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TOXICOLOGICAL REQUIREMENTS FOR A MENSES-INDUCER

A. HERXHEIMER

Department of Pharmacology and Therapeutics, London Hospital Medical College, London E1 2AD, England

SUMMARY

What toxicological data does a clinical investigator require for a new type of oral contraceptive, before he studies it in women? This question is considered for an anti-progestin with weak oestrogenic activity which would be given from Day 24 to Day 28 of the menstrual cycle. Routine toxicity studies (which would be needed for any new drug) would have to be supplemented by special studies of cyclical administration in a primate species, and of possible teratological effects. The reversibility of the drug effects would also need to be tested. It is important that the clinical investigator should understand and be satisfied with the toxicological data; he should not hesitate to discuss the data with the toxicologists concerned or with the appropriate expert in the drug-regulatory agency.

The first part of this symposium considers a new type of hormonal contraceptive that acts by a mechanism quite different from those of the contraceptives in use until now. A compound of this type can be called a menses-inducer. It would have antiprogestin activity with only weak oestrogenic activity, and would be taken from the 24th to the 28th day of the cycle.

Such a drug is not just an armchair invention. Two substances have been described in the literature that may have the right properties, though further work is needed to show how selective their antiprogestin effect is. One is a naphthofuran developed at the Central Drug Research Institute in India [1], the other, known as R 2323, is a steroid resembling norgestrel, but having three double bonds [2]. In principle these substances might be tried in humans, and some clinical results with R 2323 have in fact been reported at this congress by Sakiz and his colleagues [3], though the drug was given on days 15–17, not days 24–28 of the cycle.

Anyway, let us suppose that we have a drug that is a pure anti-progestin, and blocks the effects of progesterone on the endometrium. In an ordinary ovulatory cycle in which conception has not occurred this would make no difference, because the corpus luteum has regressed by the 28th day and the level of progesterone in the blood is then very low. However, in a conceptual cycle the corpus luteum is kept in being by the secretion of chorionic gonadotrophin and continues to produce progesterone. At the same time the infant trophoblast probably also produces some progesterone [4]. Now in this situation the antagonism of progesterone by the drug should have a dramatic effect: the endometrium would be deprived of its support, and shed. The drug would then have induced menstruation. What toxicological data ought we to have before we begin to test such a new kind of drug in humans? This question compels us to think out from scratch what we really need to know about the drug. The need to get back to principles is at the same time an opportunity that we would not have if we had to consider a new compound that was merely another one of a familiar kind, say a new continuous progestagen contraceptive.

As soon as we ask ourselves what we need to know, we realise that this depends on the type of study we are planning to do. For a brief study in a small number of volunteers we shall require rather less toxicological data than for a larger trial involving prolonged administration of the drug. Drug studies in humans are conventionally categorised as Phase I, II, or III [5]. Phase I denotes an initial exploratory study of toxicity, metabolism and pharmacology in a few subjects given the drug for up to 10 days; phase II is the phase of early small-scale clinical trial, in up to about 50 subjects given the drug for up to 3 months, or in the case of contraceptives for 3 menstrual cycles; phase III comprises definitive clinical trials in the clinical situations for which the drug is ultimately intended.

In considering the toxicological requirements for human studies in the different phases, it is useful to distinguish between routine toxicological investigation, which would be required for any new drug, and special toxicity studies that we may think necessary for our particular drug, the menses-inducer.

Routine toxicity studies [6] have the twofold function of demonstrating what toxic effects the drug produces, and of assessing how safe the drug is likely to be in use. Ideally a drug should be tested in a species which metabolises the drug and responds to the drug in the same way as man, but of course this cannot be known before the drug has been studied in man. In practice therefore toxicity is tested in several widely differing species, and the bulk of the work is then done in the two that are most accessible and seem most relevant. In the case of the menses-inducer these are probably the rat and the

Rhesus monkey. It is worth investigating the metabolism of the drug in these species, so that later, when comparable observations have been made in women, one can check how appropriate the choice of test species was, as far as metabolism of the drug is concerned. If both species differ greatly from man, one may need to do further toxicity tests in other species before continuing with studies in humans. The appropriate route of administration would be by mouth, since this is how the drug is intended to be given in man. In each species, at least four dose-level groups are needed for conventional routine toxicological work. A high dose-level in the sublethal range will show what toxicity the drug is capable of; some deaths would be expected in this group. A low dose-level, comparable on a body-weight basis to the highest dose envisaged for humans, or a little higher, will show whether such quasi-therapeutic doses are devoid of toxicity. Two or more intermediate dosages will show how high a dose can be given without producing toxicity. However, in the case of many hormonal steroids it may be impossible to give lethal doses, and the highest dose that seems likely to give meaningful results has to be determined on other grounds.

The frequency of administration must also be considered. If it seems possible that the drug is inactivated more rapidly in the test species than in man, it may be useful to include groups given the drug twice a day, as well as the usual once-a-day schedule—even though twice daily administration to humans is not envisaged. The duration of dosing must provide a reasonable safety margin for the proposed duration of administration to humans, e.g. before a Phase I study the U.S. Food and Drug Administration (FDA) requires 3-month toxicity studies. The observations to be made should include some record of behaviour, measurements of body weight, food and water intake and whatever laboratory tests seem appropriate. At one or more points in time, animals are killed and organs and tissues examined macroscopically and histologically.

Now let us turn from the routine toxicity tests to special studies. To model the cyclical use of a menses-inducer in women, we would need observations on Rhesus monkeys dosed for the last five days of each of three consecutive menstrual cycles, preferably cycles in which successful mating had occurred. In these studies the lowest two dose-levels used in the routine toxicology would probably suffice. Rhesus monkeys and women have similar menstrual cycles, and the timing of progesterone secretion is also similar in the two species [7]. It may be useful, though not essential, to examine a vaginal smear for spermatozoa after mating, but it is of course important to perform a pregnancy test before the end of the cycle. The three cycles with drug administration should be followed in some of the animals by continued mating in the three following cycles, unless a pregnancy occurs before then. This will indicate whether a drug effect persists beyond the cycle of medication, and if so, how long it is before fertility returns to normal. In any pregnancy that occurs it is of course desirable to examine the fetus for possible drug effects.

This leads on to consideration of testing for teratogenicity. With a drug of this kind, which can in effect be regarded as an abortifacient, there is an obvious risk that if it is ineffective the surviving embryo may be affected. Teratogenicity must therefore be looked for before the drug is given to women. Tests in non-human primates are likely to have the greatest relevance, and should be performed at various times up to about twelve weeks pregnancy. It is still uncertain which primate species is the most appropriate [8]. The doses to be used need to be low enough not to produce abortion; in the majority of animals it would also be desirable to use doses somewhat below that required to induce menstruation reliably. Such a lower dose might possibly affect development of the embryo or fetus.

Mutagenicity is at present difficult to predict. Many test systems have been tried, but it is not known which of them should be recommended [9].

At this point we have enough data to give the drug to a few volunteers in a Phase I study. If it is at all possible, this study will include some investigation of the metabolism of the compound and, as already mentioned, this can provide an important check on the relevance of the species used in the toxicity studies.

What further toxicological data do we need before embarking on Phase II and Phase III studies of the drug in women? We clearly must have the results of routine toxicity studies carried on for longer. Before Phase II studies of hormonal contraceptives the F.D.A. requires 1-year studies in rats, dogs, and monkeys, and before Phase III, 2-year studies in these species [5]. In the case of a menses-inducing drug I am not sure that continuous administration to monkeys would be very meaningful, and cyclic administration would probably provide a better estimate of toxicity and safety. Tests for carcinogenicity take much longer, and the present practice is to start these long-term tests, but to allow Phase III trials to begin before they have been completed. Special tests for a menses-inducer again need to include observations on the reversibility of the drug effect after long-term cyclical use; i.e. for 1 year and 2 years.

Having decided what information we would like before giving the menses-inducer to a woman, we must consider whose responsibility it is to provide this information.

When an investigator gives a drug to humans, he has the responsibility of ensuring that all proper and reasonable precautions are taken to minimise the risks. He must satisfy himself that adequate toxicological studies have been made, and he can only do so if he reads and understands the toxicologist's report. There will often be parts of a report that are difficult to understand or to interpret, and it is important to try to clarify these by discussion with

the author of the report or with experienced colleagues. Though the clinical investigator himself has the primary responsibility, this is usually shared by people working in the pharmaceutical company that has produced the drug and carried out the basic research on it. The scientist/administrator in the regulatory agency equally has to be satisfied that the toxicity data are adequate before he approves a proposal to give the drug to humans. The physician in charge of clinical research in the drug company will also have satisfied himself on this before he offers the drug to the investigator. And of course the toxicologist himself will have produced all the evidence that seems to him necessary before the drug is used in man. It seems particularly important before undertaking the first Phase I study of a drug, that the clinical investigator should wherever possible discuss the toxicity data with the toxicologist himself, and with the expert in the regulating agency, and not only with the company's doctor. The geographical separation of the participants unfortunately means that such meetings often do not happen, for the only one who travels much tends to be the company physician. Direct and uninhibited discussion can help all concerned to make sound decisions.

REFERENCES

1. Kamboj V. P., Chandra H., Setty B. S. and Kar A. B.: *Contraception* **1** (1970) 29–45.
2. Sakiz E., and Azadian-Boulanger G.: *Proc. 3rd Int. Congr. Hormonal Steroids*, Hamburg. Excerpta Med. Amsterdam (1971) 865–871.
3. Sakiz E., Azadian-Boulanger G., Laraque F. and Raynaud J. P.: *J. steroid Biochem.* **5** (1974) 378.
4. Shearman R. P.: *Human Reproductive Physiology.* Blackwell, Oxford (1972) p. 84.
5. Berliner V. R.: *Acta endocr., Copenh.* suppl. **185** (1974) 240–253.
6. Noel P. R. B.: *Acta endocr., Copenh.* suppl. **185** (1974) 17–33.
7. Knobil E.: *Acta endocr., Copenh.* suppl. **166** (1971) 137–144.
8. Tuchmann-Duplessis H.: *Acta endocr., Copenh.* suppl. **185** (1974) 203–223.
9. WHO: *Wld. Hlth. Org. techn. Rep. Ser.* **482** (1971).