Animal Experiments and Adverse Drug Reaction Reports

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3.1 Animal Experiments

The history of teratology experiments with laboratory animals goes back to the early twentieth century. They should be looked upon as tools to disturb the normal development in order to get a better understanding of the forces which control embryogenesis. Much of the early studies referred to the effect of hypovitaminosis, beginning with the studies on pigs given an A-vitamin-deficient diet which resulted in anophthalmia in the offspring (Hale 1933). In the 1940s, many studies were published on the effect of antimetabolites which could cause malformations.

Baxter and Fraser (1950) demonstrated that treatment of pregnant mice with cortisone could induce cleft palate, and similar effects have been found in some other – but not all – species studied. A strong variability in sensitivity between different inbred mouse strains was demonstrated by Kalter (1965), 12 % in the CBA and 100 % in the A/J strain. Extensive studies of this model have been made during the decades, and also other types of corticosteroids have been tested. The relatively constant findings have resulted in a belief that the same should be true also for man, and many studies have tried to investigate whether the use of corticosteroids during early pregnancy increases the risk of orofacial clefts and notably of median cleft palate in man. The results of epidemiological studies have varied, but it is possible that a slight over-risk exists but it actually seems to be valid mostly for cleft lip/palate and not for cleft palate. Anyway, there is a clear contrast between the relatively constant findings in the animal experiments and the uncertain relationship suggested in man. The animal data, however, for a long time resulted in a warning for the use of corticosteroids during pregnancy, also when they were used as an inhaled drug at asthma, a situation on which large amounts of data are now available without any certain signs of a teratogenic effect. The odds ratio for any major malformation after maternal use of inhaled glucocorticoids in early pregnancy was 1.03 (95 % CI 0.94–1.14) (Källén 2009).

Another drug which belongs to the classical animal teratogens is acetylsalicylic acid which caused cleft lip and other defects in mice (Trasler 1965) and is also

B. Källén, *Drugs During Pregnancy*, DOI 10.1007/978-3-319-40697-8_3

teratogenic in other species. In spite of the fact that single cases of gross malformations have been associated with maternal use of aspirin, proper epidemiological studies have in most cases found no such effect. An example is a prospective study with 8371 exposed infants where the odds ratio for a congenital malformation was 0.94 (95 % CI 0.83–1.06) (Källén 2009).

On the other hand, when thalidomide was identified as a strong teratogen, it took about half a year until anyone succeeded in producing a similar malformation with the drug in animals, white New Zealand rabbits (Somers 1962). Further studies have shown that primates could react in the same way as humans but that results of experiments with rodents and other common laboratory animals gave unclear results. Primates seem to be remarkably sensitive to the teratogenicity of this drug, and it is doubtful if the present protocols for preclinical testing of drugs for teratogenicity would have identified the risk associated with thalidomide.

After the thalidomide tragedy, various actions were taken to prevent a repetition. One was that it became compulsory to test new drugs in animal reproduction experiments, and such experiments were to a large extent standardized. Such tests try to identify reproductive toxicity effects which are judged likely to be valid also for humans, and drugs with such properties will not be brought to clinical testing and marketing. There will thus be no way to evaluate how predictive the tests are for the human situation. Very few drugs with a strong human teratogenicity have been identified after thalidomide – this may be the result of a high effectiveness of the preclinical testing or just an effect of the fact that such drugs actually are rare. Some drugs with positive animal tests are brought to the market, but their use in pregnant women is discouraged in order to be on the safe side. Some exposures will occur nevertheless, but obviously a moderately strong teratogenicity will be difficult to detect because of a low statistical power.

Relatively few drugs are demonstrated as definitely teratogenic for the human embryo. As pointed out above, it took some time before the teratogenic property of thalidomide (identified by alert clinicians) could be demonstrated, and the best animal models use primates, hardly suitable for routine screening procedures. Another example of a human teratogen is methimazole with a typical teratogenic activity (see above). No teratogenic activity was detected in mice or rats even at high dosage, and a preclinical testing had probably not identified the human teratogenic effect (Mallela et al. 2014). The quoted paper tended to draw the conclusion that methimazole lacked teratogenic properties; a more likely interpretation is perhaps that mice and rats are not suitable animals for a study of this problem. In other circumstances, animal studies identified risks which were later verified in human studies; the best example is perhaps isotretinoin. As mentioned above, high doses of vitamin A have long been used in experimental teratology and can cause CNS malformations, notably exencephaly, but also, for instance, eye and limb defects and facial clefts. Among different retinoids, all-trans retinoic acid seems to be a stronger teratogen than 13-cis-retinoic acid (isotretinoin), and it is thought that the teratogenic activity of the latter is the result of its metabolism to all-trans retinoic acid, a process which differs between species (Adams 1993). The high teratogenic activity in some species (man and other primates) may also be due to slow elimination and

metabolism and high placental transfer (Nau 2001). In order to mimic the human teratogenic process, experiments on other primates are needed. Actually, the teratogenic effect of high doses of vitamin A (for instance, in the mouse and rat) may not be related to the rather specific teratogenicity of isotretinoin in man.

Animal studies are useful in order to clarify the mechanism of a human teratogenic effect, seen with a certain drug. For this purpose, a suitable species or strain should be identified reacting in a similar way as man does. Experiments in rabbits and other animal species suggested various explanations of the teratogenic activity of thalidomide.

Another example is the very strong association between maternal use of valproic acid and hypospadias in male offspring. Male genital phenotype is due to an androgenic stimulation of the genital rudiments, and hypospadias can be regarded as the result of an incomplete masculinization of the genital organs. As expected, hypospadias can be obtained in animals with antiandrogenic drugs. Even high doses of valproic acid did not affect genital development in rats in experiments where an antiandrogenic drug (flutamide) did (Källen 2004). The effect of valproic acid most likely has another explanation than an antiandrogenic effect.

3.2 Adverse Drug Reaction Reports

A second result of the thalidomide tragedy was an intensification of the reporting of suspected adverse reactions of drugs, including observations of congenital malformations or other adverse effects in infants whose mothers had used drugs. Such information is collected by the World Health Organization, at present by the branch in Uppsala, Sweden. Obviously reproduction anomalies are only a small part of all adverse reactions reported. I have some experience of this system because reports in Sweden on suspected adverse reactions associated with congenital malformations or other reproductive abnormalities have to some extent been referred to me for evaluation.

There are different questions which can be raised by such a report. In some instances, the reported association is known from the literature; sometimes it is not. Given the large number of drugs used and the large number of different reproductive abnormalities which can occur, many random associations between drug use and outcome are to be expected. In many cases, this can be made likely, for instance, if the exposure has occurred too late to be able to have caused the reported malformation (see Chap. 12). In other cases, there is a possibility that a causal association exists. It *could* be a case of an "alert clinician" noting the first known teratogenic effect of a drug. The probability for an association increases, of course, if independent reports on a specific association are obtained. All such reports should therefore be stored for reference purposes. A rather high level of detail is needed in the report, notably with respect to the outcome, for instance, a detailed diagnosis of the malformation involved. Unfortunately, such details are often missing, and one has put the malformations into groups like "limb malformations" or "musculoskeletal malformations." If used correctly, this is an effective way to get an indication that a new

association between maternal drug use and infant abnormality has appeared which should initiate a study of the association in an independent material.

When the report refers to an association which is already known from the literature, another aspect may be relevant, the question of causality in the individual case. If the association is very strong, e.g., the association between use of valproic acid and spina bifida or that between methimazole and choanal atresia, the question of causality is relatively simple. If the risk increase is 20 times, 19 out of 20 exposed infants with that malformation will be caused by the drug and only one will be coincidental. If, on the other hand, the risk is only doubled, half of the associations between exposure and outcome will be random and half causal. If the risk increase is lower than that, it is more likely that an individual case is random than causal.

It does not matter if it is a rare malformation or a common malformation – the important point is the strength of the association. In the evaluation of an individual case, one also has to consider the exposure time. For example, if the drug exposure occurred during the organogenesis of the malformation in question, it is compatible with causality, if it occurred outside the period of organogenesis it speaks for randomness. Later in this book, we will come back to the problem of timing and risk estimates which are not always as simple as they may seem (Chap. 12).

There are problems in the individual case with statements that a specific drug has caused a specific abnormality. First, as just stated, it is always a question of probabilities. Second, if a drug is pointed out as the cause of an abnormality, other possible etiological factors may be ignored, e.g., a genetic risk. If an infant's microcephaly is blamed on maternal use of a drug and it really is an autosomal recessive condition, the couple may disregard the recurrence risk of 25 % and just avoid the pinpointed drug.

In Table 3.1, 27 adverse reports are summarized which I have evaluated the last year or so. It can be noted that ten of them refer to antidepressants. The use of such drugs during pregnancy is relatively common and associated with some problems related to the offspring. The risk for a congenital malformation is usually low, perhaps higher with a tricyclic antidepressant (TCA) than with a selective serotonin reuptake inhibitor (SSRI). When used in the later part of the pregnancy, temporary effects on the neonate are common, e.g., respiratory problems.

There are rather good evidence that at least use of SSRI increases the risk of a rare but serious complication, persistent pulmonary hypertension of the newborn, PPHN. In a study from Sweden (Reis and Källén 2010), a risk increase of 2–3 times was found in infants born after week 33. Among the cases listed in the table, four refer to PPHN, two of them after exposure to sertraline (an SSRI) and two after exposure to venlafaxine (an SNRI). The two cases with sertraline exposure and PPHN were more likely caused by the drug than they were coincidental. An association between venlafaxine use and PPHN has never been proved, but due to the low rate of this complication, a very large number of exposures are needed to detect an association and causality in the reported cases cannot be dismissed. There is a need for a large-scale study of maternal use of venlafaxine.

Long-term effects on the development of the child may exist but are difficult to demonstrate and interpret (Källén et al. 2013). The association between use of paroxetine

Table 3.1 Summary of some recently evaluated adverse drug reports concerning reproduction outcome

Number	Drug	Outcome	Known association
1	Venlafaxine	PPHN	No
2	Venlafaxine	PPHN	No
3	Sertraline	PPHN	Yes
4	Sertraline	PPHN	Yes
5	Sertraline	Infant liver affection	No
6	Sertraline	Neonatal respiratory problem	Yes
7	Citalopram	Megaloureter/hydronephrosis	No
8	Citalopram	Positional foot defect (2 cases)	No
9	Paroxetine, fluoxetine	Late language development	No
10	Duloxetine, fluconazole, oxazepam	Miscarriage	No
11	Valproic acid	Epilepsy, ADHD, developmental delay	Yes
12	Valproic acid	Late language development	Yes
13	Lamotrigine	TGV	No
14	Dixyrazine	ADHD and epilepsy	No
15	Perphenazine	Autism	No
16	Oxycodone, diclofenac, ondansetron	Cardiac defect	Yes
17	Misoprostol	Neonatal asphyxia	No
18	Misoprostol	Anencephaly	No
19	Magnesium before delivery	PAS, hypotonia	Yes
20	Mesalazine	TGV	No
21	Nitrofurantoin	Severe ear malformation	No
22	Fingolimod	Lissencephaly	No
23	Glatiramer	Severe brain malformation	No
24	Adalimumab	Severe cardiac defect	No
25	Vaccination against H1N1 influenza	Autism	No
26	Vaccination against H1N1 influenza	Autism	No
27	Mercaptopurine (paternal exposure)	Schizencephaly	No

ADHD attention deficit and hyperactivity diagnosis, *PAS* pulmonary adaption disturbances, *PPHN* persistent pulmonary hypertension of the newborn, *TGV* transposition of great vessels

and fluoxetine and delayed language development in the infant agrees with the results of a study by Skurtveit et al. (2014) which found an effect on language development from long-term use of SSRI during pregnancy. In the present case, exposure occurred only during months 2–3, and according to Skurveit et al. short-term use was not found to affect speech development, which speaks against causality in the reported case.

Three cases refer to use of citalopram and relatively minor and common malformations. No certain teratogenicity of citalopram has been demonstrated even though an association with cardiac septum defects has been suggested in some studies but not in other. It is likely that these cases represent coincidental associations.

Two cases refer to exposure to valproic acid and long-term effects on child development. Such effects are known to occur (Banach et al. 2010) and causality is likely. Two other cases (#14, 15) refer to long-term effects on child development from psychoactive drugs. Large studies of maternal use of such drugs and autism, ADHD, and other developmental deviations in the offspring are badly needed but offer large methodological difficulties.

One case represents the association of maternal use of lamotrigine and infant TGV. Many anticonvulsants increase the risk of cardiovascular defects, but data for lamotrigine indicate no such strong association but more information is needed. To detect a three times increase in the rate of TGV (supposing a base rate of 1/2000), one would need information on about 5800 exposures and compare them with a large control material. A similar situation exists for report #20. Mesalazine has been associated with a moderate teratogenic risk, notably cardiovascular defects. In the largest available study (Källén 2014), only 2050 exposures were included, less than half of what would be needed to have a reasonable chance to demonstrate a significant association with TGV.

The case reporting the use of misoprostol and infant anencephaly is interesting (#18). Misoprostol, when used to induce a miscarriage, has been linked to some congenital malformations but as far as I know not to anencephaly. In this case, exposure occurred in week 6 which is too late to cause anencephaly (under the assumption that dating was correct).

Case #16 represents a complex exposure situation. The nature of the cardiac defect was not known, but the child was operated upon for its malformation. Among the mentioned drugs, notably ondansetron has been linked to congenital malformations in the offspring and notably to cardiovascular defects (Danielsson et al. 2014), but this finding mainly refers to septal defects. The total risk increase for a cardiovascular defect in that study was 1.6 which means that it is more likely that the association in the reported case was random than causal.

Two adverse reports (#23, 24) refer to drugs used for multiple sclerosis and severe but different brain malformations. One infant, whose mother had previously used fingolimod, had lissencephaly; the other infant whose mother had used glatiramer during pregnancy had a frontal encephalocele, agenesis of corpus callosum, and a suspected optic nerve hypoplasia. Use of such drugs during pregnancy is very rare, and both drugs are contraindicated during pregnancy. The first exposure to glatiramer in Sweden was registered in 2003 – up to the end of 2013 there were only 39 exposures registered. Among these, three had a malformation diagnosis: one had an atrium septum defect, one a larynx abnormality, and one an unstable hip. No significant teratogenicity was thus seen in this small material – but it would take a long time to collect enough data to evaluate the reported association.

No woman giving birth had reported the use of fingolimod during early pregnancy up to 2013. In the reported case, the woman had stopped using the drug about

2 years before the pregnancy, and even if the drug has a long half-life, it seems improbable that it could cause a malformation such a long time after stopping the drug. The case is complicated by the fact that the woman had a previous pregnancy with fingolimod when a cardiac malformation (tetralogy of Fallot) was detected which resulted in an induced abortion. There is very little information on the use of fingolimod during pregnancy – a study of 28 live births showed two malformed ones, one with anencephaly, the second with a bowing of the tibia (Karlsson et al. 2014). The drug is contraindicated during pregnancy mainly because of animal data.

The association between maternal use of nitrofurantoin and infant ear malformation (#21) is probably random. No teratogenic effect of adalimumab (#24) has been demonstrated. The first exposure during pregnancy in Sweden occurred in 2008, and up to and including 2013, 55 exposures in early pregnancy are known. Two of the infants had an anomaly; one had a hydronephrosis and one a tongue tie. To get a better idea of possible risks, one would need many more exposed cases.

Two reports (#25 and 26) concern autism in children born by women who were vaccinated against H1N1 influenza during pregnancy. These reports cannot at present be evaluated because there are no studies on the subject available. It would be possible to follow a large number of infants after such maternal vaccinations in order to identify autism cases as information on such vaccinations during pregnancy is available (Källén and Olausson 2012).

The last case in the table (#27) refers to paternal exposure for mercaptopurine. We will discuss the risks with paternal exposures later on in the book. A possible effect could be an increased mutation rate caused by the drug. This could result in a congenital malformation caused by a dominant gene. In the literature, only small studies are available of male mercaptopurine exposure (Hoeltzenbein et al. 2012). The genetic background of the observed malformation (schizencephaly) is unclear, and a dominant mutation cannot be excluded.

These examples illustrate the complexity in the evaluation of reported suspected adverse reactions of drug use in connection with pregnancy. It is often not possible to state if the association between drug use and outcome was causal or not. The important thing is to collect the data and search for the repeated occurrence of associations between specific drugs and specific malformations and to follow-up such observations with proper epidemiological studies, when possible.

References

Adams J (1993) Structure-activity and dose–response relationships in the neural and behavioral teratogenesis of retinoids. Neurotox Teratol 15:193–202

Banach R, Boscovic R, Einarson T, Koren G (2010) Long-term development outcome of children of women with epilepsy, unexposed or exposed prenatally to antiepileptic drugs a meta-analysis of cohorts studied. Drug Saf 33:73–79

Baxter H, Fraser FC (1950) Production of congenital defects in offspring of female mice treated with cortisone. McGill Med J 19:245–249

Danielsson B, Norstedt-Wikner B, Källén B (2014) Use of ondansetron during pregnancy and congenital malformations. Reprod Toxicol 50:134–137

Hale F (1933) Pigs born without eyeballs. J Hered 24:105–106
Hoeltzenbein M, Weber-Schoendorfer C, Borisch C, Allignol A, Meister R, Schaefer C (2012) Pregnancy outcome after paternal exposure to azathioprine/6-mercaptopurine. Reprod Toxicol 14:364–369
Källen B (2004) Valproic acid is known to cause hypospadias in man but does not reduce anogenital distance or causes hypospadias in rats. Basic Clin Pharmacol Toxicol 94:51–54
Källén B (2009) Drugs during pregnancy. Nova Biomedical Books, New York
Källén B (2014) Maternal use of 5-aminosalicylates in early pregnancy and congenital malformations in the offspring. Scand J Gastroenterol 49:442–448
Källén B, Olausson PO (2012) Vaccination against H1N1 influenza during pregnancy and delivery outcome: a Swedish register study. BJOG 119:1583–1590. doi:10.1111/j.1471-0528.2012.03470.x
Källén B, Borg N, Reis M (2013) The use of central nervous system active drugs during pregnancy. Pharmacoceuticals 6:1221–1286
Kalter H (1965) Experimental investigation of teratogenic action. Ann N Y Acad Sci 123:287–294
Karlsson G, Francis G, Koren G, Heining P, Zhang X, Cohen JA et al (2014) Pregnancy outcomes in the clinical development program of fingolimod in multiple sclerosis. Neurology 82:674–680
Mallela MK, Strobi M, Poulsen RR, Wendler CC, Booth CJ, Rivkees SA (2014) Evaluation of developmental toxicity of propylthiouracil and methimazole. Birth Def Res (Part B) 101:300–307
Nau H (2001) Teratogenicity of isotretinoin revisited: species variation and the role of all-trans-retinoic acid. J Am Acad Dermatol 45:183–187
Reis M, Källén B (2010) Delivery outcome after maternal use of antidepressant drugs in pregnancy: an update using Swedish data. Psychol Med 10:1723–1733
Skurtveit S, Selmer R, Roth C, Hernandez-Dias S, Handal M (2014) Prenatal exposure to antidepressants and language competence at age three: results from a large population-based pregnancy cohort in Norway. BJOG 121:1621–1
Somers GF (1962) Thalidomide and congenital abnormalities. Lancet 2:912–913
Trasler DG (1965) Aspirin-induced cleft lip and other malformations in mice. Lancet 1:606