ABSTRACT

Tamoxifen, a nonsteroidal antiestrogen used widely in the treatment of breast cancer, was tested in a conventional 2-year carcinogenicity bioassay in rats, a species in which tamoxifen acts variably as a partial agonist and antagonist on different target tissues. Groups of 51 males and 52 females were given 5, 20, and 35 mg/kg of tamoxifen/day by gastric intubation in 0.5% hydroxypropyl methylcellulose at 5 ml/kg dose volume. There were 102 male and 104 female controls dosed with vehicle alone.

Growth rate and food consumption were reduced in all treated groups. The major finding was a dose-related increase in the incidence of hepatocellular tumors which were first observed after 31 weeks of treatment in the top dose group. The majority of the neoplasms were hepatocellular carcinomas showing a well differentiated trabecular pattern. Some tumors were glandular in type. Mortality was increased in the 20 and 35 mg/kg dose groups compared with controls as a result of these tumors. By contrast, survival was greater than controls in rats given 5 mg/kg tamoxifen despite the presence of hepatocellular tumors due to a reduction in the number of pituitary tumors in females and less chronic renal disease in males.

The mechanism of hepatic tumor induction by tamoxifen in rats is unclear. In view of the lack of genotoxic activity in conventional genotoxicity studies and lack of similar effect in mice or in humans, the findings may relate to a particular constellation of effects in rats. All other drug-induced changes in this study were nonneoplastic in nature and most appeared to be the result of hormonal perturbation since they were confined to endocrine organs or have been seen previously in rats treated for long periods with tamoxifen.

INTRODUCTION

Tamoxifen (tamoxifen citrate or Nolvadex; ICI 46, 474) is a triphenylethylene nonsteroidal antiestrogen that has been used for the treatment of breast cancer since 1969. It represents a widely used endocrine treatment for patients with all stages of breast cancer (1, 2).

It is a well tolerated drug with a low level of adverse effects, particularly when compared with more conventional cytotoxic anticancer drugs which produce damage to rapidly proliferating cells in the gastrointestinal tract and bone marrow (3). Concerns about long-term treatment with tamoxifen relate more to the potential adverse health consequences of hormonal manipulation than to effects on rapidly proliferating cells (4, 5).

This side effect profile of tamoxifen in female patients broadly reflects the low level of toxic effects observed in the early preclinical safety studies (6). Tamoxifen showed low acute and chronic toxicity in conventional studies performed in rats, mice, and dogs. Most of the pathological findings were found in the endocrine and reproductive systems in these species and appeared related to the exaggerated pharmacological activity at high doses.

Following long-term treatment of Alderley Park outbred mice with tamoxifen, treatment-related neoplasms were confined to hormone-sensitive tissues. These were believed to be the consequence of long-term derangement of endocrine status in this species in which tamoxifen is classified as a full estrogen (6, 7).

While the findings in these earlier preclinical studies fully support the use of tamoxifen in palliative treatment of breast cancer, consideration of tamoxifen for use in treating nonmalignant disease and breast cancer prophylaxis among women at high risk of developing mammary cancer led to the initiation of a conventional rat carcinogenicity study in 1986. Although some studies have addressed the carcinogenicity of tamoxifen in the rat after exposures up to 1 year (8, 9), this conventional 2-year rat carcinogenicity bioassay is now reported in view of the potential importance of tamoxifen in breast cancer prophylaxis.
Mortality was similar in all male groups up to week 36 and in all female groups to week 27. Thereafter, rats given 35 mg/kg/day died or were killed for humane reasons between weeks 37 and 71. Likewise, rats given 20 mg/kg/day died or were killed for humane reasons between weeks 43 and 87. By contrast, mortality among male and female rats given 5 mg/kg/day was lower than in the comparative control group (Fig. 1 and 2).

From week one growth of all rats given tamoxifen was impaired compared with controls and this was associated with lower food consumption with males being generally more affected than females (Fig. 3 and 4). A number of clinical signs were associated with treatment in a dose-related manner. These included an increased prevalence and severity of alopecia, most marked in females, waxy pelage, and excessive salivation. Abdominal masses related to the liver were also palpated in life. The majority were detected between weeks 27 and 52 in rats given 35 mg/kg/day, weeks 52 and 78 in rats given 20 mg/kg/day, and weeks 79 and 107 in rats given 5 mg/kg/day.

Ophthalmoscopic examination revealed an increase in the prevalence of crescentic cataracts in rats treated with tamoxifen. This is the most common form of spontaneous cataract in this strain being crescentic in shape with a broad base at the equator and extending along both the posterior and the anterior lens capsule. An increased prevalence of crescentic cataracts was recorded in rats given 35 mg/kg/day compared with controls from the time that they were first noted at week 26. By week 52 crescentic cataracts were seen in about 50% of the eyes of females given 35 mg/kg/day and 25% males at the same dose, compared with about 10% in control female and 2% of control male rats. Similar but less marked differences were seen at lower doses with the exception of female rats given 5 mg/kg/day. These had a slightly lower incidence of crescentic cataracts than control female rats.

In addition, cataracts of other types may also have been potentiated by treatment. Anterior cortical radial lines were seen in two rats given 35 mg/kg/day, two rats given 20 mg/kg/day, and in only one control. Posterior suture cataracts developed predominantly in rats given 5 mg/kg/day as they developed in significant numbers only after 18 months. They were not seen in controls but were found in three rats treated with 35 mg/kg/day, one treated with 20 mg/kg/day, and by the end of the study, 17% of males and 25% of females treated with 5 mg/kg/day.

Hematological examination at the end of the study revealed no meaningful biological differences between groups. Serum hepatic enzyme values in some individual treated rats were markedly higher than the reference range. Comparison of blood chemistry group means at weeks 51 to 53 showed that alkaline phosphatase activity was about three-fold higher in all treated groups compared with controls and the differences were statistically significant. Mean alanine aminotransferase activity was also increased.

<table>
<thead>
<tr>
<th>Table 1 Dose levels and numbers of animals</th>
</tr>
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<tbody>
<tr>
<td>Dose level of tamoxifen (mg/kg of free base)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>I  (control)</td>
</tr>
<tr>
<td>II 5</td>
</tr>
<tr>
<td>III 20</td>
</tr>
<tr>
<td>IV 35</td>
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</tbody>
</table>
ferrase and aspartate aminotransferase activities were highly variable between groups although the mean alanine aminotransferase was higher in males treated with 35 mg/kg/day than in controls (difference statistically significant, \( P < 0.01 \)).

The outstanding histopathological finding was the dose-related increase in incidence of hepatocellular tumors (Table 2). Liver tumors were first found at necropsy after 31 weeks of study. The majority of these tumors were hepatocellular carcinomas with a well differentiated trabecular pattern (Fig. 5). Some showed more variable histological features with glandular, cystic, or papillary appearances (Figs. 6 and 7). Most were locally invasive. A small number showed clear evidence of extracapsular spread and 11 had developed metastatic deposits in lungs or lymph nodes (Fig. 8). Some tumors were benign in character and these also showed both hepatocellular and glandular differentiation patterns. Some nodular lesions retained intact portal structures and were designated nodular hyperplasia (Table 3).

Much of the nonneoplastic hepatic tissue was disrupted by the presence of these large hepatic neoplasms. Hence, critical assessment of focal nonneoplastic alterations was not possible in this study. Foci of hepatic cellular alteration were not commonly found in any groups. Zones described as peliosis hepatis, composed of relatively normal hepatocytes separated by widened sinusoids and centered mainly around central veins were more common in treated animals. Proliferation of biliary epithelium was found in nonneoplastic liver but this seemed particularly marked in livers containing large neoplasms (Table 3).

Some other tumor types were reduced in treated groups when compared with controls and with the background incidence of tumors in this strain of rat. Reductions were recorded in the incidence of pituitary adenomas in both sexes, mammary tumors in females, and parathyroid adenomas in males (Table 4). This reduction could not be entirely explained by reduced survival because this effect also occurred in rats treated with 5 mg/kg/day which had better survival than controls.

A variety of nonneoplastic pathological changes were also recorded in other organs. Those findings which occurred in increased incidence in treated rats included small foamy macrophage aggregates in lungs, degenerative lenticular alterations characteristic of advanced cataracts, atrophy of seminiferous tubules, epididymides, prostate gland, seminal vesicles, uterus, cervix, vagina, ovaries, and mammary glands. A slightly higher incidence of hypertrophy of the zona fasciculata of the adrenal cortex and thickening of bone trabeculae was noted in some rats given 20 and 35 mg/kg/day. A lower incidence of chronic renal disease (chronic glomerulonephropathy) was also noted in treated rats compared with controls (Table 3).

The principle cause of death or factor which necessitated early termination of treated rats was the presence of hepatocellular tumors. The main cause of death among female controls was pituitary adenomas and in control males was chronic progressive glomerulosclerosis.

### DISCUSSION

The principle finding in this study was the increase in the number and the early onset of hepatocellular neoplasms in rats treated with tamoxifen compared with controls. This is consistent with the results of findings in recent 12-month studies in Sprague-Dawley rats (8, 9). Thus, tamoxifen must be regarded as a hepatic carcinogen in rats. Hepatic tumor pathology was the principle cause of poor survival in rats given 20 or 35 mg/kg/day and the early termination of these groups by weeks 87 and 71 of the study, respectively. There was no correlation between high serum levels of tamoxifen and premature deaths. Although hepatocellular tumors caused the early death of some
Carcinomas can be induced rapidly in rats, as in this study, without the periods of time, usually over 1 year (17, 18). Nevertheless, hepatic where chronic administration gives rise to highly malignant hepatic metastatic deposits confirming their malignant biology. In certain cases, there were also widespread pulmonary metastases showing a highly glandular appearance. Hematoxylin and eosin × 170.

Fig. 8. Intravascular pulmonary metastasis of a solid hepatocellular carcinoma in a high dose group male rat. Hematoxylin and eosin × 170.

Rats given 5 mg/kg/day, overall survival in this group was better than controls. This was mainly because of a lower level of renal disease in males and reduced number of pituitary adenomas in females compared with respective controls. Pituitary adenomas and renal disease are the principle causes of death in Alderley Park rats (13). It is recognized that the suppression of growth which occurred in rats treated with tamoxifen is capable of reducing the incidence of renal disease and pituitary adenomas (14). This reduction in growth is believed to be a consequence of the pharmacological activity of tamoxifen and related changes in hormonal status (15, 16).

Many of the hepatic tumors showed highly malignant histological and cytological features and some showed glandular differentiation patterns. In certain cases, there were also widespread pulmonary metastatic deposits confirming their malignant biology. This pattern of hepatic tumor pathology was analogous to that induced by the powerful genotoxic agents such as diethylnitrosamine where chronic administration gives rise to highly malignant hepatic tumors within periods of about 6 months. By contrast, many nongenotoxic agents such as clofibrate, phenobarbital, and sex steroids give rise to nodules and tumors of relatively low malignancy after longer periods of time, usually over 1 year (17, 18). Nevertheless, hepatic carcinomas can be induced rapidly in rats, as in this study, without the administration of genotoxic agents. For instance, a choline deficient diet induces a highly malignant spectrum of hepatic tumors in the rat liver within 6 months by a mechanism not involving genotoxic carcinogens possibly through the formation of free radicals, chronic cell damage, and increased cell turnover (19, 20).

Although there were some hyperplastic nodules, nonneoplastic liver tissue of tamoxifen treated rats contained surprisingly few foci of cellular alteration. However, no histochemical study was performed to detect foci. Biliary alterations as well as spionosis hepatis and peliosis hepatis and foci of necrosis were seen. However, some of these changes were related to the disruption caused by the presence of large tumors because the hepatic parenchyma in low dose animals without neoplasms showed relatively little evidence of focal nonneoplastic alterations. Hence, it was not possible to assess tumor progression from findings in nonneoplastic hepatic tissue in this study. Thus, firm conclusions about whether tamoxifen acts as a genotoxic carcinogen cannot be based simply on the nature and time course of development of hepatic tumors and focal nonneoplastic lesions.

Tamoxifen showed no evidence of mutagenic potential in several in vitro assays performed in accordance with OECD guidelines, both with and without a rat liver metabolizing system. These were tests with Salmonella typhimurium (5 strains), the CHO/HRPT locus assay, a metaphase analysis with human lymphocytes and unscheduled DNA synthesis in HeLa cells (6). Moreover, a dominant lethal test in rats also showed no evidence of mutagenic potential. Nevertheless, recent studies have suggested that DNA adducts of tamoxifen form in the liver of treated Sprague-Dawley (21) and Fischer rats (22). From this work it has been postulated that hepatocellular carcinoma develops in the rat through an accumulation of these adducts and consequent DNA damage.

It has been suggested that tamoxifen exerts its tumorigenic effect by nongenotoxic mechanisms because it has been shown to act as a promoter in the rat liver model when initiated with diethylnitrosamine (23). Although tamoxifen inhibits DNA synthesis and the appearance of γ-glutamyltranspeptidase-positive foci induced by estrogens in the rat liver, tamoxifen alone can promote the appearance of foci in diethylnitrosamine initiated rat liver (23). In view of the current debate on the role of chemically induced hepatocyte proliferation in rodent hepatocarcinogenesis, it is important to know whether tamoxifen can act as a liver mitogen and to define any dose and time dependency of such responses. One possibility is that tamoxifen promotes by virtue of its pharmacological activity at high doses in the rat. The pharmacology of antiestrogens is complex (24, 25). Tamoxifen itself has variable agonist and antagonist properties in different target tissues and in different species. It is believed that by virtue of the shape of the molecule, tamoxifen wedges into the estrogen receptor complex and prevents the full range of conformational changes required for receptor activation (7). In the liver, tamoxifen is believed to exert agonist effects through the estrogen receptor system by causing translocation of estrogen receptors from the cytosol to the nucleus of hepatic cells (26). Like the estrogen-receptor complex, the tamoxifen-receptor complex moves to the nucleus. However, as it initiates only some of the estrogen responses, it is postulated that the precise nuclear effects are different (27).

Estrogens themselves have well recognized importance in protein synthesis and cell division and are capable of promoting hepatic neoplasia in rodents. It has been suggested that modification of receptor pathways associated with hepatic growth control may be factors in the clonal expansion of preneoplastic cells during estrogen-induced promotion (28). Hence, tamoxifen could produce hepatic tumors through analogous effects on cell division and protein synthesis mediated via the estrogen receptor complex.

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2 J. C. Topham and T. C. Orton, unpublished ZENECA reports.
Tamoxifen has been shown to increase drug metabolising capacity in the rat liver notably activities of cytochromes P450, ethoxycoumarin deethylase, ethoxyresorufin deethylase, and epoxide hydrolase (29). Such metabolic alterations may be linked to its hepatocarcinogenicity, perhaps by inducing enzymes responsible for its own activation (22). Tamoxifen has also been shown to have weak peroxisomal proliferating properties (8). Moreover, it has been suggested that tamoxifen disturbs lysosomal function because of its ability to cause intralysosomal storage of polar lipids and induction of generalized lipidosis in rats as a consequence of its amphipatic cationic properties (30).

The relevance of these rat finding for the therapeutic use of tamoxifen in humans is unclear. In contrast to the rat, the mouse does not develop hepatic tumors following long-term treatment. In two previous carcinogenicity studies in the Alderley Park mouse conducted with tamoxifen at doses of up to 50 mg/kg/day, hepatic findings were limited to fatty change and enlargement of hepatocytes (6). Most other effects in the mouse including an increase in interstitial tumors of the testis were similar to those produced by estrogens in this strain (6). While it is well recognized from general comparisons of carcinogenicity of a wide range of chemicals in rat and mouse that some are only carcinogenic in the rat and that over 30% of chemicals active in one species produce tumors at different target organs in rat and mouse (31), this species difference is particularly difficult to explain in the case of tamoxifen.

Although in this particular rat study, blood levels of tamoxifen correlated broadly with dose, the range of values was very large, presumably because of the variable health status of the rats and particularly the fact that samples were available only at different times after dosing. Hence, the exposure achieved in these rats cannot be easily correlated with exposure in therapy. The metabolism of tamoxifen is complex and results in a wide range of metabolites in all species studied, some of which are estrogenic and others antiestrogenic (32, 33). Excretion is largely via the feces and hepatic exposure to drug and metabolite is similarly high in both rat and mouse (34, 35). No clear differences have been identified in the disposition of tamoxifen that are sufficient to explain the species differences and facilitate the risk assessment (34, 35).

Moreover, a recent comparative study has not only shown accumulation of modified DNA bases in Fischer rats treated with tamoxifen but also in both DBA/2 and C57BL/6 mice (22). Apart from the fact that the levels of the adducts were shown to be 30–40% lower in the livers of mice than in rats, this information provides no ready explanation for the differences in tumorigenicity between the two species.

Tamoxifen was also shown to potentiate the appearance of cataracts in the rat at high doses. An association between the development of cataracts in rats and the administration of estrogens has been well described (36–38) although these agents have not been generally associated with the development of cataracts in humans (39). One striking lenticular change was the crescentic cataract, a term used to describe a characteristic cortical cataract which develops spontaneously from about 6 months in the Alderley Park strain of rat with a high prevalence among females (40). The etiology of this type of cataract is uncertain but it is probably the result of age-related degenerative or metabolic factors in the lens epithelium or bow area. However its prevalence can be modulated by alterations in sex hormone status (40). Thus, the high incidence of this form of cataract may reflect the excessive estrogenic activity of high doses of tamoxifen in rats.

The accumulation of foamy macrophages in the lungs of rodents treated with tamoxifen has been previously reported and it has been postulated that this change is related to its amphipatic cationic properties (30). Atrophy of ovaries, testes, secondary sex organs, and mammary glands as well as the hypertrophy of the adrenal cortex and bone changes are believed to be the result of the hormonal activity of tamoxifen (6, 11, 24, 41).

Whereas it can be concluded that most of the changes induced by long-term administration of tamoxifen to the rat can be considered a result of prolonged perturbation of hormonal status in this species, a

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**Table 3 Main Nonneoplastic findings**

<table>
<thead>
<tr>
<th>Males (mg/kg/day)</th>
<th>Females (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>No. of animals examined</td>
<td>102</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Bilary cystic dilatation</td>
<td>0</td>
</tr>
<tr>
<td>Proliferative cholangiomatic component</td>
<td>0</td>
</tr>
<tr>
<td>Nodular hyperplasia</td>
<td>3</td>
</tr>
<tr>
<td>Peliosis hepatis</td>
<td>0</td>
</tr>
<tr>
<td>Spongiosis hepatis</td>
<td>10</td>
</tr>
<tr>
<td>Enlarged centrilobular hepatocytes</td>
<td>1</td>
</tr>
<tr>
<td>Altered foci of hepatocytes</td>
<td>0</td>
</tr>
<tr>
<td>Hepatocyte necrosis</td>
<td>6</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Chronic glomerulonephropathy</td>
<td>90</td>
</tr>
</tbody>
</table>

---

**Table 4 Incidence of principal neoplasms in other tissues**

<table>
<thead>
<tr>
<th>Male (mg/kg/day)</th>
<th>Female (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Mammary glands</td>
<td>(102)</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>(102)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>14</td>
</tr>
<tr>
<td>Parathyroid gland</td>
<td>(93)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>10</td>
</tr>
</tbody>
</table>

* Trend test P value.

a NS, not significant at P < 0.05.
similar mechanism does not readily explain the rapid development of hepatocellular carcinomas. Although these findings may relate to the particular constellation of effects in rats, they should be considered in any clinical risk-benefit analysis of the extended use of tamoxifen into breast cancer prophylaxis.

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Two-Year Carcinogenicity Study of Tamoxifen in Alderley Park Wistar-derived Rats

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