Nonclinical Evaluation of Endocrine-Related Drug Toxicity

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2015
Pharmacology and Toxicology

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Contains Nonbinding Recommendations

Nonclinical Evaluation of Endocrine-Related Drug Toxicity Guidance for Industry[1]

This guidance represents the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not create any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance provides recommendations to sponsors of investigational new drug applications, new drug applications, and biologics license applications regulated by the Center for Drug Evaluation and Research (CDER) regarding nonclinical studies intended to identify the potential for a drug to cause endocrine-related toxicity.[2]

The goals of this guidance are to:

- Describe how endocrine-related toxicity is assessed using the standard battery of nonclinical tests
- Identify situations in which additional studies should be considered to more fully characterize the endocrine-related toxicity of a drug

This guidance focuses on nonclinical testing designed to assess the potential for a drug to cause endocrine effects that are unintentional and adverse. It does not provide guidance relating to the development of drugs that are intended to interfere with the endocrine system to prevent or treat a particular disease or condition, and it does not provide detailed recommendations regarding clinical studies to investigate adverse endocrine-related activity. Environmental assessment issues are not covered in this guidance.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only

[1] This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration.

[2] For the purposes of this guidance, all references to *drugs* include both human drugs and therapeutic biological products unless otherwise specified.

as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Some compounds can interfere with the endocrine system of an organism or its progeny, resulting in adverse effects in one or more sensitive tissues. Compounds can do this through multiple mechanisms, including: (1) mimicking or enhancing the action of an endogenous hormone; (2) blocking a hormone receptor, thereby preventing the action of an endogenous hormone; or (3) affecting the synthesis, transport, metabolism, or excretion of an endogenous hormone. These effects may be intended, as in the case of oral contraceptives, or they may be an unintended side effect. For biologically significant effects to be produced, exposure needs to be sufficiently high at the target site and the substance needs to be of sufficient potency. It is noted that not all perturbations of the endocrine system are adverse. For pharmaceuticals, only effects seen at clinically relevant exposures are of concern.

Test articles that affect the endocrine system include, but are not limited to, those that alter the function of the sex hormones (e.g., estrogen and androgen), the hypothalamic-pituitary-adrenal hormones, and thyroid hormone, and the hormones involved in feedback regulation of endocrine systems (e.g., the gonadotropin-releasing hormone, corticotropin). The perturbation of endocrine systems potentially can cause a wide variety of adverse effects on development. Appropriate hormonal exposure during the different developmental stages is critical to normal development. Increased or decreased estrogenic or androgenic activity at inappropriate times can produce transient or permanent alterations in end-organ structure and/or function. For example, animal studies have shown that males exposed to antiandrogens during a specific perinatal period can be demasculinized in morphology, physiology, and behavior.

Endocrine-active compounds also can affect the nervous system through receptors expressed on neuroendocrine cells or on neurons. In rodents, for example, early developmental exposure to estradiol or testosterone establishes permanent sex-dependent brain morphology. Sexual differentiation is also regulated by metabolism of steroid hormones in the brain via enzymes controlling steroidogenesis such as CYP19A1 (P450 aromatase), which converts testosterone to estradiol. For example, in rodents, compounds that alter aromatase activity can cause an imbalance in sex hormones that result in altered sexual behavior.

It is important to assess drugs being developed for human use for their potential effects on hormonal systems. Nonclinical testing is appropriate for this assessment because endocrine systems are similar among vertebrates, and many species respond similarly with regard to their sensitivity to hormones. Based on review of recent literature and experience with drugs being developed for human use, including those that target the endocrine systems, it is possible to identify potential endocrine effects in drugs not intended to possess those activities. Endocrine effects in patient populations have been successfully predicted by standard nonclinical test batteries. The potential need for additional testing under specific circumstances is discussed in section IV.

III. OVERVIEW OF NONCLINICAL ASSESSMENTS

As a part of the safety evaluation of drugs for human use, CDER recommends a standard battery of toxicity tests using study designs intended to identify potential toxicity (see the ICH guidances for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* and *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*).[3] A well-designed nonclinical testing program conducted using the recommended paradigm will detect clear adverse effects related to endocrine perturbation by drugs under development. If endocrine effects are identified, other factors — such as the indication; target population; and route, duration, and level of exposure relative to the expected clinical exposure — will determine how to proceed (e.g., whether further nonclinical testing or clinical monitoring is appropriate or not warranted).

The typical nonclinical studies conducted during drug development are discussed below, with a focus on the information that the studies can provide about potential endocrine-related toxicity.

A. Receptor-Binding Assays

Binding assays that include endocrine receptors can serve as an initial screen. A lack of binding does not rule out a potential endocrine effect (e.g., a drug that does not bind to a receptor still could affect synthesis or metabolism of a hormone, resulting in an indirect adverse endocrine effect). Similarly, if binding does occur, this does not provide conclusive evidence that the interaction will lead to a significant biological change. If a drug binds to an endocrine receptor, then additional testing — including in vitro studies and nonclinical in vivo functional assays — can be used to characterize the interaction, if warranted.

Enzyme assays also can be used to screen for the potential of a drug to interfere with the endocrine system by altering the activity of certain enzymes. For example, some of the cytochrome P450 isozymes are involved in anabolism and catabolism of steroid hormones. These include CYP 11A1, 11B1, 11B2, 17A1, and 21A1, which act in steroid biosynthesis. Similarly, uridine diphosphate-glucuronosyltransferases in the liver metabolize circulating thyroid hormones.

B. Pharmacology Studies

Sponsors typically conduct nonclinical studies to assess intended pharmacologic effects, including studies on the effects of the drug in in vitro and in vivo models of disease. In some cases, the intended pharmacologic target of the drug can be within an endocrine-related pathway. The pharmacology studies can help characterize this endocrine activity.

[3] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance Web page at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

C. Repeat-Dose Toxicity Studies

Nonclinical safety assessments for most new drugs include repeat-dose toxicology studies in two species.[4] For drugs intended to be used chronically, this typically means studies exposing two animal species to a wide range of doses of the test drug for up to 6 (rodent) or 9 (nonrodent) months. These studies generally include a variety of endpoints that can identify adverse endocrine-related activity. Changes in organ weight, gross organ pathology, clinical chemistry, and histopathology can all be indicative of particular endocrine effects.

Organs typically collected for gross and histopathological evaluation include endocrine-sensitive organs such as the thyroid gland, adrenal glands, reproductive organs, and pituitary gland. Changes observed can indicate potential adverse endocrine effects. For example, estrogenic drugs can produce effects such as increased ovarian and uterine weight, endometrial stimulation, mammary gland stimulation, decreased thymus weight and involution, and increased bone mineral density. Other hormone activities produce characteristic histological changes in target tissues. Sometimes a carefully selected hormonal analysis can provide a biomarker for clinical monitoring and risk assessment.

D. Developmental and Reproductive Toxicity Studies

For most drugs, sponsors routinely conduct a battery of tests designed to determine if a drug potentially impairs fertility or affects embryo/fetal and neonatal development.[5] Some developmental stages (e.g., gestational, neonatal, peripubertal) are particularly sensitive to endocrine effects. Sensitive endpoints can include vaginal patency, preputial separation, and anogenital distance; for male offspring, sensitive endpoints can include nipple retention. The reproductive performance of offspring also can be important for detecting neuroendocrine effects. Standard developmental and reproductive toxicity studies generally capture gestational developmental time points effectively, but might not be adequate for evaluation of effects on postnatal development unless the neonates were dosed directly during the lactation period.

E. Carcinogenicity Studies

Carcinogenicity studies conducted in rats and mice are another important source of information about the effects of chronically used drugs. Although the goal of these studies is to assess the drugs' carcinogenic potential, organ changes assessed by histopathology also give information about potential endocrine effects. For example, persistent disruption of the hypothalamic-pituitary adrenal/gonadal/thyroidal axis(es) can result in various neoplasms. Histologic, organ weight, clinical chemistry, or hematologic data evaluated together can form recognized patterns of changes associated with hormonal effects that precede the neoplasm. Often the available data are sufficient to identify the hormonal axes involved.

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[4] See the ICH guidances for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* and *S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.*

[5] See the ICH guidance for industry *S5 Detection of Toxicity to Reproduction for Medicinal Products.*

IV. WHEN ADDITIONAL ASSESSMENT OF ADVERSE ENDOCRINE-RELATED EFFECTS MAY BE WARRANTED

Standard nonclinical testing programs conducted in accordance with CDER's recommended paradigm generally are adequate to detect whether a drug has the potential to interfere with the endocrine system. As a part of this overall assessment, there are specific factors to consider that could suggest a need for additional investigation.

Sponsors should consider the following factors when deciding if the results of the standard nonclinical evaluation discussed above are adequate to evaluate the drug's adverse endocrine-related effects or if additional assessment is warranted:

- Are there data on related compounds indicating that the drug or related compounds may have adverse endocrine-related effects?
- Is the drug proposed for use in a population not studied in standard toxicity studies?
- Is the clinical systemic exposure to the drug near or above the exposure at the no observed adverse effect level (NOAEL) for endocrine effects in animals?

These questions are further addressed below.

A. Are There Data Indicating Adverse Endocrine Activity?

When data (e.g., class effects, receptor binding) show a potential adverse endocrine-related effect, the sponsor should consider whether additional investigation is warranted. If the endocrine effect was expected based on the primary pharmacology or class of the drug and those class effects have been well characterized previously, additional endocrine-specific investigation might not be necessary. However, if it is not known whether under conditions of use the endocrine-related finding will be relevant to humans, additional studies sometimes can be warranted.

B. Is the Drug Being Used in a Population not Studied in Standard Toxicity Studies?

Standard nonclinical studies are adequate for detecting adverse effects following in utero exposures and exposures to adults, but might not be adequate for detecting effects following exposures during the early postnatal period. Direct dosing during the early postnatal period should be conducted to ensure exposure during the postnatal period when insufficient exposure occurs during the lactation period. The endocrine changes experienced by juvenile animals can have long-term consequences not adequately demonstrated by studies conducted only in adult animals. When only adult animals have been tested, additional studies designed specifically to evaluate toxicity in juvenile animals can be important

C. Is the Clinical Systemic Exposure to the Drug Near or Above the Exposure at the NOAEL for Endocrine Effects in Animals?

Sponsors should assess the exposure-response relationship of any potential endocrine activity identified in standard nonclinical studies. Those endocrine activities that occur in animals only at exposures substantially above (e.g., greater than 50 times) the human exposure usually do not warrant additional investigation. Additional assessment is likely to be appropriate only if human exposure is comparable to (or exceeds) the exposure level at which endocrine-related activity is observed in standard nonclinical studies and relevance to humans is not understood. Note that assessment of human exposure should take into consideration possible exposure to multiple drugs containing the same active agent (e.g., over-the-counter monograph active ingredients) or exposure to multiple agents having similar endocrine effects. This potentially can increase the risk compared to that of a single drug.

V. ADDITIONAL STUDIES FOR FURTHER ASSESSMENT OF ADVERSE ENDOCRINE-RELATED ACTIVITY

As previously discussed, the FDA has concluded that the standard battery of nonclinical tests for new drugs detects relevant adverse endocrine-related effects in almost all cases. Based on a thorough evaluation of the totality of data from the standard battery of nonclinical tests and the factors identified in section IV, sponsors should determine whether the assessment of adverse endocrine-related activity is adequate or whether additional studies should be conducted to further characterize potential endocrine effects. Sponsors should consider whether risk for adverse endocrine-related activity can be expected at clinical exposures. In situations where there is a large exposure margin, no additional studies may be necessary.

If further studies are warranted, sponsors can consider the following additional assessments:

Mechanistic Studies. Results of standard studies demonstrating evidence of adverse endocrine effects may trigger questions on mode of action and human relevance that could be addressed by mechanistic studies. For example, if changes in endocrine tissues are noted in a standard study, the role of inhibition or induction of P450 isozymes might be appropriate to explore, because such inhibition or induction can be a mechanism by which drugs alter hormone levels in animals. These mechanisms might not be relevant for the drug in humans. If the relevance to humans of an endocrine signal is important for a regulatory decision, the sponsor can consider whether mechanistic studies in alternative animal models may be informative.

Nonclinical Juvenile Studies. Juvenile animal studies can be helpful in addressing the long-term endocrine effects from acute or continuous exposure for those drugs administered to neonates and pediatric patients, when these effects cannot be adequately predicted from adult animals.

Clinical Studies. As an alternative to, or in addition to, supplemental nonclinical assessment of endocrine toxicity, inclusion of endocrine endpoints (e.g., hormone levels) in clinical

studies can be important to clarify the relevance of adverse endocrine activity identified in nonclinical studies. Planning for these assessments should be discussed with the FDA.

In summary, the standard comprehensive test battery is generally sufficient to identify endocrine-related toxicity. Depending on the outcome of a standard battery of nonclinical tests, additional nonclinical studies may be warranted to more fully characterize the endocrine-related toxicity potential of a drug. For particular situations, the review division should be contacted for further guidance.