Toxicity of Antiestrogens

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Abstract: The object of this article is to review briefly the preclinical and clinical safety of some antiestrogens. Tamoxifen, toremifene, droloxifene, and idoxifene are polyphenylethylene antiestrogens, whereas the pure antiestrogen, ICI 182,780 or faslodex, as well as raloxifene, is of a different structure. Tamoxifen has been shown to be genotoxic in several studies. It induces unscheduled DNA synthesis in rat hepatocytes and micronuclei in MCL-5® cells in vitro. Tamoxifen also induces aneuploidy in rat liver in vivo and chromosome aberrations and micronuclei in mouse bone marrow. Toremifene has also shown to be genotoxic, but to a far lower extent, by inducing micronuclei in MCL-5® cells in vitro and by inducing aneuploidy in rat liver in vivo. Tamoxifen has been shown to be hepatocarcinogenic in the rat in at least four independent long-term studies. The initiation of tumors in the rat is the result of metabolic activation by cytochrome P450 isoenzymes to an electrophile(s) that binds irreversibly to DNA. The other antiestrogens have not been shown to be carcinogenic in rodents. In several independent clinical studies, the risk of endometrial cancer has increased among tamoxifen-treated women. After reviewing the available data, the International Agency for Research on Cancer concluded that there was sufficient evidence to show that tamoxifen is a class I human carcinogen. The increased risk for endometrial cancer occurs predominantly among women who are 50 years old or older and who have been treated with tamoxifen. It is not yet clear whether the uterine tumor formation is a result of genetic mechanisms, analogous to those seen in the rat liver or due to the estrogen agonist action of tamoxifen. However, the other antiestrogens with a more or less similar intrinsic estrogenic potential have not been shown to be carcinogenic in humans.

Key Words: antiestrogen, toxicity, endometrial cancer, tamoxifen, toremifene, raloxifene

Antiestrogens act as estrogen antagonists; that is, they bind to and compete with endogenous estrogen for estrogen receptors in the nuclei of estrogen-responsive tissues. In clinical practice, antiestrogens are used for ovulation induction (clomiphene, Clomid®) in the treatment or prevention of breast cancer (tamoxifen, Nolvadex® and toremifene, Fareston®) and are also recently used for the prevention of osteoporosis (raloxifene, Evista®)(1). Preliminary results indicate that raloxifene may also have efficacy in breast cancer prevention. The drug is being compared with tamoxifen for this indication. Tamoxifen, toremifene, droloxifene, and idoxifene are polyphenylethenes (Fig. 1). Tamoxifen was introduced in 1971 and toremifene in 1988, whereas droloxifene and idoxifene are newer antiestrogens. The pure antiestrogen, ICI 182,780 or faslodex, and raloxifene are still newer antiestrogens (Fig. 2). The antiestrogens have also been called selective estrogen receptor modulator (SERM) drugs (1,2).

During recent years, convincing preclinical evidence has shown that tamoxifen has genotoxic properties (3–5). The most severe clinical manifestation of this property is the increased risk for secondary endometrial cancer among women using tamoxifen. New longer treatment options, such as chemoprevention and treatment of early breast cancer (adjuvant treatment), targeting healthy women or women with long life expectancy led to an increased alertness toward the safety aspects of antiestrogens. Improved safety is a high priority when developing new SERM drugs (2,5).
PRECLINICAL STUDIES

Genotoxicity

The genotoxicity of tamoxifen and toremifene is well documented in the literature, whereas documentation of the other antiestrogens in this respect remains poor. Tamoxifen has been shown to be positive in all genotoxicity studies. Tamoxifen induces unscheduled DNA synthesis in rat hepatocytes and micronuclei in MCL-5\(^3\) cells in in vitro studies. In in vivo studies, tamoxifen induces aneuploidy and is clastogenic in rat liver and leads to chromosome aberrations and micronuclei formation in mouse bone marrow (Table 1). Toremifene has shown far less noxious genomic effects in preclinical studies than tamoxifen. Williams et al. reported that toremifene is not genotoxic when tested in three standard in vitro assays (6); reversion of bacterial point mutations, unscheduled DNA synthesis in rat hepatocytes from two rat strains, and chromosome aberration assays of human lymphocytes. Similar to idoxifen (7) and even to estrogen (8), toremifene has been reported to induce micronuclei in MCL-5\(^3\) cells in vitro, and in in vivo studies, it induces aneuploidy. It is not, however, clastogenic in the rat liver as is tamoxifen (Table 2). Multiple studies have shown that tamoxifen induces DNA adducts in high levels in rat liver. No adducts or 300-fold less adducts have been reported with toremifene (4).

Carcinogenicity

Tamoxifen has been shown to be hepatocarcinogenic in the rat in several independent, long-term studies (17,18). In a 2-year carcinogenicity study that was conducted by Greaves et al. (19), in Wistar rats, a dose of 5 mg/kg/day induced hepatocellular carcinoma in 16% of the males (controls 1%) and in 12% of the females (controls 0%). Toremifene is not carcinogenic.

Table 1. Genotoxicity Studies with Tamoxifen

| System            | End point                        | Result     | Study |
|-------------------|----------------------------------|------------|-------|
| Tamoxifen         | In vitro                         |            |       |
| Rat hepatocytes   | Unscheduled DNA synthesis        | Positive   | (9)   |
| MCL-5\(^3\)       | Micronuclei                      | Positive   | (10)  |
| In vivo           | Rat liver                        | Aneuploidy | Positive (7,11) |
| Clastogenicity    | Positive                         | (7,8)      |       |
| Mutagenicity      | Positive                         | (12,13)    |       |
| Rat liver         | DNA adduct formation             | Positive   | (4,5,16,23) |
| Mouse bone marrow | Chromosome aberrations           | Positive   | (14)  |
|                   | Micronuclei                      | Positive   | (15)  |
Table 2. Genotoxicity Studies With Toremifene and Raloxifene

| System   | End point                      | Result          | Study |
|----------|-------------------------------|-----------------|-------|
| Toremifene |                               |                 |       |
| In vitro | Micronuclei                   | Positive        | (10)  |
| Rat liver| Aneuploidy                    | Positive        | (7,11) |
| Rat liver| Clastogenicity                 | Negative        | (7,8)  |
| Rat liver| Mutagenicity                   | Negative        | (12,13) |
| Rat liver| DNA adduct formation          | Negative or very low | (4,5,16,23) |
| Raloxifene |                               |                 |       |
| Rat liver| DNA adduct formation          | Negative        | (16)  |

* Metabolically competent lymphoblast clone.

in rodent tests (17,18,20). The initiation of tumors in the rat is the result of metabolic activation by cytochrome P450 isoenzymes (CYP) to an electrophilic(s) that binds irreversibly to DNA. The ultimate reactive carcinogenic metabolite of tamoxifen is the sulfate-conjugated derivative of the α-hydroxylated parent drug or its N-desmethyl metabolite (5,21–23). Toremifene and raloxifene molecules cannot be activated by this α-hydroxylation pathway in vivo because of their different molecular structure (16). Tamoxifen but not toremifene induces endometrial cancers in adult rats as well (24), although the estrogenic/antiestrogenic effects of these drugs on the endometrium are equal (25). Neonatal tamoxifen exposure also leads to endometrial cancers in rats and mice (26,27). No data are available on carcinogenicity tests with raloxifene.

**CLINICAL STUDIES**

In general, antiestrogens are associated with a low incidence of serious adverse effects. There is no increase in the incidence of liver cancer with any of the drugs. It is well established, however, that tamoxifen is associated with an increased risk for endometrial cancer in women (5,28). When compared with placebo, the increase in the relative risk to develop secondary endometrial cancer varies twofold to sevenfold in different studies, depending on the length of tamoxifen exposure.

Rutqvist et al. (29) found an average of fourfold increase in endometrial cancer as compared with untreated controls among 4,900 Scandinavian breast cancer patients. The follow-up time was 8 to 9 years (29). A continuous divergence in the cumulative incidence of endometrial cancer between the tamoxifen and the control group in this study, even several years after cessation of treatment, suggests that tamoxifen initiates some of the observed events.

In the National Surgical Adjuvant Breast and Bowel Program in the United States, which involved 2,823 patients with node-negative, estrogen receptor-positive breast cancers, tamoxifen treatment over a 5-year follow-up period resulted in a relative risk of 7.5 over the placebo group (30).

A case-control study in the United States of the Surveillance, Epidemiology and End Results showed an approximately 1.6-fold increase in the incidence of uterine tumors in the group receiving tamoxifen 20 mg/day (31).

A total of 13,388 healthy women at high risk for developing breast cancer were included in National Cancer Institute founded Breast Cancer Prevention Trial. The 4-year follow-up revealed a 4.97-fold increase in the relative risk of developing endometrial cancer among tamoxifen-treated women. Unfortunately, the study was interrupted too early to be able to conclude the long-term effects of tamoxifen treatment in this respect (32).

Reviewing the available data, the International Agency for Research on Cancer concluded that there was sufficient evidence to show that tamoxifen is a class I human carcinogen (28). An increased risk for endometrial cancer is predominantly found in women 50 years old or older. There is a comparatively rapid onset of these secondary tumors, often within 2 to 5 years of the start of treatment (5). However, some data are available that show that late onset, 8 to 10 years after cessation of tamoxifen treatment, is possible (29).

The most common type of endometrial carcinoma, endometrial adenocarcinoma, develops from endometrial hyperplasia in the setting of excess estrogen exposure (33). Relatively few articles have focused on the descriptive morphology of the tamoxifen-associated lesions. In a review of 102 cases using hormone replacement therapy-related endometrial specimens and conventional polyps as the control group, the most characteristic findings of tamoxifen-associated lesions included polarized glands along the long axis of polyps (40%), a cambium layer (72%), frequent and diverse metaplasias, staghorn glands (36%), myxoid degeneration (12%), and small glands (36%). Similar morphologic features were identified in the hormone replacement therapy and control groups but to a variable, lesser extent. The tamoxifen group consisted of 18 cases of hyperplasia and 1 case each of adenofibroma, adenosarcoma, endometrial stromal...
sarcoma, and leiomyosarcoma (34). Estrogen receptors are usually expressed in leiomyosarcomas (35) and also in some adenomas (36).

Available long-term clinical data on tamoxifen with 3 to 5 years of exposure and nearly 10 years of follow-up indicate that there is no increase in endometrial cancer incidence or that the risk is considerably lower than with tamoxifen (37,38). However, further reports and a longer follow-up are needed to confirm this.

The available reports on raloxifene suggest that it has no proliferating or carcinogenic action on human endometrium (39,40). Only long-term data will be able to verify these assertions (39).

It is still not clear whether the mechanism of formation for these uterine tumors is through genetic mechanisms, analogous to those seen in the rat liver, or as a result of the estrogen agonist actions of tamoxifen. Conclusive evidence on the endometrial effects of the newer antiestrogens may be obtained only by further long-term clinical studies.

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