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Feature Articles

Should Clinicians be Concerned About the Carcinogenic Potential of Tamoxifen?

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DURING THE past year, the media has reported stories on the safety of tamoxifen. The stories emanate from publications concerning tamoxifen and ocular problems [1], tamoxifen and liver tumours in rats [2] and tamoxifen and endometrial cancer [3] in the scientific literature. Examination of the titles of the articles alone [1–3] is enough to cause alarm for anyone who is already taking tamoxifen as a breast cancer therapy. However, the benefit of tamoxifen as a cancer therapy is clearly proven. The concern really centres on the use of tamoxifen as a breast cancer preventive in healthy women. Naturally, stringent tests must be conducted on any new drug being evaluated in healthy women and concerns are valid. However, extensive clinical experience is able to guide the applications of tamoxifen in the setting of a prevention trial. Three clinical trials are currently evaluating the worth of tamoxifen in preventing breast cancer in high-risk women. Recruitment to a vanguard study at the Royal Marsden Hospital [4] is now complete so that national and international recruitment can progress. In the United States, 11 000 of the required 16 000 volunteers have been randomised to a National Cancer Institute-funded prevention trial [5], and in Italy 3000 volunteers in a 20 000-volunteer study have been recruited [6].

However, the debate and objections raised about the use of tamoxifen in organised clinical trials with healthy female volunteers [7–9] has had the effect of producing concerns in women who are being treated with tamoxifen for breast cancer, despite the fact that tens of thousands of patients have benefited from tamoxifen during treatment. The fear of side-effects and toxicities create a dilemma for both the patient and physician as there is currently no simple way to place the risk of toxicity in perspective.

There are a number of current reviews [10–13] that consider the advantages and disadvantages of tamoxifen therapy in the context of clinical trials and clinical practice. The aim of this review is to focus specifically on the reports of the carcinogenic potential of tamoxifen in the rat and human, and provide a synthesis of the published evidence.

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As our title suggests, our primary concern is for the patient with breast cancer being treated with tamoxifen.

TAMOXIFEN AND RAT LIVER CANCER

High daily doses of tamoxifen will produce hepatocellular carcinoma in the rat (Table 1) if administered for up to half the animal's lifetime. This is particularly true at a 45.2 mg/kg dose, when tumours are formed within 6 months in 29% of the animals [2]. There is general agreement that high daily doses of tamoxifen result in the premature death of rats. In the study by Greaves and coworkers [14], 50% of control female rats were alive and well at about 104 weeks (2 years), but treatment with 35 mg/kg tamoxifen daily produced 50% deaths by 42 weeks. Interestingly, the low dose of 5 mg/kg/day increases the survival of male and female rats at 2 years (males: 30% deaths in treated versus 70% deaths in controls; females: 25% deaths in treated versus 50% deaths in controls). The authors note [14] that their low tamoxifen dose (5 mg/kg/day) completely inhibited the incidence of adenomas in the pituitary gland and adenocarcinomas of the mammary gland in female rats, and almost completely inhibited adenomas of the pitituary gland and parathyroid gland in male rats.

The published studies indicate (Table 1) that there is a threshold level for liver carcinogenicity, which is approximately 3 mg/kg/day [2]. However, the study by Dragan and coworkers [16], using a different rat strain and experimental design, observed no hepatocellular carcinomata after 15 months of treatment. The design of the study divided carcinogenesis into initiation and promotion. Carcinogenesis was initiated with diethylnitrosamine (DEN 10 mg/kg oral) in partially hepatectomised Fischer F344 rats, and promotion to carcinogenesis was completed with tamoxifen in the feed at 250 ppm. Blood levels of tamoxifen were 230 ± 30 ng/ml (i.e. in the range of clinical experience [16]). It can be estimated that a 200-g rat consumes 10 g of food containing 2.5 mg tamoxifen per day, so a rat recevied a daily dose of 12.5 mg/kg, which is within the 10–30 mg/kg/day dosing regimens of other studies [14, 15]. No hepatocellular carcinomata were observed if DEN, the initiator, was omitted, but tumours were seen if DEN was given with tamoxifen, leading the authors to conclude that tamoxifen is a promoter of hepatocellular carcinoma in the Fischer rat. However, all the other studies, mainly using Sprague–Dawley strains of rats and bolus administration of drug by lavage, suggest that tamoxifen is a complete carcinogen at high doses.

Table 1. The occurrence of hepatocellular carcinoma in various rat strains during long-term tamoxifen treatment

Strain of rat	Daily dose (mg/kg)	*n*	Duration (months)	Hepatocellular carcinoma %	Hepatocellular carcinoma (*n*)	Reference
1. Sprague–Dawley (Crl:CD(BR))	2.8	57	15	0	(22)	[2]
	11.3	57	15	45	(11)	
	45.2	55	12	75	(4)	
2. Wistar (Alpk:APfSD)	5	52	24	16	(51)	[14]
	20	52	24	64	(51)	
	35	52	24	64	(51)	
3. Sprague–Dawley (Crl:CD(BR))	11.3	84	12	44	(36)	[15]
	22.6	75	12	100	(24)	
4. Fischer F344	12.5*	20	15	0	(8)	[16]

The numbers of rats (*n*) given is the number at the start of the experiments, but scheduled and unscheduled deaths occurred during treatment. The % hepatocellular carcinomas only refers to the % at the scheduled kill. * 250 mg tamoxifen per kg diet. Animals weighed 200 g and ate 10 g of food per day.

TAMOXIFEN AND DNA ADDUCT FORMATION

Carcinogenesis requires genotoxicity so it is important to correlate the formation of DNA adducts with the formation of tumours in a particular organ for a sensitive species. Mani and Kupfer [17] first showed that in human and rat liver microsome systems *in vitro* [^{14}C] tamoxifen was metabolised by an NADPH-dependent cytochrome P_{450}-mediated activation system to intermediate(s) which covalently bound to microsomal proteins. Han and Liehr [18] subsequently showed that the administration intraperitoneally (i.p.) of tamoxifen (20 mg/kg/day) to Sprague–Dawley rats resulted in two DNA adducts after only 1 day and up to six adducts after 6 consecutive days of treatment. A simliar result was observed by Hard and associates [15] using 48 mg/kg/day tamoxifen for 7 days in Sprague–Dawley rats.

It is clear that large doses of tamoxifen can produce DNA adducts, but White and coworkers [19] have investigated the dose adduct relationship in rats. Seven days of dosing with between 5 and 45 mg tamoxifen/kg/day produced an almost linear dose-dependent increase in DNA adducts in the Fischer 344 rat. At doses of less than 5 mg/kg/day, tamoxifen did not alter the chromatograph from ^{32}P post-labelled DNA from treated rats. It would appear, therefore, that there is a threshold for the appearance of adducts with tamoxifen, and the induction of liver tumours.

White and colleagues [19] have also examined whether adduct formation occurs in the mouse, which does not produce liver tumours in response to tamoxifen. There is DNA adduct formation in both C57B1/6 and DBA/2 mice; however, this is approximately 30% of that observed with a similar dosing schedule in the Fischer rats [19], raising questions about the correlation between adduct formation and clinically evident tumours.

In humans, DNA adducts have not been observed in the livers of tamoxifen-treated women; however, only limited samples have been screened. A recent study *in vitro* [20] demonstrates the ability to form DNA adducts with human and rat liver microsomes using 100 μM tamoxifen. Although the levels of DNA adducts are low and in the range of the studies *in vivo* with mice, the human liver was two to three times more effective at producing DNA adducts than the rat. Obviously, further comparisons with mice and rats of different strains are important. The Sprague–Dawley rat livers used in these studies *in vitro* [20] are from the strain that is extremely sensitive to the carcinogenic actions of tamoxifen *in vivo*. Adduct formation *in vitro* can be dramatically altered by adding different cofactors [20], and the level of DNA adduct formation that is required for carcinogenesis may be dose related, as in the rat *in vivo* [19]. The level of adducts, $1–3 \times 10^8$ nucleotides, observed in the study of rat liver microsomes *in vitro* [20] is not in the carcinogenic range *in vivo* [19], although caution must be used when comparing *in vivo* and *in vitro* studies.

Overall, these data demonstrate that DNA adducts can be formed *in vitro* and *in vivo*, but the level of adduct formation is critical for carcinogenesis. Adduct formation using human microsomes is very low, but this can be enhanced into the mouse range using cumene hydroperoxide as a cofactor [20]. However, mice do not produce liver tumours after long-term treatment. A recent study *in vitro* has shown that rat liver microsomes produce more tamoxifen epoxides that could be responsible for adduct formation and rat liver carcinogenesis [21, 22]. Thus, the most important issues to resolve are the species differences, the correlation between liver carcinogenesis and DNA adduct formation, the effect of the rate of repair of DNA in different species, and the relative doses used to demonstrate the carcinogenic effects of tamoxifen.

DOSES OF TAMOXIFEN IN ANIMALS AND MAN

A key argument made regarding rat liver carcinogenesis studies is that since the serum concentrations of tamoxifen obtained in the rat (Table 2) are within the range of serum concentrations achieved during the treatment of breast cancer, then the results are clinically relevant. It is generally believed that toxicology testing should be conducted to mimic human pharmacokinetics. However, the rat and mouse clear tamoxifen from the body at a much faster rate than the human so that higher doses must be administered to maintain the blood level in the human range used for treatment. Examination of the relative dosage regimens in different species and the resulting

Table 2. The levels of circulating tamoxifen achieved with the dosing regimens used in rats during carcinogenesis experiments

Rats	Dosage regimen (mg/kg)	Tamoxifen concentration (ng/ml)	Liver tumours	Reference
1. Mature Wistar	5	166	Yes	[14]
	20	644		
	35	636		
2. Mature Sprague–Dawley	11.3	138 ± 41	Yes	[15]
	22.6	172 ± 103		
3. Mature Fischer	12.5*	230 ± 30	No	[16]

* Based on an estimate of daily food intake of 10 g per day of 250 mg tamoxifen/kg feed.

serum levels of tamoxifen illustrates the point (Table 3). Serum levels of tamoxifen during the treatment of breast cancer with 10 mg twice daily (approximately 285 μg/kg daily for a 70 kg postmenopausal woman) are usually between 100 and 200 ng/ml [23]. In contrast, the administration of 50 or 100 μg tamoxifen daily to ovariectomised mature mice (approximately 2.5 mg/kg for a 20 g mouse) or immature rats (approximately 3 mg/kg for a 35 g rat) for 7–10 days results in pharmacological effects, but produces serum levels of tamoxifen often below the level of detection by high performance liquid chromatography [24]. Only by giving high doses of tamoxifen (200 mg/kg) to animals can one adequately study circulating levels of drug [24]. We have studied the circulating levels of tamoxifen in patients receiving high daily doses of tamoxifen. Increasing the daily dose to the limits of toxicity (10 mg/kg) [26] in humans reaches the dose range (5–35 mg/kg) used to treat rats in the liver carcinogenesis studies (Table 2). However, the blood levels are 10-fold higher in the human (Table 3).

Comparable serum levels in the rat and human during tamoxifen treatment can only be produced by treating rats with high doses of tamoxifen. The schedules that are used to demonstrate liver carcinogenesis in the rat (5–40 mg/kg) are 20 times greater (Table 2) than the standard treatment regimen in women (20 mg daily or 285 μg/kg).

TESTING AT COMPARABLE THERAPEUTIC LEVELS

Tamoxifen, at a daily dose of 50 μg (250 μg/kg), inhibits the growth and development of dimethybenzanthracene-induced rat mammary tumours [27]. This is equivalent to the therapeutic dose used to treat metastatic breast cancer and as an adjuvant therapy in node positive and node negative disease. The duration of therapy for the treatment of breast cancer can be indefinite in some clinical trials [28, 29], but most treatment plans use 5 years of adjuvant tamoxifen at a dose of 20 mg daily. With the life expectancy of most women being 80 years of age, this translates into about 6% of a woman's lifetime and most women are treated during their postmenopausal years. In contrast, studies of rat liver carcinogenesis employ a test system that starts at 6 weeks of age (just post-puberty) and treats daily with approximately 20 times the human dose for the rest of the animals' life. At a dose of 11.3 mg/kg, approximately half the rats develop liver tumours within a year [15].

It is important to state that the general need for carcinogenic testing is to establish whether an agent is carcinogenic *per se* not just at therapeutic levels. To achieve this, animals are tested with a high dose, with lower doses approaching the therapeutic range. A positive result in the animal test does not mean that human therapeutic levels will be carcinogenic but provides a warning of such a possibility.

A therapeutically equivalent carcinogenocity test is illustrated in Figure 1. A treatment regimen of tamoxifen, 0.25 mg/kg daily, for 2–3 months during the second year of the rats' life would be an equivalent bioassay. This approach would give a realistic view of the toxicological risks observed in patients. Since the doses to be used are far below the level that causes adduct formation [19], and repair mechanisms occur after the cessation of therapy, there is little probability that animals will develop liver tumours, thus duplicating clinical experience.

Table 3. Circulating serum levels obtained with different dosage regimens in the rat, mouse and human (70 kg postmenopausal women)

Species	Dosage per day (mg/kg)	Duration	Tamoxifen concentration (ng/ml)	Reference
Human	0.28	> 2 years	148	[23]
Rat	3.0	7 days	< 1	[24]
Rat	200	7 days	1000	[24]
Mouse	2.5	7 days	< 10	[24]
Mouse	200	10 days	300	[24]
Human	4.9	1 year	1300	[24, 25]
Human	Approx. 10	11 days	1855	[26]

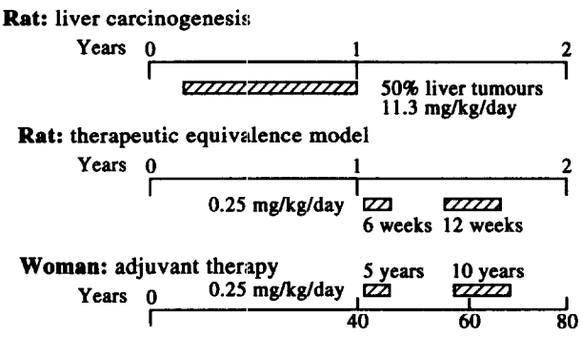

Figure 1. A therapeutic equivalence model to determine rat liver carcinogenesis using similar doses of tamoxifen per kg for the same relative time periods. Adjuvant tamoxifen is usually employed for 5 years in late menopausal or postmenopausal patients. In contrast, rat liver carcinogenesis experiments use 10–30 times the dose from the time of puberty for half the animal's life. An equivalent therapeutic model would establish the real risks for women.

Toxicological testing of new drugs in development to reduce the risks to patients is crucial, but tamoxifen has received extensive clinical testing over the past 20 years without producing major toxicities. Although it is argued that a decade is required for iatrogenic carcinogenesis in patients [30], there is currently little or no information to demonstrate that tamoxifen is a strong liver carcinogen in the human, as has been demonstrated for the rat [2]. We suggest that this is because of differences in the dose, duration and timing of tamoxifen treatment, differential metabolism, and consequently the susceptibility of some inbred strains of rat to hepatocellular carcinogens.

TAMOXIFEN AND ENDOMETRIAL CARCINOMA

In contrast to the concerns raised in the laboratory regarding tamoxifen and hepatocellular carcinoma, the association between tamoxifen and endometrial carcinoma is based upon clinical observation during the past decade. There is believed to be an increased incidence of endometrial carcinoma associated with breast cancer, therefore physicians need to take extra precautions for the routine care of their patients. Tamoxifen is known to have oestrogen-like properties in the uterus of some patients [31–33], so treatment would be expected to encourage the growth of pre-existing disease, a principle which was first illustrated in the laboratory. When a breast tumour and an endometrial carcinoma are co-transplanted into athymic mice, tamoxifen will block the oestrogen stimulated growth of the breast tumour while stimulating the endometrial carcinoma to grow [34, 35], a demonstration of target site specificity.

When evaluating reports of tamoxifen induced endometrial carcinoma, it is important to appreciate that the incidence of occult endometrial tumours found in autospy specimens is approximately five times the reported incidence in the general population [36]. The oestrogen-like properties of tamoxifen can cause uterine hyperaemia and proliferation, facilitating the growth of occult disease and leading to symptoms such as spotting and bleeding. Deaths from endometrial carcinoma have occurred during tamoxifen therapy for breast cancer raising the possibility that an aggressive form of the disease could be caused by tamoxifen. However, it should be remembered that only one-third of metastatic endometrial cancer is hormonally responsive, so tamoxifen would not be expected to control the majority of advanced disease.

DEATHS FROM ENDOMETRIAL CARCINOMA

Magriples and coworkers [3] completed a computer search of the Yale New Haven Hospital tumour registry for the decade 1980–1990 and identified 53 patients with a history of breast cancer who subsequently developed endometrial cancer. 15 of these patients received tamoxifen and 38 did not. A total of 3457 women were initially identified with breast cancer, but the proportion receiving tamoxifen was not stated. Interestingly enough, all of the tamoxifen-treated patients received 40 mg tamoxifen daily rather than the standard 20 mg daily. 5 patients died of endometrial carcinoma during tamoxifen therapy (Table 4), and the tumours from tamoxifen-treated patients were in general (67%) poorly differentiated endometrial carcinomata. The authors concluded "it appears that women receiving tamoxifen as treatment for breast cancer who subsequently develop uterine cancer are at risk for high-grade endometrial cancers that have a poor prognosis". Examination of the duration of tamoxifen therapy received by women before detection and subsequent death from endometrial carcinoma demonstrates that 3 of the patients received tamoxifen for 12 months or less (Table 4).

Deaths in women taking tamoxifen for relatively short time periods were also reported in the Stockholm study [37] and the NSABP study B14 [38]. In the Stockholm study, 931 patients were randomised to receive either 2 or 5 years of tamoxifen 40 mg daily. 17 patients have been diagnosed with endometrial carcinoma with a follow-up time ranging from 3.5 to 14.5 years. 3 patients died of endometrial carcinoma (Table 5); however, examination of patient records shows that each of the women received tamoxifen for less than 2 years, and the reported tumours were grades 1 and 2. One of the major conclusions of the study was that the probability of developing endometrial carcinoma was increased with duration of tamoxifen therapy [39]. However, examination of the 17 cases of endometrial

Table 4. Clinical and pathological features of tamoxifen treated breast cancer patients who died of endometrial carcinoma in the Yale New Haven Cancer Survey [3]

Patient	Age	Months on tamoxifen	Endometrial histology	FIGO stage
1	87	3	Papillary Serous	NS
2	71	12	MMT	IVB
3	60	12	Endometrioid FG3	NS
4	85	96	Endometrial	IIIC
5	71	120	Adenosquamous FG3	NS

NS, Not stated; MMT, mixed Mullerian tumour.

Table 5. Clinical and pathological features of tamoxifen-treated patients who died of endometrial carcinoma in the Stockholm trial [37]

Age	Months on tamoxifen	Patient	Endometrial histology	FIGO stage
70	11	1	NS grade II	IV
69	13	2	NS grade II	I
68	24	3	NS grade I	I

NS, not stated.

carcinoma detected in the nearly 1000 patients demonstrates that 13 of the women who developed endometrial carcinoma received less than 2 years of tamoxifen treatment [37].

In the NSABP study [38], 1419 patients were randomised to receive 20 mg tamoxifen daily for 5 years and 1220 patients were recruited and registered to receive at least 5 years of tamoxifen. 23 women developed endometrial carcinoma with an average time of evaluation of 8 years and 5 years for randomised and registered patients, respectively. 6 patients in the tamoxifen-treated arms have died after a diagnosis of endometrial carcinoma (Table 6). Three of the six women took tamoxifen for less than 2 years and one women never took tamoxifen, although she was included in the analysis based on intention to treat. Overall, 8 of the total of 23 women taking tamoxifen received the drug for less than 2 years.

It is now possible to address the question [3] of whether an aggressive high-grade disease develops during tamoxifen therapy by analysis of current clinical trials data.

TAMOXIFEN AND THE STAGE OF ENDOMETRIAL CARCINOMA

The discovery that high doses of tamoxifen will cause adduct formation in rat liver DNA [18] occurred at the same time that Magriples and coworkers [3] reported tamoxifen was associated with high-grade endometrial carcinoma. This naturally lead to the possibility that tamoxifen may be causing progression of pre-existing disease. However, a survey of randomised clinical trials [38] and an epidemiology study [40] do not support this proposition, although, in each case, the authors state that the numbers are too low to draw any definitive conclusions. Fisher and coworkers [38] have compared the stages of endometrial carcinoma and tumour grades found in their study and in the Yale Tumour Registry Study and the Swedish Trial. The comparisons are summarised in Table 7. A recent epidemiology study from the Netherlands Cancer Institute is included for comparison [40]. It is difficult to make absolute comparisons of these data, but several points can be made. The studies all find that the majority of tumours reported are stage 1 endometrial carcinoma. The percentage of low-grade tumours is variable with 78, 33, 53 and 52% for the NSABP, Yale, Swedish and Netherlands studies, respectively. For comparison, a Gynaecologic Oncology Group Study [41] of 222 patients found the distribution of cases to be 82% low-grade cases (FIGO 1 and 2) and 18% high-grade cases (FIGO 3). The Yale group has the highest proportion of high-grade tumours, with 67%. However, the fact that the events are so low, and patients with already advanced endometrial carcinoma are being given tamoxifen to treat breast cancer, make this not unexpected. At present, there is insufficient evidence to support the statement "women receiving tamoxifen as treatment for breast cancer who subsequently develop uterine cancer are at high risk for high-grade enodmetrial cancers that have a poor prognosis" [3].

INCIDENCE OF ENDOMETRIAL CANCER WITH TAMOXIFEN

It is impossible to give a precise rate for the incidence of endometrial carcinoma in tamoxifen-treated patients. This, in part, is because the rate is low and also the data base is dependent upon the anecdotal reports in the literature. The first clinical report in 1985 [42] described 3 patients treated for breast cancer with tamoxifen for 7, 12 and 14 months, respectively. In the decade that followed, more than a hundred cases have appeared in the literature. In a recent review, Friedl and Jordan [43] described the then known clinical characteristics of 94 patients. The mean length of tamoxifen treatment was 3.6 years and ranged from 3 months to 10 years. The clinical stage was

Table 6. Characteristics and pathological feature of tamoxifen-treated breast cancer patients who died of endometrial carcinoma (EC) in the NSABP B14 trial [38]

Patient	Age	Months on tamoxifen	Off tamoxifen to diagnosis (months)	Histology	FIGO stage	Cause of death
1	66	0	0	Endometrioid	1BG1	EC
2	63	9	0	Endometrioid	1BG2	EC
3	68	5	0	Endometrioid	1A	CV disease
4	58	22	73	Papillary	1BG3	EC
5	54	42	23	Carcinosarcoma	11BG3	EC
6	68	65	0	Papillary	IVG1	PE

CV, cardiovascular; PE, pulmonary embolus.

Table 7. Comparison of uterine cancers in tamoxifen-treated and control patients [36–38, 40]

	NSABP		Yale tumour registry				Swedish trial				Netherlands Cancer Institute			
	Tamoxifen $n=25$		Tamoxifen $n=15$		No tamoxifen $n=38$		Tamoxifen $n=17$		No tamoxifen $n=5$		Tamoxifen $n=23$		No tamoxifen $n=75$	
	Events	%	Events	%	Events	%	Events	%	Events	%	Events	%	Events	%
Stage														
1	21	88	7	78	23	88	14	82	4	100	17	85	62	87
II–IV	3	12	2	22	3	12	3	18	0	0	3	15	9	13
Total no. staged	24		9		26		17		4		20		71	
Histological grade														
Low (good)	18	78	5	33	26	74	8	53	4	100	12	52*	24	32*
High (poor)	5	22	10	67	9	26	7	47	0	0	11	48	51	68
Total no. graded	23		15		35		15		4		23		75	

* Calculated from a statement made by the authors in the discussion of the paper [40]. No breakdown of histological grade was presented in the results, although the morphological classification for users and nonusers of tamoxifen was in the same proportions. The proportion of well-differentiated tumours in the no tamoxifen group of this study is very low in comparison to all other studies and the survey in [41].

reported in 39 cases and the grade of the tumour was reported in 48 cases. In only 22 cases do the authors specify that there was no prior oestrogen therapy. It is, however, possible to estimate the incidence from the clinical trials and provide an estimate of the elevation in risk associated with tamoxifen treatment. The Stockholm Study [37] reports 17 endometrial carcinomas at 10 years in the 931 women receiving tamoxifen. The association between the months of tamoxifen treatment and the years after the diagnosis of breast cancer is illustrated in Figure 2. Thirteen of these women received only 2 years or less of tamoxifen. Eight of these women were diagnosed with endometrial carcinoma after the tamoxifen was stopped at 2 years. But in the 465 women who continued the tamoxifen for another 3 years, there was half the incidence of endometrial cancer, i.e. 4 new cases. On the face of it, these results imply that continuing tamoxifen past the 2-year point reduces the incidence of endometrial cancer by 50%. However, a longer observation time may be necessary to determine the eventual rate of endometrial carcinoma in the decade after 5 years of adjuvant tamoxifen therapy. The five

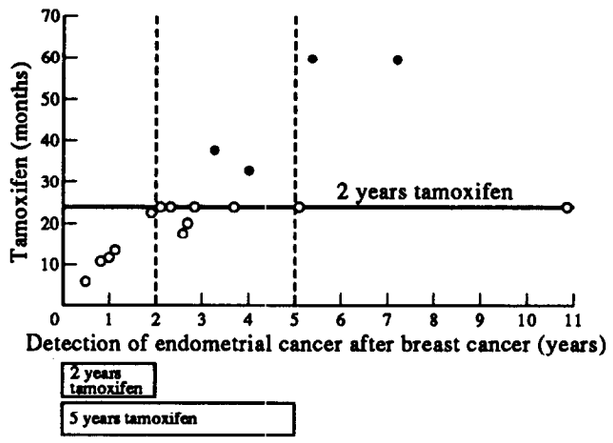

Figure 2. The occurrence of endometrial carcinoma after the detection of breast cancer in patients treated with varying lengths of adjuvent tamoxifen (40 mg daily) in the Stockholm Trial [37]. Patients were treated with up to 2 years of tamoxifen and then randomised to stop or receive an additional 3 years of tamoxifen.

women in the control group who developed endometrial carcinoma did so between 4 and 13 years after the diagnosis of breast cancer. The result implies that tamoxifen is encouraging the detection of occult disease because the majority (77%) of women who developed endometrial carcinoma had only 2 years of adjuvant tamoxifen therapy. Alternatively, increased sampling is occurring in the tamoxifen-treated group as a medical precaution. There is no doubt, however, that detection of endometrial carcinoma occurs at a higher incidence in both the Stockholm [37] and NSABP studies [38]. The incidence of endometrial carcinoma is difficult to compare in the studies because of the variable durations of tamoxifen treatment and the detection of endometrial carcinoma after adjuvant therapy has been stopped, but an estimate of two to three women per 1000 per year is reasonable.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

Tamoxifen has been shown to be a liver carcinogen in the rat. Although the dose range tested is outside the human therapeutic range, it points to the possibility that tamoxifen could be a liver carcinogen in humans. Unlike studies in the rat, human liver from patients treated with tamoxifen has not been found to contain DNA adducts [44]. However, it must be stressed that only limited studies with biopsies are possible, and there could be subgroups of women who will be susceptible to liver carcinogenesis because of novel pharmacogenetic traits. The most powerful tool to examine the hypothesis that tamoxifen is a liver carcinogen in women is epidemiology. A recent report [45] has demonstrated that there has been no increase in liver carcinogenesis since the introduction of tamoxifen in the United States in 1977. However, the rarity of the tumour requires monitoring for longer periods. What is encouraging is the paucity of reports since the possibility was raised as an issue in 1989 [39]. In contrast, it is clear that tamoxifen treatment is associated with a modest increase in endometrial carcinoma based upon current clinical trials and epidemiological information.

Tamoxifen, an anti-oestrogen, does not prevent the development of endometrial carcinoma, although the Stockholm Study (Figure 2) does suggest that continuing tamoxifen past two years

reduces the incidence of endometrial carcinoma detected by 50%. Nine deaths from endometrial carcinoma have occurred in randomised clinical trials, but some patients had stage IV endometrial cancer at the start of tamoxifen treatment. However, the cases of endometrial cancer occurring within 2 years of instituting tamoxifen raises the possibility that tamoxifen may accelerate the development, and ultimately the detection, of endometrial carcinoma. Clinicians must determine that a patient does not have pre-existing, advanced endometrial cancer when adjuvant therapy is started. There is little evidence that tamoxifen causes aggressive endometrial cancer, as the majority of patients who develop endometrial carcinoma have low grade, early stage disease. The rate of detection of endometrial carcinoma is only two to three women per 1000 per year, therefore it would be unreasonable to suggest a screening programme with regular endometrial sampling without some evidence of a favourable cost–benefit ratio. A programme of patient education and physician vigilance, through a detailed history and annual pelvic examination, can detect the signs and symptoms which warrant evaluation by more invasive procedures.

Adjuvant tamoxifen therapy is known to confer a survival advantage on breast cancer patients, and it reduces the incidence of second primary breast cancers [12, 13]. Developing information suggests that adjuvant tamoxifen therapy will help to maintain bone density and reduce the risks of fatal myocardial infarction in long-term breast cancer survivors [13]. The benefits from adjuvant tamoxifen therapy therefore exceed the risks of developing liver or endometrial carcinoma.

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Control of Oncogene Expression by Antisense Nucleic Acids

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INTRODUCTION

TRANSFORMATION OF normal cells into malignant cells is a multistep process involving the activation of proto-oncogenes and the inactivation of tumour-suppressor genes and of DNA repair genes [1]. The discovery of oncogenes, tumour suppressor and mutator genes has opened new areas of research in oncology aimed at discovering drugs that could selectively inhibit the biological effects of oncogene products and/or restore the function of tumour suppressor and DNA repair genes.

Most of the drugs presently available act at the level of proteins, the products of gene expression. Even DNA intercalating anticancer drugs exert their biological effect via inhibition of DNA processing enzymes such as topoisomerases. During the last decade, new approaches have been developed to selectively inhibit gene expression. The simplest way to control nucleic acids is to use nucleic acids themselves [2]. Short nucleic acid fragments, called oligonucleotides, can be designed to bind selectively to a complementary sequence on a single-stranded nucleic acid, for example, a messenger or a viral RNA, using the molecular code discovered by Watson and Crick in 1953, when they proposed the structure of the DNA double helix. Upon binding to the RNA target, these antisense oligonucleotides can block translation or reverse transcription. An oligoribonucleotide can also be designed to induce a catalytic cleavage of its RNA target. Such ribozymes bind to a complementary sequence on the RNA, as do the antisense oligonucleotides, but they contain an additional sequence that is responsible for the cleavage activity.

At the end of the 1980s, a new strategy was developed, which we called the antigene strategy [3], where the oligonucleotide is targeted to double-helical DNA to form a local triple helix. This triple-helical complex can block transcription, the first step of gene expression.

Oligonucleotides can also be used to control gene expression in the so-called sense approach. An oligonucleotide decoy can be designed to trap, for example, a transcription factor. It will therefore alter the expression of all genes which depend on this transcription factor for their activity. Compared with the antisense, ribozyme or antigene approaches, the sense strategy is expected to be less selective. However, the oligonucleotide decoy can be used to trap a viral or a parasitic protein which is involved in controlling the expression of viral or parasitic genes. Therefore, its effect should be selective for the virus or the parasite.

Another potential application of oligonucleotides has been more recently described. Oligonucleotides can be selected on the basis of their binding to proteins whose normal function does not involve any interaction with nucleic acids. This aptamer approach [4] leads to the design of oligonucleotides as a special class of ligands for enzymes, receptors, growth factors etc.

In all the approaches mentioned above, the oligonucleotide can be obtained through organic synthesis. This chemical approach has led to the development of oligonucleotide analogues, where the nucleic acid backbone or the bases are modified to confer upon the oligonucleotide additional properties when

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