after filtration, Watanabe and Kurita [19] and Watanabe and Ohsawa [41] noted that 99.9998% of particles measuring 50 nm or more were eliminated. However, no information on the instrument or method for measurement of this elimination rate was available in their papers. They also described that gravimetric measurements of the particulate matter were conducted daily using an automatic β-ray dust mass monitor, and measurements of particle sizes with a particle fractionating sampler confirmed that more than 90% of

the particulate matter in the diesel exhaust measured less than 0.5 μm [19] or 0.05 μm [41]. These methods are unmeasurable for the elimination rate of 99.9998%. The number concentration and size distribution nanoparticles can be determined by Condensation Particle Counter (CPC) and Differential Mobility Analyzer (DMA), respectively [82,83]. It is imperative that an appropriate methodology including instrument be selected in order to obtain reliable information on sample characterization.

Oxidative stress resulting from increased generation of reactive oxygen species (ROS) has been involved in the induction of developmental toxicity and sperm/testicular toxicity [84–86]. ROS are among the major causative factors for the toxicity of nanoparticles [4,87]. Nanoparticles have a potent effect on ROS production and increase inflammation, and DEPs and CB increase ROS production [87]. It is suggested that up-regulated mRNA levels of HO-1 by DEPs in Leydig cells were caused by DEP-absorbed chemicals rather than particle itself [72]. Sagai et al. [88] reported that DEPs produced oxygen radicals such as superoxide and hydroxyl radical but DEPs washed with methanol did not produce oxygen radicals and suggested that oxygen radicals were produced by the organic solvent extract from DEPs. These findings indicate that DE including DEPs and components of the gaseous phase could generate ROS resulting in oxidative stress.

Effects of exposure to DE are a concern because DE contains toxic compounds in the gaseous phase and DEPs including nanoparticles whose toxicity cannot be predicted from the toxicological effects of larger substances of the same composition [5]. It is important to diminish emissions of toxic components and DEPs including nanoparticles to reduce the exposure risk and protect human health. Diesel technology has advanced during the last decade and the emissions from post-2006–2007 diesel engines and from earlier model diesel engines retrofitted with exhaust after-treatment devices (filters and catalysis) and using ultra-low sulfur fuels are termed "New Technology Diesel Exhaust (NTDE)" [13,14,89,90]. The NTDE was quantitatively, qualitatively and distinctly different and the concentration of DEPs was markedly lower than for DE from earlier model diesel engines [13,90]. Toxicity, including developmental toxicity, studies of NTDE are expected to fill a data gap.

Many findings on the developmental toxicity of DE have been reported by the same research group. To validate the experimental findings, it is necessary to involve other research groups. The risk assessment of the developmental toxicity of DE is difficult because there are many variables in the manifestation of adverse effects of pre- and postnatal exposure to DE and poor reproducibility of the developmental toxicity of DE due to indirect exposure of offspring. Confirmation of adverse effects on development in animals exposed by the anticipated route of human exposure and relevant concentrations closely reflecting expected levels of human exposure would be important for risk assessment in humans.

Conflict of interest

None.

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Impact of planning of pregnancy in women with epilepsy on seizure control during pregnancy and on maternal and neonatal outcomes

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ABSTRACT

Purpose: To investigate whether planning of pregnancy in women with epilepsy affects seizure control during pregnancy and to compare the maternal and neonatal outcomes in planned and unplanned pregnancies.
Methods: This was a retrospective cohort study of 153 pregnant women with epilepsy who were treated at the University of Tsukuba Hospital and Hokkaido University Hospital between 2003 and 2011. Twenty-one pregnancies were excluded due to insufficient data. Data of patients followed by neurologists during their planned pregnancies (planned-pregnancy group, $n = 51$) were compared to those of patients referred to neurologists after conception for managing epilepsy during pregnancy (unplanned-pregnancy group, $n = 81$). The treatment profile for epilepsy, seizure control, and maternal and neonatal outcomes in both groups were compared using Chi-square test or Fisher's exact test and Mann–Whitney *U* test.
Results: Compared to the unplanned-pregnancy group, the planned-pregnancy group showed a significantly greater proportion of patients receiving monotherapy with antiepileptic drugs (80% vs. 61%: planned vs. unplanned, $P = 0.049$) and those not requiring valproic acid (77% vs. 56%, $P = 0.031$). Furthermore, the frequency of epileptic seizures (16% vs. 35%, $P = 0.018$) and changes in antiepileptic drugs (24% vs. 41%, $P = 0.042$) were significantly lower in the planned-pregnancy group than in the unplanned-pregnancy group. No significant intergroup differences were noted in the obstetric complications and neonatal outcomes, including congenital malformations.
Conclusion: For women with epilepsy, planning of pregnancy is associated with good seizure control during pregnancy and less fetal exposure to antiepileptic drugs.

1. Introduction

Population-based studies indicate that the prevalence of epilepsy in pregnant women is 0.7%, whereas registry-based studies suggest a range of 0.2–0.4%.[1] Thus, obstetricians often encounter cases of pregnancy in women with epilepsy (WWE) and can be involved in the management of the pregnancies in these women as well as offer preconception counseling. In general, although the pregnancy outcomes in most WWE are favorable, epilepsy poses additional risks for the mother and fetus, including the potential effects of teratogenic antiepileptic drugs (AEDs), the effects of maternal seizures on the fetus, and genetic risks, all of which contribute to a two- to three-fold increase in the risk of adverse outcomes.[2] These adverse outcomes include major congenital malformation (MCM) in the fetus and long-term developmental delay.

A maternal seizure can directly affect the fetus and cause fetal hypoxia and distress. Additionally, adverse effects on the mother include falls; superficial abdominal hematomas; burns or other accidents; and significant obstetric sequelae, such as placental abruption and premature labor and delivery. Various complications of pregnancy have been reported in WWE. These women are at an increased risk of spontaneous abortion, induction of labor,

Abbreviations: WWE, women with epilepsy; AED, antiepileptic drug; MCM, major congenital malformation; VPA, valproic acid; PB, phenobarbital; CBZ, carbamazepine; PHT, phenytoin; VSD, ventricular septal defect.

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cesarean section, and postpartum hemorrhage[3]. Infants of WWE exposed to AEDs in utero are at a high risk of fetal growth restriction, preterm birth, low birth weight, and low Apgar scores.[3]

The incidence of fetal MCM in women without epilepsy is 1.6–2.2%,[4–6] whereas that in WWE is higher, at 2.8–3.6%[7–9] who take AEDs and at 4.2–6.7% who do not take AEDs.[4,5,7–9] Furthermore, the risk of fetal MCM increases with the number of AEDs administered.[5,6,9,10] In particular, treatment with valproic acid (VPA) in combination with AEDs is associated with a high risk of fetal MCM.[11] The teratogenic effects of most AEDs appear to be dose dependent,[7,12] and numerous reports have confirmed this dose dependency in the case of VPA.[7,9,10,13–15] Hence, transitioning from VPA to another AED should be considered before conception, and the lowest possible dose of the most appropriate AED should be administered before conception in WWE.

The Japanese guidelines for the management of WWE, as stipulated by the Societas Neurologica Japonica, recommend preconception counseling to provide detailed information about pregnancy and delivery in WWE and emphasize the importance of the medication during pregnancy, along with antenatal management involving the use of the lowest possible dose of the most appropriate AED, avoiding VPA, possibly withdrawing AED before conception, and supplementation with folic acid before and after conception to prevent neural tube defects.[16] International guidelines recommend monotherapy with lamotrigine owing to its low risk of teratogenicity; however, this treatment option is currently not permitted by the Japanese national health insurance policy.[16]

Although various guidelines recommend planned pregnancy for WWE, the effects of planning remain largely unclear. Preconception counseling for WWE has been reported to be effective for reducing fetal MCM in children born to WWE receiving AEDs.[17] However, the effects of a planned pregnancy on seizure control in WWE during pregnancy and maternal and neonatal peripartum outcomes have not been evaluated; in the present study, we aimed to evaluate these effects. Moreover, we aimed to compare the maternal and neonatal outcomes in planned and unplanned pregnancies in WWE.

2. Methods

We retrospectively reviewed the hospital records of 153 pregnancies in WWE who were treated at the University of Tsukuba Hospital (98 pregnancies) and Hokkaido University Hospital (55 pregnancies) between 2003 and 2011. Planned pregnancy was defined as the completed process of planning and preparing for pregnancy, during which the doses and numbers of AEDs and maternal physical health prior to conception were optimized by neurologists or neurosurgeons. According to the Japanese Healthcare system, all WWE taking AEDs should be followed up by their neurologists or neurosurgeons and not by general physicians, even if their seizures are well controlled, and some WWE who remain free of seizures without taking AEDs for a long duration are normally not followed by any medical doctor. Among WWE in the planned-pregnancy group, those who were regularly reviewed by their neurologists or neurosurgeons before conception received preconception counseling, epilepsy reassessment, AED dose adjustment; after successful execution of the planned pregnancy processes, their neurologists or neurosurgeons considered their conception appropriate. For WWE in the planned-pregnancy group who were not followed by medical doctors, neurologists or neurosurgeons consulted their conception, reviewed their condition, and considered pregnancy to be appropriate. The unplanned-pregnancy group included all the pregnancies not included in the planned-pregnancy group. WWE in the unplanned pregnancy group who were regularly followed up by their neurologists or neurosurgeons included those with unexpected pregnancies (for both WWE and medical doctors) and those in whom the pregnancy had occurred during planning but not when conception was considered appropriate by the neurologists or neurosurgeons. The unplanned pregnancy group also included pregnancies of WWE who were not followed up by medical doctors and who did not consult neurologists or neurosurgeons about their pregnancy before conception. The planned pregnancy process was recorded on their medical chart or the introduction form administered at our hospital. The pregnancy was planned according to the discretion of the neurologist or neurosurgeon and was not structured, but it was in accordance with the guidelines for doctors authorized to treat patients under the Japanese government medical insurance system, as well as with the Japanese guidelines for the standard management of epilepsy. Data regarding the history of preconception treatment were unavailable in the case of 21 pregnancies, including medical letters from neurologists or neurosurgeons and medical interview forms; hence, these pregnancies were excluded from the study analysis. We classified the remaining 132 pregnancies on the basis of whether the pregnancy was planned or unplanned and compared the seizure control in pregnancy and maternal and neonatal outcomes in the 2 groups. Data were analyzed using the Chi-square test or Fisher's exact test and the Mann–Whitney *U* test, as appropriate. A P value of <0.05 was considered statistically significant.

The approval of the institutional review board and ethics committee was obtained at the University of Tsukuba Hospital (number: H23-62) and Hokkaido University Hospital (number: 011-0133). Informed consent was obtained from all patients.

3. Results

The planned-pregnancy group consisted of 51 pregnancies (39%), whereas the unplanned-pregnancy group comprised 81 pregnancies (61%).

3.1. Maternal backgrounds

Table 1 shows the patient demographics. Women in the planned-pregnancy group were significantly older (median age, 32 years; range, 17–42 years) than those in the unplanned-pregnancy group (median age, 28 years; range, 22–38 years; $P = 0.012$) (Table 1). The 2 groups showed no significant difference with respect to parity and gravidity (Table 1). Most women in both groups were Japanese. None of the women in the 2 groups had health conditions that were likely to affect maternal and neonatal peripartum outcomes, such as diabetes mellitus, hypertension, and hyperthyroidism. Only 1 woman in the unplanned-pregnancy group had asthma, which was well controlled. None of the women in the 2 groups had exposure to other teratogens except AEDs. Eight women in each group were overweight (BMI > 25), and the difference between the 2 groups in the proportion of overweight patients was not statistically significant ($P = 0.32$). Socio-economic

Table 1
Maternal characteristics.

	Planned	Unplanned	*P*-Value
Number	51	81	
Age	17–42	22–38	0.012[a]
Nullgravida	20 (39.2%)	37 (45.7%)	0.47
Nullipara	29 (56.9%)	53 (65.4%)	0.32
BMI	16.9–38.3	14.3–32.8	0.15
Japanese	49 (96.1%)	79 (97.5%)	0.64
On welfare	1 (2.0%)	4 (4.9%)	0.65

[a] $P < 0.05$, BMI: body mass index.

statuses of the women in the 2 groups were not significantly different.

3.2. Treatment profile for epilepsy

Table 2 shows the epileptic treatments administered to the patients. Approximately 75% of WWE in both groups received some kind of AED during pregnancy. Compared to the unplanned-pregnancy group, the planned-pregnancy group showed a significantly higher proportion of WWE who received monotherapy with AEDs ($P = 0.049$). For monotherapy, phenobarbital (PB) was the most common medication used in the planned-pregnancy group (10/31), followed by carbamazepine (CBZ) (8/31), VPA (8/31), clonazepam (3/31), diazepam (1/31), and primidone (1/31). In comparison, the primary drugs used for monotherapy in the unplanned-pregnancy group were VPA (17/37), CBZ (10/37), PB (3/37), phenytoin (PHT) (3/37), clonazepam (2/27), clobazam (1/37), and zonisamide (1/37), in that order of frequency. Eight women required polytherapy in the planned-pregnancy group, of which 1 took VPA. In the unplanned-pregnancy group, 24 women required polytherapy, and 10 of them took VPA. VPA was required in significantly fewer cases in the planned-pregnancy group than in the unplanned-pregnancy group (9 vs. 27, $P = 0.031$). No significant intergroup difference was noted with regard to folic acid supplementation.

3.3. Seizure control and AEDs during pregnancy

A significantly high rate of seizures during pregnancy was recorded in the unplanned-pregnancy group than in the planned-pregnancy group (Table 3). Due to concerns of teratogenicity, changes in the AED schedule immediately after the diagnosis of pregnancy were required in 21 WWE who belonged to the unplanned-pregnancy group (Table 3). Of these 21 WWE, 8 requested the change themselves, whereas in 13 WWE, the change was recommended by neurologists. Eleven women (52.4%) had seizures even after altering the AED schedule. In particular, the rate of seizures was high (66.7%) in 12 women who took VPA before alteration of the AED schedule after pregnancy detection. With regard to these 12 women, seizures were noted in 1 of 3 women for whom VPA was switched to other AEDs, 1 woman whose VPA dosage was reduced, and 6 of 8 women for whom VPA was discontinued.

The rate of non-compliance with the AED treatment schedule was higher in the unplanned-pregnancy group than in the planned-pregnancy group (Table 3). In the planned-pregnancy group, 3 women opted to discontinue AEDs before conception of their own accord and then resumed these medications after completion of the high-risk period of fetal malformations according to the recommendations of their neurologists. They did not experience any seizure during the AED-free period. In the unplanned-pregnancy group, 13 women discontinued AEDs by themselves after the detection of pregnancy. Eight of them subsequently experienced seizures, which made it necessary to restart AED treatment, but 1 WWE refused to resume the AED treatment despite developing seizures. Of the remaining 5 women without seizures, 4 resumed AED treatment after the teratogenic period, whereas 1 did not resume AED treatment throughout the pregnancy, as per the advice of the neurologist.

Table 2
Treatment profile for epilepsy.

	Planned	Unplanned	*P*-Value
AED use	39 (76.5%)	61 (75.3%)	0.88
Monotherapy[a]	31/39 (79.5%)	37/61 (60.7%)	0.049[b]
VPA included[a]	9/39 (23.1%)	27/61 (44.3%)	0.031[b]
FA supplementation	23 (45.1%)	30 (37.0%)	0.36

[a] Mothers not receiving AEDs were excluded.
[b] $P < 0.05$, AED: antiepileptic drug, VPA: valproic acid, FA: folic acid.

Table 3
Seizure control and AEDs during pregnancy.

	Planned	Unplanned	*P*-Value
Epileptic seizure	8 (15.7%)	28 (34.6%)	0.018[a]
AEDs after seizure			
Increased	6	20	
Stable	2	7	
Discontinued	0	1[b]	
SUDEP	0	0	N.A.
Adjusted AEDs following pregnancy diagnosis	0 (0.0%)	21 (25.9%)	<0.001[a]
Switched	0	5	
Decreased	0	5	
Discontinued	0	11	
AEDs discontinued by patients themselves[c]	3/39 (7.7%)	13/61 (21.3%)	0.07
Stable dose and drugs during pregnancy	39 (76.5%)	48 (59.3%)	0.042[a]

[a] $P < 0.05$.
[b] A woman chose to discontinue AEDs against medical advice.
[c] Mothers not reaching AEDs were excluded, AED: antiepileptic drugs, SUDEP: sudden unexpected death in epilepsy.

The percentage of patients receiving stable antiepileptic treatment, i.e. no change in the type and dosage of AEDs or no additional requirement of AEDs, and was significantly higher in the planned-pregnancy group than in the unplanned-pregnancy group (Table 3). In the planned-pregnancy group, 12 women required medication adjustment, and all of them required an increase in the dosage of AEDs because of seizures (6/12), abnormal electroencephalogram findings (2/12), or for restarting previously withdrawn AEDs after the organogenetic period (4/12). In comparison, 33 women in the unplanned-pregnancy group required alterations in the AED schedule during pregnancy; the changes were required because of pregnancy detection in 21 of these women and because of seizures (increased dosage, 10 women) or for unknown reasons (increased dosage, 2 women) in 12 of them.

3.4. Peripartum outcomes

Peripartum outcomes are shown in Table 4. There was no significant difference in the frequency of preterm birth between the 2 groups (4/51 in planned vs. 8/81 in unplanned, $P = 0.77$). Similarly, no significant differences were noted in the frequency of termination of pregnancy and the modes of delivery.

3.5. Neonatal outcomes

Table 5 shows the neonatal outcomes. There was no significant difference in the birth weight and Apgar scores of the live-born neonates between the planned- and unplanned-pregnancy groups. The rates of congenital malformations were nearly identical in the 2 groups. In the planned-pregnancy group, the congenital malformations included 2 cases of ventricular septal defects (VSDs), 1 case of cryptorchidism, and 1 case of congenital diaphragmatic eventration. Exposure to AEDs in utero was noted in 2 cases: one infant with VSD was exposed to VPA (600 mg) and clonazepam (10 mg), whereas the one with cryptorchidism was exposed to CBZ (400 mg). In the unplanned-pregnancy group, 6 neonates had congenital anomalies, with 1 case each of fetal hydantoin syndrome (exposure to VPA [500 mg], PB [30 mg], and PHT [100 mg]), stenosis of the external acoustic meatus (exposure to VPA [800 mg] and PB [90 mg]), choroid plexus cyst (exposure to PB [60 mg]), hypotonia with widely spaced nipples (exposure to VPA [400 mg]), duodenal atresia with hypospadias (no AED

Table 4
Peripartum outcomes.

	Planned	Unplanned	*P*-Value
Induced abortion	0 (0%)	4 (4.9%)	0.16
Spontaneous abortion	2 (3.9%)	5 (6.2%)	0.71
Stillbirth	0 (0%)	1 (1.2%)	>0.99
Live birth	49 (96.1%)	71 (87.7%)	0.13
PIH[a]	2/49 (4.1%)	3/72 (4.2%)	>0.99
NRFS[a]	7/49 (14.3%)	6/72 (8.3%)	0.3
FGR[a]	3/49 (6.1%)	4/72 (5.6%)	>0.99
IUFD[a]	0/49 (0%)	1/72 (1.4%)	>0.99
TOP[b]	9/49 (18.4%)	16/71 (22.5%)	0.58
Uncontrollable seizure	2	5	
Obstetric indications	5[c]	8[d]	
Elective	2	3	
Cesarean delivery[b]	16/49 (32.7%)	23/71 (32.4%)	0.98
Uncontrollable seizure	0	5	
Obstetric indications	12[e]	12[f]	
Cerebral AVM	3	3	
Moyamoya disease	0	1	
Elective	1	1	
Others	0	1	
Operative delivery[b]	3/49 (6.1%)	1/71(1.4%)	0.30
Obstetric indications	3[g]	1[h]	
Normal vaginal delivery[b]	30/49 (61.2%)	47/71 (66.2%)	0.58

PIH: pregnancy-induced hypertension, NRFS: non-reassuring fetal status, FGR: fetal growth restriction, IUFD: Intra-uterine fetal death, TOP: termination of pregnancy.
[a] Cases of abortion were excluded.
[b] Cases involving stillbirths and abortions were excluded.
[c] Four cases with NRFS and 1 case with FGR were included.
[d] Four cases with FGR, 3 cases with NRFS, and 1 case with PIH were included.
[e] Five cases with NRFS, 4 cases involving breech presentations, 1 case involving a footling presentation, 1 case involving a low-lying placenta, and 1 case with prior cesarean delivery were included.
[f] Three cases with NRFS, 2 cases involving breech presentations, 2 cases with prior cesarean delivery, 2 cases with prior myomectomies, 1 case of placenta previa, 1 case of obstructed labor, and 1 cases involving a twin pregnancy were included.
[g] Two cases with NRFS and 1 case of obstructed labor were included.
[h] One case with NRFS was included.

exposure), and VSD (no AED exposure). There was no significant intergroup difference in the neonatal complications.

4. Discussion

In the present study, we investigated whether planned pregnancy in WWE affected seizure control in pregnancy and improved maternal and neonatal outcomes. We found that planning a pregnancy with alterations in the treatment regimen based on the currently accepted guidelines reduced the frequency of seizures during pregnancy and fetal exposure to AEDs, but did not significantly improve the maternal or neonatal outcomes.

Compared to the unplanned-pregnancy group, the planned-pregnancy group had a significantly higher rate of monotherapy and lower rate of VPA use, which reflects the clinical practice of the treating neurologists in adjusting the lowest possible dose of the most appropriate AED and switching from VPA to another AED according to the currently followed guidelines. Furthermore, compliance to medication was greater in the planned-pregnancy group than in the unplanned-pregnancy group; this may be partly attributed to the fact that the mothers were provided precise information about the risks of AEDs for the fetus and the necessity of taking AEDs to control seizure during the preconception counseling. Three women in the planned-pregnancy group chose to discontinue AEDs before conception, but restarted the AEDs according to the advice of their neurologists. Thus, preconception counseling and the active involvement of neurologists play important roles in improving seizure control in WWE during pregnancy.

In 21 cases in the unplanned-pregnancy group, the woman or the neurologist attempted to change AEDs (5/21), decrease the dose of AEDs (5/21), or discontinue the AEDs (11/21) immediately after the detection of pregnancy because of concerns regarding the teratogenicity of AEDs, which led to seizures in 11 cases (52.4%). Seizures after changing the treatment schedule occurred particularly in women who had been taking VPA (66.7%). These cases were only observed in the unplanned-pregnancy group; it is possible that the seizures would have been avoided and the fetal exposure to AEDs would be minimized if the AEDs had been adjusted before conception in a carefully planned manner. If these 11 women had not had seizures, the rate of seizures during pregnancy in the unplanned-pregnancy group would be reduced to 21.0%, which is nearly equal to that in the planned-pregnancy group. Moreover, the absence of preconception counseling may have led to the non-compliance to AED intake noted in 13 women in the unplanned-pregnancy group, 8 of whom experienced seizures (61.5%). The non-compliance and subsequent occurrence of seizures may have been prevented if these women had received appropriate preconception counseling. We would like to emphasize that adjusting medications for reducing teratogenicity of fetuses during pregnancy poses a risk of inducing maternal epileptic seizures.

A decrease in the doses of AEDs during pregnancy was required only in the unplanned-pregnancy group. Pregnancy may reduce the plasma concentrations of AEDs,[18] and therefore, any dose adjustment that is required, usually involves increasing the concentration of AEDs as the pregnancy progresses. Since this may be counterintuitive to the natural physiology of pregnancy, the neurologists may reduce the AED dose in an unplanned pregnancy, as observed in 5 cases in this study. The occurrence of pregnancy may have been so unexpected in these cases that the doses of AEDs were decreased to reduce possibility of congenital malformation of fetuses. These changes resulted in a high rate of seizures, with 40% of these women subsequently experiencing seizures. Although the exact reasons for decreasing the dose of AEDs are unknown, we recommend a decrease in the dose before conception in cases where pregnancy is planned.

There were several limitations to this study. We focused on seizures during pregnancy, and the type of seizure and its direct

Table 5
Neonatal outcomes.

	Planned	Unplanned[a]	P value
Congenital malformation[b]	4/49 (8.2%)	6/74 (8.1%)	>0.99
Birth weight (g)[c]	1467–3718	472–3985	0.66
Apgar score (1 min)[c]	1–10	3–10	0.58
Apgar score (5 min)[c]	9–10	6–10	0.11
Withdrawal syndrome[c]	0/49 (0%)	2/73 (2.1%)	0.52
Neonatal hemorrhage[c]	0/49 (0%)	0/73 (0%)	N.A.
NICU/GCU admission[c]	8/49 (16.3%)	16/73 (21.9%)	0.45

[a] Four neonatal cases involving monochorionic, diamniotic twins were included.
[b] Cases involving abortions were excluded.
[c] Cases involving stillbirths and abortions were excluded, N.A.: not analyzed.

impact on fetal or maternal outcomes were beyond the scope of this investigation. Nevertheless, we noted that epileptic seizures warranted termination of pregnancy in 2 cases among the planned-pregnancy group and in 5 cases in the unplanned-pregnancy group. Furthermore, cesarean delivery was performed in the unplanned-pregnancy group due to uncontrollable seizures (Table 4). The unplanned-pregnancy group had a higher rate of seizures during pregnancy and required more obstetric interventions due to epileptic seizures than the planned-pregnancy group. Thus, a planned pregnancy may result in reduced seizure activity and consequently require fewer obstetric interventions, such as termination of pregnancy or cesarean delivery. This study is retrospective in nature, and the women seeking preconceptional counseling are different from those with unplanned pregnancies.

The primary objective of a planned pregnancy in WWE is to avoid the use of AEDs, including VPA, which pose high teratogenic risks. In the present study, although VPA could be avoided in a significantly greater proportion of women in the planned-pregnancy group than in the unplanned-pregnancy group, the rate of congenital malformations in the 2 groups was similar. This may be explained by the following 2 possibilities. First, the other AEDs are also teratogenic, and therefore, even if VPA is switched with another AED, not all congenital malformations can be prevented. A recent study on live-born infants revealed that exposure to new-generation AEDs, such as lamotrigine, oxcarbazepine, topiramate, gabapentin, and levetiracetam, during the first trimester was not associated with an increased risk of major birth defects.[19] If older AEDs could be switched to newer AEDs before conception in a planned pregnancy, then it may be possible to decrease the risk of developing congenital malformations. Second, our hospitals are general perinatal medical centers. Many fetuses with congenital malformations diagnosed on antenatal ultrasonography are referred from nearby clinics and hospitals to our centers, and incidental detection of epilepsy in such cases may skew the results of this study. At our hospitals, the incidence of MCM is 10.7% of all deliveries, which is much higher than the incidence (1.6–2.2%)[4–6] in the general population and also higher than the percentage of fetuses exposed to AEDs (4.2–6.7%).[4,5,7–9] Hence, this institutional bias limits our study findings on fetal congenital malformations.

Although a greater proportion of WWE in the planned pregnancy group received monotherapy of AEDs and required less VPA than the unplanned pregnancy group, possibly because of the adjustments made to AED dosage by neurologists or neurosurgeons, folate prescription levels were the same in the 2 groups. The situation on the supplementation of folic acid for women of the reproductive age in Japan may be related to these results. Folic acid intake from the Japanese diet is appropriate and neural tube defects in Japan are less frequent than that in the other countries.[20] Therefore, the awareness of folic acid supplementation for women of reproductive age among medical doctors, even among the obstetricians, are lower in Japan than that in countries in Europe or Oceania as well as in other Asian countries, such as Korea or Taiwan, despite promotion by Japanese Ministry of Health, Labour and Welfare.[21] Because of these factors, neurologists or neurosurgeons may be less aware of folic acid supplementation for WWE in the planned pregnancy process, which may influence the results. Furthermore, we analyzed only WWE receiving folate prescription of 5 mg/day in the present study, and not WWE receiving over-the-counter drugs of folate supplementation (0.4 mg/day), which may also influence our results.

5. Conclusions

In WWE, planned pregnancy is associated with good seizure control during pregnancy and less fetal exposure to AEDs. In cases of unplanned pregnancy, the doses of AEDs are adjusted immediately after pregnancy is determined, which results in a higher rate of seizures during pregnancy. Although we noted that planned pregnancy may also improve maternal and neonatal peripartum outcomes, we were unable to confirm this finding in the present study.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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実地医家が知っておくべき共通知識と留意点

エビデンスに基づいた妊婦と授乳婦の薬の選択と使いかた

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はじめに

妊娠中に限らず薬剤使用は慎重にすべきなのは当然のことである．しかし，妊娠していると知らずに使用してしまった場合や診療のために薬剤の使用が必要なときには，薬剤が胎児にどのような影響を与えるのかを正しく理解して対応すべきである．妊娠中に薬剤を使用するかしないかは，使用するリスクと使用しないリスクのバランスで判断すべきであるが，リスクの評価のもととなる情報がきわめて少ないのが現状である．ここでは，妊娠・授乳中の薬剤の使い方に関する基本的な考え方を述べることとし，各論はそれぞれの項にゆずる．

そもそも薬は安全か？

薬剤を投与すべきかどうかは，その効果とリスクのバランスをみて判断される．妊婦・授乳婦に対しても「リスクを考慮しても薬剤を投与することにより得られる効果が病態の改善にとって必要である」と判断したときにのみ処方するという点では同様である．妊婦・授乳婦で特別なのは，妊婦・授乳婦へ薬剤投与を行うことにより薬剤を必要としていない胎児・乳児にも薬剤が投与されることである．すなわち，児にとっては副作用のリスクのみが負荷されることになるので特に慎重さが求められる．

薬剤を使用しなければすべての妊娠はうまくいくのか？

流産の自然発生率は15%前後，奇形の自然発生率は3%前後といわれている．また，奇形の方からみた場合，薬剤が原因の奇形は1%未満といわれている．この中には催奇形性がわかっていながらも継続しなければならない抗てんかん薬も含まれていることを考えると，催奇形性のはっきりした薬剤以外が原因となる確率は非常に低いものと考えられる．

妊娠の時期で薬剤の影響が異なるか？

妊娠週数と薬剤が胎児へ及ぼす影響との関係を図1に示す．

妊娠中に薬物治療した際の胎児への影響には，大きく分けて催奇形性(妊娠4～15週ごろの投与)と胎児毒性(妊娠16週以降の投与)がある．

a. 妊娠3週まで(all or none)

受精から14日間(本によって18日間との説明もある)は「all or none(全か無か)」の時期と呼ばれている[1]．この時期に胎児に影響を及ぼす可能性のある薬剤を使用したことにより有害な影響があった場合には，受精卵は着床しないかまたは流産の結果となり，逆に流産にならなかった場合には奇形の形で影響が残ることはないと考えられている．

b. 妊娠4～15週(器官形成期)

妊娠4～7週までは重要臓器が発生する絶対

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■ 薬剤を投与すべきかどうかは，その効果とリスクのバランスをみて判断される．
■ 流産の自然発生率は 15% 前後，奇形の自然発生率は 3% 前後といわれている．
■ 受精から 14 日間は「all or none (全か無か)」の時期と呼ばれている．

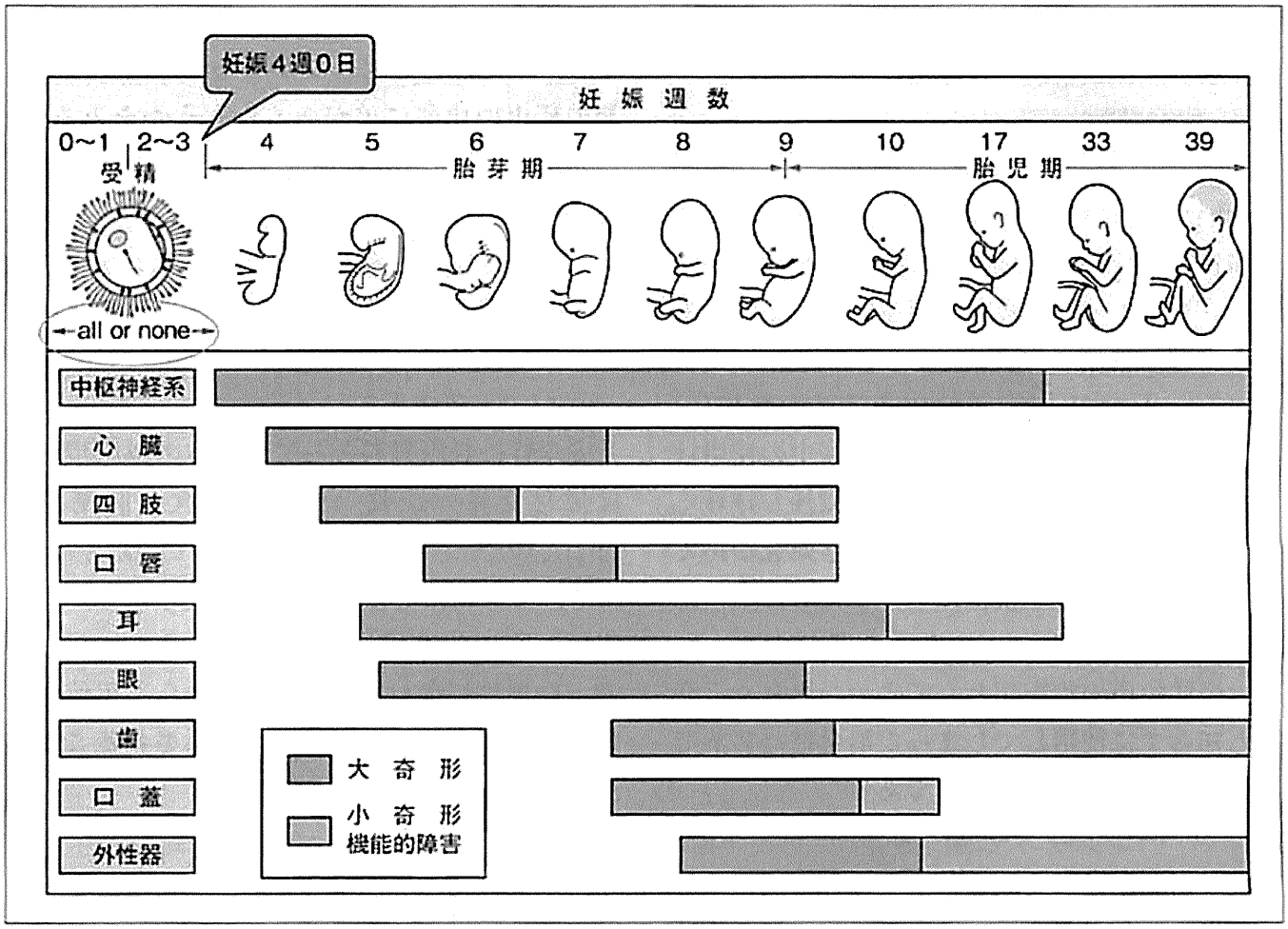

図1　胎児の発生における危険期

過敏期であり，催奇形性に対して最も過敏な時期となる．妊娠 8～15 週ごろは薬剤に対する過敏性は低下する時期だが，外性器の分化や口蓋の閉鎖が起こる時期であり，薬剤投与にはまだ注意を要する．

c.　妊娠 16 週～分娩まで

薬剤による催奇形性の心配はなくなるが胎児毒性が問題となる時期である．

妊娠中の薬剤の安全性についてどう評価するのか？

妊娠中の薬剤使用に関する安全性については，倫理上，その臨床試験を行えないため，エビデンスレベルの高いデータを出すことはむずかしい．すなわち，「この薬剤は妊娠中に使用しても安全である」ということは現実には不可

■ 動物実験の結果をヒトに応用することはむずかしい.
■ 疫学研究が動物実験よりも信頼性が高い.
■ FDA のカテゴリー分類は廃止され, 記述式で表示されることに決定し, 現在移行中である.

能である. このような状況で判断する際に根拠としている項目について説明する.

1. 動物実験

動物実験の結果をヒトに応用することはむずかしい. ある調査で, ヒトで先天異常を起こすと報告されている薬剤の動物実験における偽陰性は3%, ヒトで先天異常がないと考えられている薬剤の擬陽性は72% であったと報告されている[2]. したがって, 添付文書で「有益性投与」であっても, 発売直後の薬剤の妊娠中の使用は控えるのが無難である. 一方, 発売後しばらくしてヒトでの使用経験ならびに疫学研究が出てきても, 動物実験を根拠にいつまでも妊婦禁忌の薬剤が存在する. このような薬剤であっても, 慢性疾患の治療薬として必須な場合や, 妊娠と知らずに使用してしまった場合にはヒトでのデータを基に判断すべきである.

2. ヒトを対象とした疫学研究

疫学研究が動物実験よりも信頼性が高いことは当然のことである. ある薬剤の安全性を評価するためにはランダム化比較試験を行うのがベストであるが, 妊婦を対象としたものは倫理的に不可能である. そのためこの分野でエビデンスの高いものといえば, ある薬剤を妊娠時に使用したケースを前向きに追跡し, 対照との間に有意差がないことを証明した「前向きコホート」である. しかし, 十分な症例数を対象とした前向きコホート研究があるのは一部の薬剤であって, 大部分のものはこのような研究さえない. 一方, 前向きコホートに比べエビデンスレベルでは劣るものの, ある事象, 例えば奇形について発生した群と発生しなかった群の間である薬剤の使用の比率に差があるかどうかをみる「症例対照研究」はまれな事象を評価するには有用な手法である.

これさえない薬剤となるとケースシリーズやケースレポートを引き合いに出さざるをえないが, これらはエビデンスとはいいがたい.

3. FDA のカテゴリー分類

安全性の判断資料として, 日本の臨床現場では添付文書の記載以外に米国食品医薬品局(FDA)のカテゴリー分類(A, B, C, D, X)が重宝されてきた. しかし, これは疫学研究, 動物実験, 臨床的有用性でランクづけされたもので, 合理性に欠ける[3]との判断から4年前, この分類は廃止され, 記述式で表示されることに決定し, 現在移行中である.

催奇形性が明らかな薬剤にはどんなものがあるか? (表1)

サリドマイドは現在日本では厳重な管理がされているので問題となることはない. ビタミンA誘導体は海外でニキビの治療薬として使用されているが, それを個人輸入して使用している女性に遭遇することがある. ワルファリンは人工弁や血栓症の既往のある患者では使用している. ワルファリンを中止してすぐに妊娠する保障はないので, 「all or none (全か無か)」の時期を利用し, 妊娠が判明したらヘパリンに変更するという方法をとっている. 抗てんかん薬は

■ 妊娠末期の非ステロイド性抗炎症薬の使用は胎児の動脈管の早期閉鎖から児の遷延性肺高血圧，心不全を引き起こす．

■ ACE 阻害薬と ARB は妊娠第 2～3 三半期の投与で無尿から羊水過少となり，胎児の手足の形成異常，頭蓋・顔面の異常，肺低形成などを起こす．

■ 慢性疾患で薬剤を使いながら妊娠する場合にはその必要性を説明し，理解してもらっておくことが肝要である．

低率ではあるものの奇形のリスクのある薬剤が多いので，妊娠の可能性が出てきたら専門医による薬剤の見直しを行う必要がある．

胎児毒性(＋新生児毒性)の明らかな薬剤にはどんなものがあるか？(表 2)

妊娠末期の非ステロイド性抗炎症薬の使用は胎児の動脈管の早期閉鎖から児の遷延性肺高血圧，心不全を引き起こす可能性がある．したがって，この時期の鎮痛解熱薬としてはアセトアミノフェンが第一選択薬となる．アンジオテンシン変換酵素(ACE)阻害薬は添付文書上，妊婦禁忌である．本剤の妊娠第 1 三半期の使用の安全性についてはまだ結論は出ていないが，ほとんどの研究と症例報告では，先天奇形と第 1 三半期使用との強い相関はみられない．しかし，妊娠第 2～3 三半期の投与では胎児の低血圧と腎血流の低下による無尿から羊水過少となり，その結果胎児の手足の形成異常，頭蓋・顔面の異常，肺低形成などを起こすこともある．アンジオテンシン II 受容体拮抗薬(ARB)は ACE 阻害薬と同様の症例報告があり，同様の扱いをすべきと考える．抗レニン薬も同様の扱いとする．

生殖年齢女性への薬剤投与の基本姿勢は？

1. 慢性疾患を持つ女性に対して薬剤を投与する場合

非妊娠時には慢性疾患の管理が優先される

表 1 疫学研究をもとに催奇形性があると考えられている薬剤

薬剤の種類ないしは一般名	
サリドマイド	アザラシ肢症
男性ホルモン	女性外性器の男性化
ワルファリン	鼻の低形成
ビタミン A 誘導体	小耳症
D-ペニシラミン	弛緩性皮膚
抗てんかん薬	神経管欠損症など
メソトレキセート	頭蓋骨早期癒合，四肢異常
ミソプロストール	メビウス症候群，四肢切断
チアマゾール	頭皮欠損，臍腸瘻など
リチウム	エブスタイン奇形
ミコフェノール酸	顔面異常

が，可能であれば，いつ妊娠してもよいような薬剤選択を行う．妊娠初期の薬剤使用は児の奇形につながるとして，不安を持つ女性や家族は少なくない．順調に妊娠を継続し，母児ともにベストな状態で出産に持って行くためには薬剤で原疾患をしっかりコントロールする必要があることを説明し，理解してもらっておくことが肝要である．また，疫学研究でリスクが否定されているが添付文書上「妊婦禁忌」である薬剤，あるいはリスクがあるとわかっている薬剤であっても，疾患のコントロールと妊娠の両立のために使用しなければならない場合もある．前者の場合には，禁忌となっている根拠と疫学研究でリスクが否定的と考えられている理由をわかりやすく説明し，患者の同意があれば使用することも可能である．後者のうち，ワルファリンのように妊娠が判明してから中止するしかない薬剤では「全か無か」説(前述)を利用した計画

- 妊娠する可能性のある女性には「より安全」で，添付文書で「禁忌」になっていない薬剤を投与する．
- 母乳のメリットを考慮すると安易に母乳を中止すべきではない．具体的には抗がん剤や，放射性物質を使用しているときの授乳は禁忌と考える．
- リチウム，フェノバルビタール，エトスクシミド，プリミドン，テオフィリン，ヨード製剤は母乳を介して乳児が摂取する薬剤の量が治療域に近づく可能性があるので要注意．

表2　胎児毒性のリスクのある主な薬剤

薬剤の種類	症候
アルコール	胎児アルコール症候群
NSAIDs	動脈管早期閉鎖による肺高血圧症，羊水減少，分娩遷延
ACE阻害薬	胎児の低血圧と腎血流低下による頭蓋冠低形成や腎機能異常
AⅡ受容体拮抗薬	胎児の低血圧と腎血流低下による頭蓋冠低形成や腎機能異常
抗甲状腺薬	甲状腺機能低下，甲状腺腫
ヨード(大量)	甲状腺機能低下，甲状腺腫
精神系薬剤	出生児の呼吸障害，出生後しばらくしての離脱症状

NSAIDs：非ステロイド性抗炎症薬，AⅡ受容体拮抗薬：アンジオテンシンⅡ受容体拮抗薬．

妊娠を行う．また，抗てんかん薬のように児へのリスクがあっても妊娠中も継続しなければならない場合には継続した場合のリスクと中止した場合のリスクについて具体的にわかりやすく説明し，安易な中止や中絶を避けるような働きかけが必要である．

2．妊娠可能年齢の一般女性，妊婦に対して薬剤を投与する場合

妊娠する可能性のある女性には「より安全」で，添付文書で「禁忌」になっていない薬剤を投与するのが無難である．疫学研究でリスクが否定されている，古くから頻用されてきているということが「より安全」と判断する根拠である．

3．妊娠していると知らずに禁忌薬を使用してしまった場合

動物実験が根拠で妊婦禁忌となっている場合が多い．したがって，このような場合には成書などからの情報を収集し，科学的根拠に基づいた解釈を行ったうえで患者に説明する必要がある．自分でむずかしい場合には後述する妊娠と薬情報センターなどの相談機関を活用されたい．

授乳中の薬剤使用についてどう考えるか？

日本ではほとんどの薬剤の添付文書に「母乳へ移行するため授乳を中止するように」と記載されている．母親に投与された薬剤が母乳栄養児に作用するためには母乳に移行する，児の消化管から吸収されるという2段階を経なければならない．わずかに母乳中に移行する薬剤であっても日本の添付文書では授乳禁忌になっているが，母乳のメリット(児における栄養面・免疫力向上・肥満の予防，認知能力向上，母体における肥満の予防など)を考慮すると安易に母乳を中止すべきではない．具体的には抗がん剤や，放射性物質を使用しているときの授乳は禁忌と考える．また，リチウム，フェノバルビタール，エトスクシミド，プリミドン，テオフィリン，ヨード製剤は母乳を介して乳児が摂取する薬剤の量が治療域に近づく可能性があるので要注意だが，それ以外の場合には母乳のメリットを考えて柔軟に対応したい．

■ 妊娠中の薬剤の安全性に関する相談は妊娠と薬情報センターで行っている.

■ 授乳中の薬剤使用に関する情報は同センターのホームページで閲覧できる.

後述する妊娠と薬情報センターのホームページには授乳可能な薬剤と，授乳を避けるべき薬剤をリストにして掲載しているので参考にしていただきたい.

相談機関

妊娠中の薬剤使用に関する主な相談機関は下記の通りである.

・国立成育医療センター「妊娠と薬情報センター」(http://www.ncchd.go.jp/kusuri/index.html)

妊娠中の薬剤使用に関する情報提供ならびに症例のデータベース構築を目的に2005年10月に厚生労働省の事業として発足した[1].

・虎の門病院「妊娠と薬相談外来」

・聖路加国際病院「妊娠と薬相談クリニック」

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