

DEVELOPMENTAL TOXICOLOGY - AN INTEGRAL PART OF SAFETY EVALUATION OF NEW DRUGS

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The thalidomide tragedy stimulated an intense research in the etiology, prevention and treatment of congenital malformations. The Government requires that drugs and food additives be evaluated pre-clinically for toxicity, including developmental toxicity, before being marketed. The number of compounds which must be tested has increased dramatically with the continuous development of therapeutic, cosmetic and food additive chemicals. Such tests include: in vitro studies which can serve as efficient pre-screens to rank chemicals for further batteries of in vivo tests on pregnant animals. However, the safety of any drug would be determined only by a post-marketing epidemiological survey. Taking into account the altered susceptibility to different drugs in a pregnant individual, it could be said that administration of any drug during the first trimester is an experiment in human teratology.

INTRODUCTION

The aim of developmental toxicology is to detect any adverse effects of xenobiotics on the pregnant female and on the development of the embryo and foetus as a consequence to exposure from starting with implantation through the entire period of gestation, parturition and maturation. The very first events began in the early years of the 20th century with experimentally-induced developmental toxicity in a mammal. In humans, the first exogenously caused (with pelvic X-irradiation) malformations were observed by Goldstein and Murphy¹. In 1941 another important discovery was made. Gregg et al were first to reported virus-caused malformations in humans (eye defects due to rubella)². Actually, clinical proof that chemical agents can induce birth defects in the human species existed before the thalidomide disaster. Some 10 years earlier, the drug aminopterin, given in high abortifacient doses, caused multiple malformations in abortuses³. Assessment of the potential for developmental toxicity has been identified as one of the key areas to be considered in the evaluation of the safety of new drugs. The field opened up after the thalidomide controversy in the 1950s when the use of thalidomide by pregnant women to reduce the discomfort of pregnancy resulted in the appearance of many cases of birth defects, particularly in Germany. Before 1960, governmental recommendations given for testing chemicals during the reproductive cycle of animals were conventional 6 weeks chronic toxicity test in male and female rodents followed over two pregnancies. Foetal survival was the main parameter measured in this so-called litter test, the tests incapable of fully demonstrating toxicity to the embryo⁴. In response to the

thalidomide tragedy, the Food and Drug Administration (FDA) and experts in the fields of reproduction and teratology issued Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use in 1966 (ref.⁵). The recommended tests were more comprehensive than the earlier ones; they specifically included testing during the teratogenic susceptibility stage of development. It has been estimated that more than 3300 chemicals have been tested for teratogenicity, with approximately 63 % having been shown to be non-teratogenic, 7 % teratogenic in more than one species, 21 % teratogenic in most species tested, and 9 % producing equivocal experimental results. In contrast, only about 35 chemicals, chemical classes, or conditions have been documented to alter prenatal development in humans⁶. A tabulation of all developmental toxicity classes of the agents considered human teratogens is shown in Table 1.

MANIFESTATIONS OF DISRUPTIVE DEVELOPMENT

We recognize four main manifestations or classes of deviant or disruptive development of the conceptus: malformations, growth retardations, embryo lethality and functional impairment. The nature and incidence of developmental defects are dependent on the developmental stage insulted. This time period "the critical period of organogenesis" varies among the species and is partly dependent on the length of gestation (for e.g. degrees of susceptibility of embryonic organs to teratogens at different developmental periods in humans are depicted in Fig. 1). Other factors influencing severity of developmen-

tal defects could be the route of administration, placental transfer, dose range and duration of treatment.

Malformations

They may be single or multiple (polymorphic). The initial teratogenic effect probably occurs through programmed cell death (apoptosis) or alteration in the rate of cell growth, but the final deformity represents not only the consequence of this direct injury, but also of secondary regenerative processes that follow it^{7,8}. The pathogenesis of malformation can usually be shown to begin as one or more overt occurrences, such as cell death, reduced biosynthesis, impaired morphogenetic movement, failed tissue interaction, or mechanical disruption. Possible mechanisms for these events include mutation, chromosomal aberration, mitotic interference, altered nucleic acid or energy sources, biosynthetic imbalance, enzyme inhibition, osmolar imbalance, or altered membrane characteristics⁹.

Growth Retardation

Foetal size is an important parameter in the assessment of potential teratogens. Reduction in size or growth retardation commonly occurs among foetuses of dams given dosages that are toxic to the dam, to the offspring, or both¹⁰. Some investigators consider overall growth retardation to constitute a state of increased susceptibility to congenital malformation¹¹. Numerous agents are capable to cause intrauterine growth retardation (IUGR) (Table 1). The limit for IUGR in humans has been defined as birth weights less than 2500 g (ref.¹²).

Embryolethality

Death of offspring is another class of developmental toxicity. Mortality may primarily be due to the direct action of chemicals on the conceptus, regardless of whether they are coincidentally malformed or not, or it may be secondary to maternal effects, and it is difficult, if not impossible, to distinguish between the two¹³. When there is undue toxicity early in pregnancy in animals, the embryo dies, is resorbed, and only the presence of the site of implantation is indicated; its counterpart in the human is miscarriage, with the product usually expelled from the uterus before the 20th week.

Functional Impairment

Birth defects can affect anatomy as well as behaviour. Historically, the first publication to examine behavioural effects from the teratological perspective was published by Haddad et al. on prenatal administration of methylazoxymethanol¹⁴. In recent years, increasing attention has been given to the more subtle, non-structural alterations produced by drugs and chemicals when given prenatally¹⁵. Examples for this kind of impairment may affect motor ability, sociability, emotionality, learning capacity etc.¹⁶.

Table 1. Developmental toxicity elicited by known human teratogens (Schardein, 2000)⁴
IUGR – Intrauterine Growth Retardation.

Chemical	IUGR	Death	Functional abnormality
ACE inhibitors	+	+	-
Alcohol	+	+	+
Aminoglycoside antibiotics	-	-	-
Androgenic hormones	-	-	-
Anticancer agents	+	+	-
Anticonvulsants	+	-	+
Thyroid-active drugs	-	-	-
Cocaine	+	+	+
Coumarin anticoagulants	+	+	+
Diethylstilbestrol	-	+	-
Lithium	-	+	-
Methyl mercury	-	+	+
PCBs	+	+	+
Penicillamine	-	-	-
Tetracycline	-	-	-
Thalidomide	+	+	+
Toluene	+	-	-
Hypervitaminosis A, retinoid analogues	-	+	-

TESTS FOR REPRODUCTIVE TOXICITY

Adverse effects of chemicals on reproductive organs and function are detected by a variety of test methods¹⁷⁻¹⁹. The most direct measurement is made from fertility trials that are part of reproduction studies. Single or multiple generation reproduction studies, having typically one or two sets of litters per generation, are most often performed in mice, rats and rabbits. The safety assessment of new drugs and other chemical substances in terms of reproduction toxicity is conducted using a one-generation reproduction toxicity test²⁰, which consists of three separate segments (Fig. 2):

- Segment I – Study of fertility and general changes during reproduction
- Segment II – Teratology study (selective embryotoxicity)
- Segment III – Perinatal and postnatal toxicological study.

These classical procedures could be followed by alternative methods^{19,21}. They are used as screening techniques for testing embryotoxic effects of the substances on in-

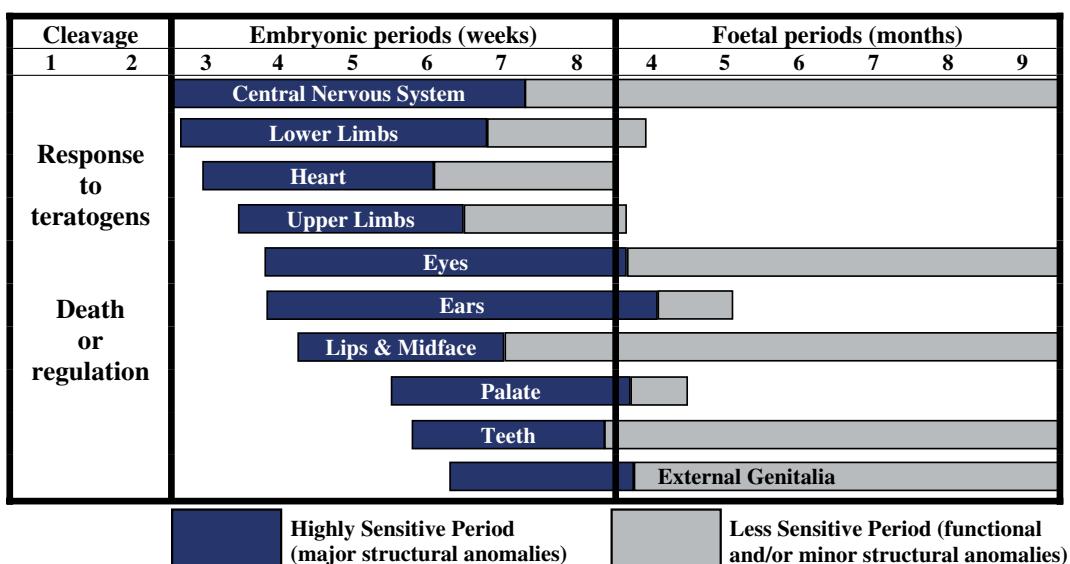


Fig. 1. Degrees of susceptibility of embryonic organs to teratogens at different developmental periods according to Wilson (1973)⁹

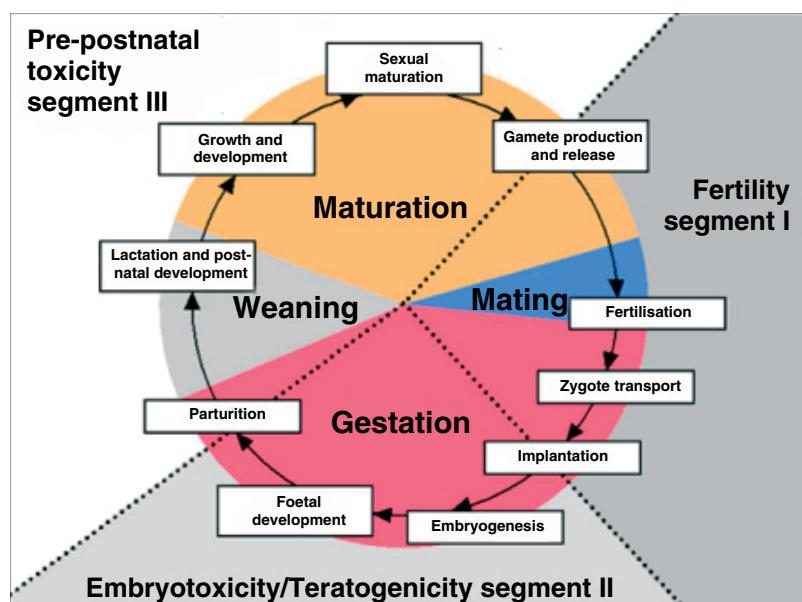


Fig. 2. Scheme of one-generation reproduction toxicity test (OECD, 1996)²⁰.

tact non-mammalian embryos (*Xenopus laevis*, *Drosophila melanogaster* and *Hydra attenuata*). Among these, the Chick Embryotoxicity Screening test (CHEST) plays an important role²². Of the test systems *in vitro* whole mammalian embryo cultures and also organ and cell cultures have to be mentioned^{17,23}.

EXTRAPOLATION FROM ANIMALS TO HUMANS

While developmental toxicants in humans produce similar responses in laboratory animals, animal studies are relatively ineffective at predicting the same type of

response in humans⁴. Many mechanisms exist by which chemicals can cause birth defects, but the critical insult which accounts for the adverse effect on the embryo is known for only a few chemicals (Tab. 1). Although it has been possible in the mouse, rat and rabbit to demonstrate teratogenic activity by all substances that have been shown to be teratogenic in humans, there is no absolute assurance that negative results obtained by testing drugs in these species can be used to predict that an agent will lack teratogenic effects in humans. Similarly, it cannot be said that agents teratogenic in high doses in these species will necessarily produce teratogenic effects in humans at therapeutic dose levels²⁴. It appears that different responses to

xenobiotics in various species, including humans, result from interspecies differences in the kinetics and biotransformation of the agent based on different enzymatic and receptor equipments²⁵.

TERATOLOGY INFORMATION SERVICES

Helping to prevent birth defects through education and research is the main goal of several organizations grouped in teratology information services. In Europe it is **ENTIS** (European Network of Teratology Information Services) helping in 16 countries, including the Czech Republic. In the USA, U.K. and Israel it is **OTIS** (Organization of Teratology Information Services). Teratology Information Services (**TIS**) are comprehensive and multidisciplinary resources for medical consultation on prenatal exposures. **TIS** interpret information regarding known and potential reproductive risks into risk assessments that are communicated to individuals of reproductive age and health care providers.

CONCLUSIONS

The reliability and extent of testing depends on the methods and experiences of the laboratory where the tests are performed. The current methodology derived from basic developmental toxicology research is able to detect the detrimental potential of the majority of substances, to identify the dose range of embryotoxicity and to determine the relationship between the dose and the effect. However, precise extrapolation will require not only knowledge of the specificity of the human xenobiotic metabolites but also the identification of the sensibility of the morphogenetic systems and their distribution in the time horizon of the development of the human embryo²⁶. Finally, safety of any drug would be determined only by the post- marketing epidemiological survey.

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