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Review Article

Preclinical Evaluation of Drugs for Evidence of Teratogenic Activity

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"If certain congenital malformations can be attributed in certain animals to dietary deficiency, anoxia, cortisone, or genetic constellations, one must not conclude without further proof that comparable malformations in man are due to similar adverse conditions. Such premature conclusions, usually not drawn by the experimentor but by a reader whose imagination and beliefs exceed his knowledge, can create superstitions in modern garb. If such a reader is also a writer with access to medical or popular journals, his unfounded beliefs are carried to millions, whereby new superstitions are established. . . . A single observation of an abnormal child born to a mother who had been in an automobile accident, becomes, if reported in a popular magazine, psychologically, a hundred thousand observations which seem to establish a causal relationship between two events. The whispered word is powerful, but the written word endures." Joseph Warkany, National Foundation Conference on Congenital Malformations, 1959 (1).

THE CIBA FOUNDATION SYMPOSIUM on Congenital Malformations (2) and reviews by Wilson (3) and Giroud and Tuchmann-Duplesis (4) refer to many agents that have been shown to have teratogenic effects in animals. These vary from anticancer drugs to essential nutritional elements as shown by the following:

TERATOGENIC AGENTS FOR ANIMALS

Alkylating Compounds
- Chlorambucil
- Busulfan (human)

Cytotoxic or Antimitotic Agents
- Actinomycin D

Nutritional Factors

Deficiency
- Oxygen
- Riboflavin
- Pantothenic Acid
- Folic Acid
- Vitamin A

Antimetabolites
- 6-Aminonicotinamide
- Methotrexate

Physical
- X-ray (human)
- Nuclear
- Ultrasonics
- Trauma

Excess
- Vitamin A
- Carbon Dioxide

Infectious Agents
- Rubella (human)
- Influenza (?) (human)

Some drugs used to treat cancer are effective because of their depressant effects on rapidly proliferating cells and, therefore, may have an adverse effect on the rapidly growing animal or human embryo. Similarly, antimetabolites, by interfering with essential nutritional materials such as vitamins, could be expected to have teratogenic properties. The effect of German Measles, or Rubella, on the human fetus is well known, but the effect of other viral agents such as the influenza virus (5) has not been substantiated.

Some other well known agents have been

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shown to have teratogenic properties in animals but do not have any proven effect on the human fetus.

TERATOGENIC AGENTS FOR ANIMALS

Penicillin	Salicylates
Chlorpromazine	Trypan Blue
Prochlorpemazine	Galactose
Hypoglycemia Sulfonamides	Thalidomide (human)
Insulin	Pilocarpine
Chlortetracycline	Sulfanilamide

Seemingly, thalidomide is the outstanding exception. Other drugs, as an aftermath of the thalidomide incident, have been accused of being teratogenic for man, but a causative relationship has not been proven to the satisfaction of all. No doubt many other drugs will be accused of producing malformations in humans.

Warkany (6) stated the problem clearly when he said:

> "We should be very cautious in making general statements about these drugs. One could easily make a mistake and accuse a drug wrongly. The cases in which a drug has been proven to be teratogenic in humans are very few. Dose and time play a role, and probably also the genetic constitution. But, if we are not careful, we may hear very soon that aspirin causes malformations. This should not happen since our animal experiences are usually very different from therapeutic attempts in man."

Murphy (7) pointed out that in the embryo the outcome of a drug effect, under the conditions of a limited number of doses—

> "Depends also on the specific drug as well as the quality and density of events taking place within the embryo. . . . The choice of a mammal with a longer gestation time might be expected to accomplish a decrease in the density of events taking place during a given time period and, therefore, decrease the probability of a foetal abnormality from a single dose of a growth-inhibiting agent. On the other hand, choice of an animal with a much shorter period of gestation would accomplish the purpose of increasing the density of events occurring during a given period and increase the probability of abnormalities."

This would suggest that, of the laboratory animals commonly used for preclinical evaluations of safety, the mouse would be the ideal species for testing for the teratogenic properties of a drug and the dog would be least valuable.

However, the conditions employed in the more academic teratogenic studies may not apply to the preclinical evaluation of safety since the drug should be administered daily in reasonable, nonstressing doses.

The problem of evaluating the safety of a drug for the fetus is not new and is similar to that routinely faced by the toxicologist and the clinician when a new compound is evaluated for its safety for initial clinical trials. The nature of the toxicity of a drug for the animals may be well known and on the basis of this knowledge and the other biological properties of the agent, a scientific judgment must be made of the relative risk for its trial in man. In the same way, as Beyer (8) pointed out—

> "Since such a substantial list of important agents can elicit fetal anomalies under laboratory conditions, the relevance of such findings to the prognostication of similar clinical outcomes requires a broad basis for judgment. Depending on methodology and experimental design, an important teratogenic action of one compound could be missed and another might be indicted unjustly."

The toxicologic and teratogenic evaluations of a drug are interrelated and require the same general considerations of its chemical and biologic properties and proposed clinical use. It may be useful then to discuss briefly the principles of the toxicologic evaluation of a new drug since these principles will apply also to the teratogenic evaluation of that drug.

The toxicologic, or safety, evaluation of new drugs in animals encompasses a great deal more than the determination of acute lethal doses and the daily administration of relatively small doses which may provide a relative "margin of safety." It is important also to produce toxicity in subacute and chronic studies. As the complexity of the biologic properties of drugs increases and as our knowledge of the mechanism of action on these drugs increases, the testing for possible adverse side effects can expand also to provide greater assurance of safety and to alert the clinician to potential toxic effects in the human. Factors which should be considered in the safety evaluation of therapeutic agents are:

- Chemical structure and properties
- Route of administration
- Reversible alterations
  - Primary and secondary biologic attributes
  - Endocrinologic properties
  - Biochemical properties
  - Organ function
  - Normal body constituents
  - Anatomical changes
    - Gross and microscopic
    - Organ weights
- Metabolism of the drug
  - Degradation
  - Conjugation
  - Elimination

Clinical
Proposed use (disease, dose, duration of treatment, etc.)
Possible misuse

Thus, the safety evaluation of a drug may now include an overall consideration of its chemical properties, its pharmacologic, endocrinologic or biochemical attributes, its effects on normal body functions and normal body constituents, the manner in which the body excretes or degrades the agent, and the potential of the drug to produce reversible changes of body function or anatomical structures. Any of these properties might be a primary attribute of the drug and the activity necessary for its therapeutic use. In addition, the proposed clinical use, and possible misuse, of the agent must be considered. In general, the more serious the disease, the more risk is acceptable; conversely, if a disease is of minor consequence, the drug must have a large safety factor.

TYPES OF UNDESIRED EFFECTS

The types of undesired effects of a drug may be grouped into four general classes: (*a*) exaggerated primary attributes, (*b*) secondary attributes, (*c*) hypersensitivity, and (*d*) "true" toxicity.

The first class includes the undesired effects due to overdosage, or to an accentuation of the desired effects of the drug. Some individuals may be unusually sensitive to the drug so that an average therapeutic dose may become an overdose. Occasionally, the interaction of two or more simultaneously administered drugs may result in an accentuation of the biologic attributes of one of the drugs.

The second class of side effects includes those related to the secondary biologic attributes of the agent. The dryness of the mouth caused by anticholinergic agents, the nausea and respiratory depression caused by the narcotics, and the sedation produced by some antihistaminics are examples of these. Such side effects are usually reversible when the drug is withheld and are not necessarily detrimental to the health of the individual. Nevertheless, they are disturbing and, thus, there is always an effort on the part of the organic chemist and the pharmacologist to synthesize new compounds (molecular manipulation) to reduce the secondary attributes, and to increase, relatively, the effect of the primary attribute.

The third class, hypersensitivity, includes a large group of undesired side effects resulting from the sensitization of an individual to the drug. These side effects may be severe, as in anaphylaxis, or may be relatively minor, as in a skin rash. Unfortunately, there is no reliable methodology by which the incidence or type of hypersensitivity can be predicted by presently known animal experiments.

The most important class of side effects is that of the "true toxicity" of a drug. This toxicity may be unrelated apparently to any known pharmacodynamic activity of the drug and, therefore, may be unpredictable from the results of the pharmacological studies. As a general rule, the production of these important side effects requires daily administration of the drug and, thus, they are not disclosed in the short term pharmacologic studies where only one or two doses may be given. Indeed, several months of daily administration of large doses may be required to produce a toxic effect. A relationship between incidence of toxic effects and magnitude of dose is usually present, thus permitting an estimation of a "margin of safety." The "true toxicity" may result in reversible or irreversible lesions and may be detrimental to the health of the individual.

One of the functions of the pharmacologist in the safety evaluation of a new drug is to set the probable clinical dose and to predict the side effects of the first two classes. The function of the toxicologist is to demonstrate the hazards related to the first three classes and, of most importance, he must determine the potentialities of the drug with regard to the production of "true toxicity" and also must make an evaluation of its effect on reproduction.

To fulfill these functions, the toxicologist assumes that all agents are toxic if examined under the proper conditions using appropriate parameters of observation. The toxicity of a drug is examined by the acute administration of lethal doses and by the daily administration of moderate and large doses for short (subacute) and long (chronic) periods of time. He may use several routes of administration but the major route is that which is to be used clinically. Two or more species of animals may be used in both the long and short term studies since there may be species differences in sensitivity to the drug. The toxicity found in these studies may be biochemical, hematologic, endocrinologic, enzymatic, anatomic, etc., or any combination of these.

The pharmacodynamic activity of the drug may be such that the primary or secondary attributes limit the toxicologic investigations because sufficiently large doses cannot be used to produce the "true toxicity." If, for example, a

sedative action is sufficiently great, the animal cannot eat or drink properly and, since prolonged fasting or starvation can cause biochemical and anatomical alterations, it may be difficult to determine if starvation effects are related to starvation or to the "true toxicity" of the drug. In other words, in the safety evaluation of a drug, the toxicologist cannot permit primary or secondary drug effects to alter the animal sufficiently that the end results may be attributed incorrectly to a toxic effect of the agent, or, that a "true" toxic effect may be hidden by these indirect effects. In these cases, the toxicologist must limit the magnitude of the doses to levels that will permit a relatively normal daily activity of the animal.

TERATOGENIC STUDIES

All of this may appear to be far from the subject of evaluation of teratogenic effects of drugs. However, the preceding remarks provide a basis for the principles of the evaluation of the teratogenic properties of an agent. Factors such as anorexia, hormonal changes, or sedation, which can be primary attributes of drugs, may have an effect on reproduction or on the fetus and must be considered. It certainly would not be reasonable to give such large doses of an agent that anorexia would occur for long periods of time and then to expect to have a normal reproduction cycle and normal fetuses. Similarly, if one is examining the teratogenic effects of a barbiturate in a study which requires dosing prior to mating, the dosage must be sufficiently small, or temporarily stopped, so that sedation will not interfere with mating.

There are three major types of teratogenic studies that can be employed for safety evaluations of new drugs:

Established pregnancy
  Single dose
  Multiple dose
Single generation (breeding) study
  Drug administration prior to mating and throughout pregnancy
  Two or more litters by same female
Multiple generation studies
  Drug administration prior to mating and throughout pregnancy
  Young animals fed medicated diet after weaning and mated when mature
  Two or more generations produced

The first is the administration of drug to animals in which pregnancy has been established. Drug administration is initiated, usually several days after conception, and may be continued for several days or as long as desired. Such studies are of relatively short duration and are particularly useful in the larger animals. Here, there is no possibility that the drug will interfere with fertility, mating, or implantation of the ova. The study can be continued through delivery, lactation, and weaning. Single doses may be of no real value at an early stage of development of a new drug. Indeed, this type of study may be hazardous because a teratogenic effect of an agent may be limited to a very brief phase of embryonic or fetal development and thus missed if the single dose is given at the wrong time.

The second type of teratogenic study includes more than just testing for teratogenic effects and may be called a "single generation study" or "breeding study." In this experiment, both the male and female animals are given the drug daily for some period prior to mating (60 days has been suggested), and drug administration is continued throughout pregnancy and, if desired, until the pups are weaned. Holding the pups until weaning may reveal types of malformations which would not be evident if the animals were sacrificed and examined at birth. The breeder animals continue to receive drug and, after the first litter is weaned, should be remated to produce a second litter. In some instances, part of the mothers may be sacrificed just before delivery and the fetuses examined. If a normal incidence of pregnancy is not obtained, the treated female animals may be mated with control male animals and treated male animals with control female animals to determine if the fertility of one sex is impaired. The large number of parameters that can be evaluated point out the usefulness of this type of study. In addition, this reproduction study can be conducted as a part of the chronic toxicity studies. Thus, in single or multiple generation reproduction studies it is possible to evaluate

Fertility of male and female,
Implantation of the ova,
Development of the embryo and fetus,
Resorption,
Abortion,
Delivery,
Live births,
Size of litters,
Teratogenic effects,
Viability of the newborn,
Growth of young,
Quality of mother's milk (nutritional and toxicity).

A third type of reproduction study is the multigeneration experiment. Here a litter is produced, the pups are permitted to mature, and are mated to produce a new generation. Drug administration is started for the original parents, prior to

maturing, and continued for each generation until the desired number of generations have been obtained. This cycle can be repeated as frequently as desired, precautions being taken to prevent mating between litter mates. The parents of each generation may be discarded when the young are weaned. An evaluation of possible genetic abnormalities produced by an agent may be detected by this type of experiment as well as the parameters listed in the second type of study mentioned previously. These studies are necessarily of relatively long duration, depending upon the reproduction cycle of the species being used. The mouse is probably the best species for this type of test because of the relatively short reproduction cycle, rapid maturation, and the large number of animals that can be employed.

In all of these studies, the young animal can be examined in a number of ways. Gross examination of the fetus, or the newborn, clearing and staining of the fixed animal, X-ray studies, and autopsy examination permit an evaluation of teratogenicity. The specimen can be prepared for microscopic examination also. Enzyme studies can be made of the fetus or the newborn. Acute toxicity studies may be conducted on the control newborn or weaned animals to determine age differences of drug toxicity.

The use of an adequately large control group of male and female animals is a must for all teratogenic, reproduction, and routine toxicity studies. Incidental lesions occurring within animal species are so frequent that, unless there are enough controls, certain types of lesions (or fetal malformations) may be difficult to evaluate with regard to drug effect.

The conduction of these studies appears to be rather straightforward. However, because environmental factors may interfere with normal reproduction cycles in some animals, it is advisable to use quiet draft-free rooms with well regulated temperature and humidity. It is advisable also to have special personnel assigned to such studies so the animals will be accustomed to them and will not be disturbed at critical times such as delivery. Variations in litter sizes, fertility, and percentage pregnancies may complicate the interpretation of results. Thus, it may be wise to obtain a first litter without treatment, although this adds considerably to the duration of the experiment. It is also important to know, if possible, the normal incidence and types of malformations that occur in the species and strains of the animals being used.

A useful collateral study is the determination of the transfer of the drug across the placental membrane. Such a test might determine the best species to be used in a teratogenic evaluation of a drug, since the value of a teratogenic study is questionable if the drug is not transferred across the placenta to the fetus. It is reassuring to be able to give large doses of a drug to a pregnant animal without evidence of any effect on the reproduction cycle, or on the fetus, when it is known that large amounts of drug, or its metabolites, actually reach the fetus.

As indicated earlier, the dosage of the drug should be selected so that the secondary pharmacodynamic effects will not interfere with conception or nutrition (9) and activity of the mother. In addition, since some agents may interfere with normal biologic functions as a primary attribute, this type of effect on the fetus must be judged carefully. In other words, the doses selected should take into consideration the therapeutic and tolerated doses and not be so far above the therapeutic dose, or so close to the toxic dose, that the reproduction studies cannot be interpreted meaningfully. For example, by using a single maximally tolerated intraperitoneal dose of caffeine, Nishimura (10) obtained an incidence of 18 to 43% malformations in the mouse. Thus, there is a possibility that the occurrence of malformations at unreasonably high doses might result, unjustly, in the rejection of an otherwise valuable agent.

Other methods of teratogenic studies may not be applicable because the conditions of these tests are not comparable to the conditions obtained in the uterus of the intact mammal. Thus, the chicken embryo (11–14), the seaurchin (15, 16), the isolated fertilized rabbit egg (17), and the zebra fish egg (18, 19) may be valuable for certain aspects of teratogenic studies, but they lack the pharmacologic response, the metabolic alterations, and the elimination of the drug which may occur in the mammal. These studies appear to be most useful for studying the mechanism of action of known teratogenic agents, but their value for determining the teratogenic properties of unknown agents in the evaluation of safety has not been determined. There is too much chance of missing a critical period in a one or two dose experiment and thus a potent teratogenic agent might be accepted as safe. As these techniques accumulate, they may be found useful as collateral studies, but we should not, at the present time, accept them in place of the mouse, the rat, or the rabbit as the major tests for teratogenic effects of a drug. Nor should positive teratogenic effects in these

specialized tests be permitted to overweigh negative results in the mammal.

In conclusion, the evaluation of new drugs for possible teratogenic properties may be conducted as a part of the toxicologic evaluations of the same agents. The same considerations of the chemical and biological properties, the metabolic fate of a drug and its proposed clinical use, as applied to an evaluation of its toxicity, may be applied also to its teratogenic evaluation. Teratogenic studies of new agents should be conducted under reasonable nontoxic daily dosage schedules in the intact mammal to permit a more valid interpretation of the results than may be obtained from single dose experiments, or than may be obtained from the results of studies in the nonmammalian species.

At the present time, if new drugs can be shown to have teratogenic potentialities in animals, such findings should, at most, serve as a warning to clinicians—

> "If such (animal teratogenicity) became a legal barrier to human consumption, patients would be denied the benefits of some antibiotics, cortisone and even aspirin" (20).

SUMMARY

The evaluation of new drugs for possible teratogenic properties may be conducted as a part of the toxicologic evaluations of the same agents. The same considerations of the chemical and biological properties, the metabolic fate of a drug and its proposed clinical use, as applied to an evaluation of its toxicity, may be applied also to its teratogenic evaluation. Teratogenic studies of new agents should be conducted under reasonable nontoxic daily dosage schedules in the intact mammal to permit a more valid interpretation of the results than may be obtained from single dose experiments, or than may be obtained from the results of studies in the nonmammalian species.

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