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Pre-clinical evaluation of contraceptive steroids: regulatory requirements and scientific expectations*

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The development of new contraceptive steroids placed great pressures on regulatory agencies. There was insufficient time to develop a novel pre-clinical safety evaluation, hence their toxicity in animals was assessed as with other drugs. The approach of regulatory agencies to toxicity and carcinogenicity testing of these steroids are discussed and evaluated.

Introduction

When the new steroidal contraceptives were introduced into practice, it soon became apparent that they were highly effective, surprisingly well tolerated and dependable even on long-term use. Thus, it was easy to predict that these agents would be taken by a large fraction of the healthy female population and for considerable periods of time.

In contrast to other chemicals to which large segments of the population are exposed, e.g. environmental pollutants and food additives, steroidal contraceptives are ingested in relatively high, pharmacologically active doses, leading to a variety of changes in sex hormone-dependent organs, metabolic alterations, and certain cardiovascular and neurobehavioural dysfunctions. Although these effects were rarely of such a magnitude that the use of contraceptive steroids had to be discontinued, they gave rise to much concern, because their long-range consequences were unknown. Table I summarizes some of the early effects of steroidal contraceptives, and the changes that had to be considered as potential hazards on long-term treatment.

Based on these scientific speculations, it was decided that long-term toxicity studies in laboratory animals were necessary to assure the safety of women taking contraceptive steroids for many years. However, because the drugs were already in the market place, a unique situation developed: experience of the toxic potential of the drugs in humans was accumulated simultaneously with the data from long-term animal safety studies. Whenever a disturbing finding was made in an animal experiment, it had immediate consequences for the use of contraceptive steroids in clinical practice. An example is the detection of mammary nodules in beagle bitches which led to the withdrawal of certain contraceptives and to extensive epidemiological surveys. *Vice versa*, clinical findings of unexpected adverse responses in women often brought about the request for development of new animal models. For example, the discovery of an increased risk for thromboembolic complications in contraceptive users stimulated much research on assay systems for detection of hypercoagulability in laboratory animals.

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Pre-clinical evaluation

Regulatory requirements

The rapid development of new contraceptive steroids and their widespread acceptance in the population put regulatory agencies under considerable pressure. A novel approach to pre-clinical safety evaluation, especially tailored to orally active steroids, could not be developed, because there was neither enough time, nor was the science of comparative endocrinology far enough advanced. For this reason, regulatory agencies decided to request essentially the same kind of animal toxicity studies that were routinely gathered with other drugs. A survey of regulatory requirements in selected countries was recently published by Rowe (1983).

Because the long-term use of contraceptive steroids was expected to be a frequent occurrence, the possibility of carcinogenic effects was particularly considered. In 1964 the Committee on Safety of Medicines of the UK (CSM) initiated a review of existing data on carcinogenicity of contraceptive steroids. Based on findings of liver tumours in rats induced with mestranol, the Committee decided in 1966 to request carcinogenicity studies in rats and mice for all marketed and new oral contraceptives (CSM, 1972).

In the USA, a new policy on animal studies with steroidal contraceptives was announced by the Food and Drug Administration (FDA) in the late 1960s. It specified the kind of animal safety data necessary for initiation of the three phases of clinical trials and for the submission of a new drug application (Goldenthal, 1969). These guidelines are summarized in Table II. In addition to a 2-year carcinogenicity experiment in rats, FDA demanded 7-year carcinogenicity studies in female beagle dogs and 10-year carcinogenicity studies in female monkeys (FDA, 1968).

A particular feature of the FDA guidelines was the specification of the doses to be used in the long-term experiments in dogs and monkeys. It was decided that the dose selection had to be based on the human dose given as prescribed clinically (2, 10 and 25 the anticipated human doses in beagle dogs, and 2, 10 and 50 times the anticipated human dose in monkeys).

Not all regulatory agencies, by far, insisted on the long-term experiments with beagle dogs and monkeys. However, since the USA is the most important market for oral contraceptives, practically all existing and many new contraceptive steroids were, and still are, submitted to the long-term studies in these two species. In developing countries which have limited toxicological expertise, the drug regulatory agencies prefer to register those drugs which are introduced in the markets of the country of origin and preferably also in the USA. Thus, the FDA guidelines are often accepted as the most desirable standard.

At present, the 7-year dog and 10-year monkey studies are mandatory in the USA and Canada. In Western Europe, steroidal contraceptives fall under the general clause of drugs administered for prolonged periods of time. This implies that carcinogenicity studies in one or two rodent species are required.

Scientific expectations

Since I have not taken part in the decision making process on

Table I. Selected effects of contraceptive steroids and potential consequences of long-term use

Organ	Effects	Potential long-term consequences
Ovary	Inhibition of ovulation	Cyst formation
Uterus	Amenorrhoea	Cystic hyperplasia, endometritis, carcinoma
Mammary gland	Tenderness, galactorrhoea	Fibrocystic hyperplasia, adenoma, carcinoma
Liver	Functional disturbances	Icterus, biliary cirrhosis, gall bladder disease, coagulopathy, tumour
Pancreas	Disturbance of glucose tolerance	Diabetes mellitus
Cardiovascular system	Hyperlipidaemia, transient blood pressure elevation, oedema	Atherosclerosis, hypertension, myocardial infarct
Nervous system	Psychic lability, anxiety, migraine	Mental depression, stroke

Table II. FDA guidelines for toxicity testing of steroidal contraceptives

Extent of clinical studies	Animal toxicity requirements
Phase I	90-day toxicity in rats, dogs and monkeys
Phase II	1-year toxicity in rats, dogs and monkeys; fertility and reproductive performance in female rodents; teratology in rats and rabbits
Phase III	2-year toxicity in rats, dogs and monkeys; 7-year dog and 10-year monkey toxicity started, peri- and postnatal toxicity in rodents; fertility and reproductive performance in male rodents
New drug application	up-to-date progress reports on long-term dog and monkey studies

See Goldenthal (1969).

Table III. Parameters monitored in 10-year toxicity studies in monkeys (FDA, 1968)

General toxicity	appearance, body weight
Sex and related hormones	menstrual cycle, vaginal cytology, urinary 17-hydroxy and 17-ketosteroid excretion, breast examinations
Metabolism	fasting blood sugar, if changed, glucose tolerance
Pathomorphology	all organs, special emphasis on sexual and endocrine organs and blood vessels
Haemostatic system (optional)	prothrombin time, platelet count, Stypven time, regular and activated thromboplastin time, fibrinogen

the toxicological guidelines of steroidal contraceptives, it is difficult for me to speculate about the scientific information which the regulatory agencies hoped to obtain. Some insight can, perhaps, be gleaned from the recommended protocols for the long-term studies in dogs and monkeys. As an example, the endpoints which, according to the FDA recommendations, should be investigated with special care in the monkey studies, are summarized in Table III.

From this list, three kinds of toxicological targets can be identified, namely: (i) general toxicity and organ-directed toxicity not related to hormonal and metabolic effects; (ii) toxicity related to hormonal effects; (iii) toxicity related to metabolic effects. For the first kind of adverse effects, the rather unsophisticated standard procedures used for other drugs and chemicals were deemed sufficient. Apparently, it was expected that direct toxicity affecting organs such as liver, bone marrow and kidney would manifest itself in the course of routine animal studies. No efforts were made to gain more detailed information on several additional potential targets such as the neurobehavioural functions, the musculoskeletal and immunological systems and the cardiovascular functions.

Exceptions were the potential adverse effects on the structure of the blood vessels and the function of the haemostatic system. The recommended investigations (detailed histopathology of blood vessels and a set of routine coagulation assays) were issued as a response to epidemiological evidence of increased risk for thromboembolic complications in a small percentage of users of contraceptive steroids. Although nothing was known about the possible mechanisms of this complication, the recommendations for laboratory investigations were very specific and taken right off the shelf of the routine coagulation laboratory. All these tests were originally developed to detect coagulopathies of various kinds (Zbinden, 1976), but the scientific expectations apparently were that they would work equally well for the demonstration of hypercoagulable states and impending thrombosis in animals. It comes hardly as a surprise that the procedures demanded by the FDA proved to be of little use. Only after extensive research on the biological mechanisms of drug-induced thromboembolism was it possible to develop realistic tests for the assessment of the thrombogenicity of steroidal contraceptives (Wessler, 1983).

That the majority of adverse effects of steroidal contraceptives were directly caused by the hormonal effects of the drugs, was well recognized. Nevertheless, the investigations required to assess these properties in the animal experiments appear to be very limited in scope (Table III). It can hardly be expected that monthly palpations of the breast, semi-annual vaginal cytology and yearly urinary ketosteroid determinations would suffice to detect all relevant changes in the endocrine equilibrium. Thus, it is difficult to understand why such exceedingly extensive studies as a 10-year carcinogenicity experiment on >60 monkeys were demanded without making use of all the available techniques for monitoring endocrine regulation and target organ responses.

A word should be said about the dose selection for long-term carcinogenicity studies. The request for high-dose treatments was most certainly motivated by the desire to detect even weak and rare toxic effects. This practice was taken over from the standard procedures for drug toxicity testing and carcinogenicity bioassays with pharmacologically inert substances. Unfortunately, the approach did not take into consideration many basic physiological facts, such as: (i) in mammals, the same reproductive processes are regulated, in part, by different sexual hormones and different interactions with peptide hormones; (ii) the effects of hormones and hormone-like drugs differ in various mammalian species; (iii) in mammals hormones are secreted in different amounts; (iv) the hormone sensitivity of target organs differs in mammalian species; (v) continued overstimulation of endocrine and endocrine-dependent organs often leads to hyperplastic and neoplastic responses (Williams, 1982).

If the marked species differences are not taken into account for dose selection, many erroneous results may ensue. For example, the optimal ratio of oestrogen to progestogen for the rat is 1:10 000 to 1:20 000 and for women 1:50 to 100. If these

Table IV. Testing strategies for new contraceptive steroids

1. Detection and characterization of toxic properties *per se*
2. Characterization of the pharmacodynamic (endocrinological) and metabolic effects in suitable animal species
3. Target organ response to repeated administrations of effective and small multiples of effective doses in suitable animal species
4. Pharmacokinetics and metabolic fate of test drug in suitable animal species
5. Detection of mutagenic and teratogenic properties
6. Characterization of pharmacodynamic (endocrinological) and metabolic properties in humans at effective doses
7. Pharmacokinetic and metabolic fate of test drugs in humans
8. Target organ responses to prolonged administration of effective doses in humans

differences are not considered, and if the optimal human ratio is used in a rat toxicity study, virtually only the oestrogen component can exert an effect (Neumann and Gräf, 1979).

It is also important to recognize that hormones act differently according to the dose. For example, in the rat, oestrogens stimulate prolactin release, and progestogens act synergistically or antagonistically, depending on the dose (Neumann and Gräf, 1979). It must also be noted that the pharmacokinetics of steroidal contraceptives differ markedly among species. For example, elimination of many progestogens in the dog is much slower than in man. For other drugs such as chlormadinone, the elimination half-life in dogs and women is comparable, but in the dog, the volume of distribution is much larger. Megestrol acetate is excreted by the biliary route in the dog, but in women mainly in the urine (El Etreby and Gräf, 1979).

In view of these and many other biological species differences which have come to light in recent years, one must conclude that the scientific expectations for the long-term animal studies were mainly a result of wishful thinking rather than a consequence of a well reasoned approach to an important public health problem.

Of the metabolic changes induced by steroidal contraceptives, only the possibility of diabetogenic effects were considered. Clinical experience has since shown that other areas should also be investigated. Among these are water and electrolyte balance and lipid metabolism.

Conclusions

Over the past 20 years, a considerable number of steroidal contraceptives have undergone extensive animal toxicity testing. The approach originally required by regulatory agencies, i.e. high-dose and long-term carcinogenicity studies in rodents, beagle dogs and monkeys, has resulted in a large number of experiments in which hyperplastic and neoplastic responses developed in a variety of organs. For many of the tumours induced in rodents, not only marked species differences but also strain differences were observed. From a recent review by Beier *et al.* (1983) it is evident that many additional factors such as living conditions, age, sex, biodynamics of the test drug, and route, duration and frequency of administration play an important role. Therefore, it is unlikely that such experiments using rodents will yield much relevant data for the safety assessment of new steroidal contraceptives. The same conclusion had already been reached in 1972 by the CSM.

In the dog, the appearance of mammary tumours has caused some anxiety. However, extensive studies of the phenomenon have demonstrated basic differences between dog and man in the response of the endocrine system to contraceptive steroids. Thus, it is highly questionable that the beagle model, at least as far as mammary tumours are concerned, has fulfilled the scientific expectations. Many toxicologists are now of the opinion that its further use would be without sound scientific justification (El Etreby and Gräf, 1979; Beier *et al.*, 1983).

The results of the long-term carcinogenicity studies in monkeys are only now being evaluated. So far, it seems that very few tumours are induced in this species. This indicates that the results of carcinogenicity studies in the monkeys are in good agreement with the clinical experience in man, despite the high doses used. The observation of endometrial carcinoma in two monkeys treated for 10 years with a high dose of medroxyprogesterone acetate has recently caused concern (Beier *et al.*, 1983). The results of further studies in monkeys exposed to other steroids will have to be awaited, before the significance of this finding can be assessed.

In view of the practical experience accumulated in animal studies, and the knowledge of the hazards of steroidal contraceptives in humans, it is necessary to reconsider the basic approach to safety testing of new fertility-regulating agents. Because of the many physiological and pharmacokinetic differences between laboratory animals and man, the straightforward application of routine toxicity procedures has given too many 'false-positive' results. It is believed that the medical community would be served better if the biological characteristics of new compounds were studied in specially designed experimental systems. The basic strategy for such an approach is summarized in Table IV. In particular, the endocrinological profile of new agents should be investigated carefully in several animal species whose target organ responsiveness to natural hormones and synthetic derivatives is well known. In addition, the pharmacokinetic behaviour of the test substances must be investigated, and the target organ responses must always be related to the plasma concentrations necessary to induce the change.

Whenever combinations of two or more steroids are tested, the ratio of the components must be adjusted to the physiological response of the species selected. It may often be different from the ratio proposed for the use in humans. Other toxicological properties which should be assayed in special test systems are mutagenicity and teratogenicity. In special cases, binding studies to cellular macromolecules and test systems designed to detect transforming or tumour-promoting activities may add useful information.

As with all new drugs, the possibility that a contraceptive steroid may be toxic *per se*, and could cause organ damage by mechanisms unrelated to its hormonal properties, must be considered. For the detection of such effects, a subchronic (90 day) standard toxicological experiment, in one or two animal species showing endocrinological responsiveness to the test drug that is comparable to man, should be sufficient. In such experiments, the metabolic effects should receive particular attention.

Finally, a determined effort must be made to monitor target organ responses in patients, not only in the subjects used in early clinical trials, but also in patients taking the drug for prolonged periods of time. Thus, the safe use of new contraceptive steroids clearly becomes a shared responsibility of the toxicologists and the clinical investigators. My scientific expectations are that this approach will significantly improve our ability to predict human hazards. In addition, it is likely to accelerate the development of new fertility-regulating drugs. It is hoped that regulatory agencies will give these safety testing strategies a chance to prove their usefulness.

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