The contribution of new findings and ideas to the old principles of teratology

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Abstract

Although the last generally accepted concept of principles of teratology was issued more than 30 years ago, the cause of less than 50% of all congenital anomalies is known and no substantial change in their incidence has been observed worldwide. In the meantime, powerful techniques of molecular biology as well as many sophisticated preventive measures have been introduced with marginal effects on the overall birth defects numbers. In this paper, we follow the history of basic concepts of teratology starting with Isidore Geoffroy Saint-Hilaire and Dareste, followed in the 20th century by James Wilson. Since that time no bright and completely new idea, which would deserve the name principle, has emerged. The advanced molecular studies support the long-existing principles and disclose the great variability of individuals and their responses to adverse exposures. In this way, the future of teratology counseling may be seen in a deep analysis of any individual case.

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Contents

1. Introduction ................................................................. 295
2. The old principles of teratogenesis ........................................... 296
3. The principles of teratogenesis revisited. ................................. 296
4. The contribution of new findings ....................................... 297
5. What we learn from the principles of teratogenesis even now ................ 298
6. Conclusion .............................................................. 299
Acknowledgement ......................................................... 299
References ..................................................................... 299

1. Introduction

Sourcing from the detailed observations in the golden age of descriptive teratology (1750–1850), and ideas of several remote and immediate predecessors (for instance Morgagni, 1682–1771, Haller, 1708–1777 and Etienne Geoffroy Saint-Hilaire, 1772–1844), teratology as a science was founded by Isidore Geoffroy Saint-Hilaire during 1832–1837 [1]. Authoring three volumes of his famous monograph, which is considered the first scientific discourse on congenital malformations, he shifted definitely the primarily theological or metaphysical issue to a purely natural one. Following his own words, teratology has become a "special branch of
the great science of organization’. Nevertheless, it still had been waiting for some unequivocal experimental evidence that was established 40 years later by Camille Dareste in his ‘Recherches sur la production artificielle des monstruosités ou Essais de tératogénie expérimentale’ [2]. Dareste was the first to formulate several conclusions of general character based upon his own experimentation on chicken embryos, providing firm grounds to the majority of future hypotheses and theories of teratogenesis. Complementing the generalizations of Dareste by Schwalbe’s [3] concept of teratogenic termination periods, and Stockard’s [4] critical moments (periods) of development, we are facing the whole theoretical basis of teratology that has not changed substantially until the present times.

It is true that teratology failed to celebrate any triumphant success even when applying powerful techniques of molecular biology and genetics, similarly as having at disposal the basic knowledge of the human genome. Many sophisticated preventive measures have been introduced, ranging from diagnostic DNA probes and microarrays, to highly efficient ultrasound devices, but the incidence of birth defects has remained practically the same all over the world. As soon as in 1981, this discouraging and long-lasting situation provoked Josef Warkany [5] to his famous and frequently quoted claim that ‘there may be something wrong with the way we now (rem. 1981) approach the problem of congenital malformations…’. Despite thousands of papers in distinguished periodicals, hundreds of workshops and conferences, and painstaking efforts of teratological information services all around the world, the Warkany’s claim is still valid, and the cause of less than 50% of all inborn defects is known. A half of the rest is classified as ‘multifactorial’, which means a pretty fuzzy definition. Either it means that some of the fundamental principle inherited in teratogenesis still escapes our attention, or that our interpretation of some of the yet known principles remains misleading. The third possibility is mostly pessimistic: individual development may be so complicated and dependent upon an intricate tangle of too many interacting factors that it is hopelessly prone to innumerable spontaneous errors, being thus unforeseen and unpreventable. Before accepting the last sentence, at least for some near future, we would like to investigate the former ones.

2. The old principles of teratogenesis

The foundations of scientific teratology were laid down in the same era as those of genetics that is at the time of Johann Gregor Mendel (1822–1884), and were apparently influenced by his thoughts. If one goes through the first two Dareste’s [2] principles, adapted somewhat to modern terminology, he will come to the third one, which sounds really ingenious. The five principles are, as follows:

1. Identical defects can be induced by administrating different agents.
2. Particular embryos react to an adverse condition in a dissimilar way.
3. The dissimilarity is caused by unequal combination of inherited gifts and extrinsic influences.
4. Type of defect depends upon the strength and time of action of adverse impulse.
5. The smaller the defect, the later it becomes apparent.

Besides the fact that all these principles are valid even today, they presume implicitly both the concept of critical periods and dose–response relationships. Beyond doubts, they inspired James Wilson to restate in more precise terms initially five [6] and then six [7] generalizations describing typical occurrences:

1. Susceptibility to teratogenesis depends on the genotype of the conceptus and a manner in which this interacts with adverse environmental factors.
2. Susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence.
3. Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis).
4. The access of adverse influences to developing tissues depends on the nature of the influence (agent).
5. The four manifestations of deviant development are death, malformation, growth retardation and functional deficit.
6. Manifestations of deviant development increase in frequency and degree as dosage increases, from no-effect to the totally lethal level.

It is easy to follow the continuity of Wilson’s generalizations with the Dareste’s principles over a century gap. As Wilson mentions ‘… with increased understanding of teratogenic mechanisms, it will be necessary to formulate additional principles and to revise those now formulated’. To our disappointment, no new principles were generally recognized, since and what more, some of the old ones either fell into oblivion or failed to be respected.

3. The principles of teratogenesis revisited

The principles of teratogenesis revisited was the title of an invited lecture read at the Second Meeting of the International Federation of Teratology Societies held on July 14–16, 1988 in Kyoto, Japan. Briefly, the concept of the author [8] was based upon the idea that the basic unit of both individual development and teratogenesis is not a single cell; however, the morphogenetic system, defined as a set of cell populations, carrying, creating and executing the program for the development of a definite part of an organism [9–11]. The definition needs, of course, some explanation. The development of any structure starts with proliferation to produce a critical mass of cells having, in the beginning, a similar
the existing problems. I wish to quote three of them: some of the principles formulated threw some new light upon from following the rest of the concept. It was a pity, because “morphogenetic system” deterred perhaps most teratologist concept of “developmental fields” [12]. An unconventional term although bearing many similarities with the Opitz’s con-

their consistent expression failed to attract any attention, and that has been probably the reason why the attempt for translated or reformulated the other principles of teratogenesis, the actual paradigm.

regeneration. Basically, this concept stays in harmony with tent stem cells unaltered as a source for replacement and/or cells develop lineages of multipotent, progenitor, precursor and eventually mature cells, leaving some of the multipo-
tent stem cells unaltered as a source for replacement and/or regeneration. Basically, this concept stays in harmony with the actual paradigm.

Upon the idea of morphogenetic systems were formu-
lated or reformulated the other principles of teratogenesis, and that has been probably the reason why the attempt for their consistent expression failed to attract any attention, although bearing many similarities with the Opitz’s con-
cept of “developmental fields” [12]. An unconventional term “morphogenetic system” deterred perhaps most teratologist from following the rest of the concept. It was a pity, because some of the principles formulated threw some new light upon the existing problems. I wish to quote three of them:

1. Independently of the limited phases of ontogeny called critical morphogenetic periods [4] when particular develop-

mental defects can be induced by exogenous agents, only, there exist the so-called sensitive periods defined as developmental phases during which differentiating cells become susceptible to a given toxic agent. The extent of any sensitive period is dependent of the dose and the nature of the agent. The agent is capable to induce the develop-
mental defect when and only when the sensitive and the critical period overlap.

2. Exposure conditions of morphogenetic systems in case of chemical agents are determined, besides the dose, strictly by pharmacokinetics and biotransformation in the mater-

nal organism exhibiting extreme interspecies, intraspecies and individual differences dependent, among others, upon genes of the mother.

3. While the manifestations of deviant development (i.e. malformation, growth retardation, death) always exhibit, in their sum, positive dose–response relationships, this is far from being true for inborn defects. The incidence and severity of the defects initially grow with the intensity of embryotoxic impulses, but after reaching a certain limit, their absolute numbers starts to drop. Malformed concep-
tuses become progressively replaced by the dead ones. Because of that it is better to prefer the universal term embryotoxicity for denoting all manifestations of deviant development.

Ad 1) The first statement was built upon the observation that the teratogenic effect of glucocorticoids in chick embryos was dependent upon the presence of glucocorticoid receptors within target tissues [13,14]. Ad 2) The second statement was derived from a large series of experiments in mice and rats concluding that a susceptible embryo may develop within a drug-resistant mother and vice versa [15–17]. Ad 3) The third statement (3), which has only adjusted the sixth Wilson’s principle was based upon a long-lasting experience with the Chick Embryotoxicity Screening Test [18]. The validity of this principle in humans was probably foreshadowed in the Seveso accident (1976), where no major birth defects [19] and more abortions were found in the most dioxin-contaminated regions.

From time to time the Wilson’s principles have been quoted, perhaps in a slightly modified form, in various mono-

graphs or papers of intended strategic value; however, none of them has been left or added [20]. The great impact of molecular biology in every branch of medicine brought about many data of key importance that have almost unequiv-

ocally confirmed the known principles of teratology and contributed to much deeper understanding of the underlying mechanisms.

4. The contribution of new findings

Now it is clear that all reactions of the cell are medi-

ated through its genome. Consequently, most birth defects have an underlying genetic basis, ranging from single genes playing dominant or recessive roles in Mendelian disorders to a majority, which are the result of interaction between multiple genes and environmental triggers in complex traits. The Mendelian heredity is common in the inborn errors of metabolism underlain by a mutation in a sequence cod-
ing for a particular enzyme. Here, the mechanism is clear, and handling with such cases is in a full competence of genetic counselors and, possibly, it may become a field for genetic engineering. The second group is, however, much more complicated. Since adverse agents can affect practically an unlimited number of genes, it seems necessary to define both the key ones, regulating development of the conceptus and those, responsible for pharmacokinetics and biotrans-
formation within the maternal organism. First steps of such painstaking procedures were already done, however, the con-
clusions do not seem very optimistic. For instance, expression of >50% of the approximately 12,000 genes and expressed sequence tags examined in one study [21] was detected in developing murine orofacial tissues and the expression of several hundred genes was up- and down-regulated. Some of the other studies brought reasonable conclusions, espe-
cially those dealing with sonic hedgehog (SHH). It was found
that perturbation of its signaling pathway can result in holo-prosencephaly [22], and such perturbation may be induced by certain alkaloids long known to produce such defects in sheep. Molecular mechanisms were proposed in several known teratogens, as thalidomide, retinoids and valproic acid [23]. It has also been suggested that genetic differences in fetal metabolism caused by methylene reductase polymorphism may account for the increased risk of neural tube defects in epileptic women treated with anticonvulsant drugs [24]. Such results are very important because they contribute in implementing such a general and therefore vague term as “multifactorially conditioned congenital malformations”. It is generally expected that deeper insight into changes in gene expression induced by teratogens will offer greater promise of better diagnosis and prevention of birth defects originating by interaction of adverse exposures and specific allelic variants.

Genetic variants have been identified in more than 20 human drug-metabolizing enzymes [25], which underlie different responses of the individuals to drug treatment and consequently also the differences in teratogenic risk for their progeny.

A significant progress has also been made in one of the often discussed topics in teratology—the existence of dose-response in exposed human beings. The low number of cases, dependence on individual body mass and variation in pharmacokinetic parameters within the narrow dose range made the human studies hopelessly difficult. Beyond doubts, a strong source of bias might be inherent in neglecting the specific character of dose-response relationships in teratology (see above). Accounting exclusively with birth defects, a positive association can be expected in the lower segment of the dose–response curve, only. Therefore, it is not surprising that the dose dependence was eventually disclosed in weak and widespread teratogens: cigarette smoking, alcohol and retrospectively with ionizing radiation in course of Hiroshima A-bombing [26].

In course of the 20th century many chemical substances were found to have teratogenic properties. The Shepard’s catalog of teratogenic agents mentions about 1200 compounds producing birth defects in experimental animals with only a log of teratogenic agents mentions about 1200 compounds were found to have teratogenic properties. The Shepard's cat-

5. What we learn from the principles of teratogenesis even now

Strictly followed they can contribute both to detection of teratogens as well as to teratology counseling. It is generally accepted that assessing the risk of a substance to humans we need today:

(a) Casuistics describing repeatedly a typical malformation pattern (syndrome).
(b) At least two large and well controlled epidemiological studies.
(c) Results of testing in laboratory mammals following the Good Laboratory Practice (GLP) and WHO criteria. This item, however, serves only for confirming biological plausibility.

Usually, we get the information in the order animal data, casuistics, epidemiological studies. In newly marketed drugs results of animal testing, basic toxicological data and those on pharmacokinetics are the only available, mostly from manufacturers. Regularly a new drug is not recommended in pregnancy and/or childbearing age because of the unknown risk to humans. If this occurs, one has a chance at least of comparing the ratio between the embryotoxic dose estimated in the experiment and the intended (exposure) dose in humans, as well as the ratio between the embryotoxic dose and the dose toxic for the dam. Although burdened with species specificity, it may bring a very rough orientation. Further, we must wait for human data, the source of which mostly is unwanted exposure. It is true that most of the existing teratogens were disclosed by casuistics published by alert physicians and not through applying proper scientific methods, however, at any rate the casuistic must be considered a signal triggering a systematic investigation. It is recommended to make sure that the defect or components of a syndrome described correspond to the time of exposure.

The most desirable source of data remains the epidemiologic study which, accurately performed, is believed reliable. However, when one goes through the nowadays favorite form of meta-analyses it becomes apparent that the results of individual studies are subjects of a large variation. To confirm this statement, it is sufficient to overview the Reprotox database (www.reprotox.org). The studies differ in size, design, overall methodology and statistical treatment, always aimed at the same purpose: evaluating thoroughly the difference between the exposed and control groups. Because of the key position of epidemiological studies in teratology risk assessment we may ask whether the past principles and new ideas may contribute effectively to their standardization, effectiveness and validation? What are their known and ever repeating flaws?

• The majority of epidemiological studies are focused on a single and least occurring manifestation of deviant development, inborn defects. Including as many indications as possible, such as elective pregnancy terminations before 20 weeks will increase the discriminating power of the studies [27].
• Only seldomly, and not very systematically the epidemiological studies take account of the existence of well-defined human critical periods in the course of which the particular inborn defects can be induced by exogenous factors. This is further complicated by the fact that the confusing term “gestational age” is generally either not defined or is used for postmenstrual age, postovulatory age or postfertiliza-
A very convenient way seems to make use of Teratology Information Services (TIS) all around the world, defined on molecular level. The more we adhere to the modern opinion explaining relevant data as possible. We must fully agree, therefore, with Merlob and Stahl[32] in that the classification of drugs for teratogenic risk must be multifactorial and could not be limited to the qualification (if known) of a single factor, only. We feel strongly the necessity of analyzing the intraspecies differences in mice. Folia Morphol (Prague) 1986;34:69–77.

References


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