[CANCER RESEARCH 63, 8461–8465, December 1, 2003]

Tamoxifen DNA Damage Detected in Human Endometrium Using Accelerator Mass Spectrometry

Elizabeth A. Martin,[1] Karen Brown,[1] Margaret Gaskell,[1] Farook Al-Azzawi,[2] R. Colin Garner,[4] David J. Boocock,[1] Elizabeth Mattock,[5] David W. Pring,[5] Karen Dingley,[6] Kenneth W. Turteltaub,[6] Lewis L. Smith,[7] and Ian N. H. White[3]

[1]Cancer Biomarkers and Prevention Group, University of Leicester, Leicester, United Kingdom; [2]Department of Obstetrics and Gynaecology and [3]MRC Molecular Endocrinology Group, University of Leicester, Leicester, United Kingdom; [4]Jack Birch Unit for Environmental Carcinogenesis, Department of Biology, University of York, York, United Kingdom; [5]York District Hospital, York, United Kingdom; [6]Lawrence Livermore National Laboratory, University of California, Livermore, California; and [7]Syngenta Ltd., Cheshire, United Kingdom

ABSTRACT

This study was aimed to establish whether tamoxifen binds irreversibly to uterine DNA when given to women. Patients were given a single therapeutic dose of [^{14}C]tamoxifen citrate orally (20 mg, 0.37 or 1.85 MBq) ~18 h prior to hysterectomy or breast surgery. Nonmalignant uterine tissue was separated into myometrium and endometrium. DNA and protein were isolated and bound radiolabel determined by the sensitive technique of accelerator mass spectrometry. Levels of irreversible DNA binding of tamoxifen in the endometrium of treated patients were 237 ± 77 adducts/10^{12} nucleotides (mean ± SE, $n = 10$). In myometrial tissues, a similar extent of DNA binding was detected (492 ± 112 adducts/10^{12} nucleotides). Binding of tamoxifen to endometrial and myometrial proteins was 10 ± 3 and 20 ± 4 fmol/mg, respectively. In breast tissue, sufficient DNA could not be extracted but protein binding was an order of magnitude higher than that seen with endometrial proteins (358 ± 81 fmol/mg). These results demonstrate that after oral administration, tamoxifen forms adducts in human uterine DNA but at low numbers relative to those previously reported in women after long-term tamoxifen treatment where levels, when detected, ranged from 15,000 to 130,000 adducts/10^{12} nucleotides. Our findings support the hypothesis that the low level of DNA adducts in human uterus is unlikely to be involved with endometrial cancer development.

INTRODUCTION

The antiestrogenic drug tamoxifen is widely and effectively used in the treatment of breast cancer and is also being evaluated as a chemopreventive agent in women (1). Recent results from the National Surgical Adjuvant Breast and Bowel Project P1 study show a 49% reduction in breast cancer incidence in healthy, high-risk women (2). Tamoxifen is now licensed in the United States as a chemopreventive agent for breast cancer in women at high risk of developing this disease. However, tamoxifen treatment has the serious side effect of increasing the incidence of endometrial cancer in women (3) and, for this reason, has been classified as a group 1 carcinogen by IARC (4). Cancer of the corpus uteri is the fifth most frequent cancer occurring in women and, postmenopause, is usually attributed to the effects of unopposed estrogen action.

Tamoxifen is carcinogenic in rat liver and long-term administration leads to a dose-dependent increase in hepatic tumors (5, 6). The mechanism by which tamoxifen causes cancer in rat liver is now well established as involving metabolism of tamoxifen to reactive intermediates, which bind irreversibly to hepatic DNA, forming DNA adducts. In rat liver, tamoxifen DNA adducts are formed in a dose-dependent manner and accumulate with time (6). DNA adducts are an early initiating event in the carcinogenic process because if they are not removed by repair mechanisms they can cause mutations by the incorporation of an incorrect base during DNA replication. Mutations present in critical genes such as tumor suppressors or proto-oncogenes may ultimately result in tumors. However, the presence of DNA adducts does not always lead to tumor formation, as illustrated by the fact that tamoxifen forms hepatic DNA adducts in mice, but this species does not develop tumors with continued tamoxifen dosing (7). This is probably because tamoxifen adducts are formed at lower levels in mice than rats, and they do not accumulate with time. It is not yet clear whether the development of endometrial tumors in women are associated with a genotoxic or epigenetic mechanism.

There is conflicting evidence for the presence of tamoxifen-DNA adducts in human endometrium (8). Using the ^{32}P-postlabeling assay, adducts in endometrial DNA have been reported as either absent or formed at low levels, close to the limits of detection achievable with this assay (9–12). Therefore, to address the key question of tamoxifen-DNA adduct formation in humans, we have used the alternative, more sensitive technique of AMS.[3] AMS is used for measuring concentrations of rare long-lived radioisotopes such as ^{14}C. It is generally accepted that AMS is the most sensitive technique available for detecting DNA adducts with a limit of detection of 1–10 adducts/10^{12} nucleotides, depending on the specific activity (13). In addition, detection is specific for ^{14}C-radiolabeled chemicals, unlike ^{32}P-postlabeling, which detects adduct formation derived from a range of known and unknown genotoxins. In this study, women due to undergo uterine or breast surgery were given a single therapeutic dose of [^{14}C]tamoxifen citrate (20 mg). DNA from uterine tissues was assayed for covalently bound radiolabel using AMS. Protein binding was also measured as a surrogate for DNA binding in breast tissue and compared with that in the uterus.

MATERIALS AND METHODS

Chemicals. Tamoxifen citrate was a gift from Dr. John Topham (Zeneca plc., Macclesfield, United Kingdom). [1-Phenyl-U-^{14}C]tamoxifen (2.03 GBq/mmol) of >98% radiochemical purity by HPLC was from Cambridge Research Biochemicals (Cleveland, United Kingdom). Gelatin capsules were prepared by the radiopharmacy of Leicester Royal Infirmary by adding either 1.85 or 0.37 MBq of radiolabeled tamoxifen to 20 mg of unlabeled drug. The committed effective radioactive dose equivalent was <180 μ Sv, which is below the natural background radiation to which people are exposed in daily life during the course of a month. Remaining chemicals were from Sigma Chemical Co. (Poole, United Kingdom) or as indicated.

Patients. Prior to these studies taking place, protocols were independently reviewed by the Human Research Ethics Committee at York District Hospital

Received 7/18/03; revised 9/16/03; accepted 9/22/03.

Grant support: AMS measurements were carried out by the United States National Resource for Development of Biomedical AMS Grant RR13461, which is operated under the auspices of Lawrence Livermore National Laboratory and the United States Department of Energy Grant W-7405-ENG-48.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Elizabeth A. Martin, AstraZeneca, Genetic Toxicology Department, Safety Assessment UK, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom. Phone: 44-1625-231279; Fax: 44-1625-230782; E-mail: Elizabeth.martin@astrazeneca.com.

[3] The abbreviations used are: AMS, accelerator mass spectrometry; HPLC, high-performance liquid chromatography.

or Leicester Health Authority, the Department of Health Committee on the Administration of Radioactive Substances to Persons–the Medicines (administration of radioactive substances) Regulation of 1978, United Kingdom, and the Institutional Review Board at the Lawrence Livermore National Laboratory. All patients who volunteered to take part were given an information leaflet describing the background and purpose of the study followed by a discussion with a member of the medical team. None of the patients had previously been treated with tamoxifen. Informed consent was obtained from all patients before studies commenced. Patients were also asked to fill in a questionnaire to obtain relevant personal information (such as age, height, and concurrent medication). Patient information can be found in Table 1.

Study 1: York District Hospital. Eleven female volunteers were recruited who were undergoing surgery for breast cancer (5 patients) or gynecological conditions (6 patients). Each subject was given 20 mg of tamoxifen citrate containing 0.37 MBq [^{14}C]tamoxifen ~18 h before surgery. Urine was collected predose and up to 24 h after treatment. At surgery, nonmalignant breast or uterine tissue was removed. Samples were frozen and stored at −80°C. Uterine tissue was separated into myometrium and endometrium before use. Control tissue samples were also taken from 3 patients who did not receive [^{14}C]tamoxifen.

Study 2: Leicester Royal Infirmary. Ten female volunteers were recruited who were undergoing surgery for hysterectomy. Each subject was given 20 mg of tamoxifen citrate containing 1.85 MBq [^{14}C]tamoxifen ~18 h before surgery. This was a 5-fold higher dose of radioactivity than that used in the first study, although the dose of tamoxifen was the same and was necessary because the 0.37 MBq dose was found to be too low to enable unambiguous detection of radiolabel bound to uterine DNA. By using a higher radioactive dose, a greater proportion of any DNA adducts formed will be ^{14}C-labeled, which increases the likelihood that they will be detectable by AMS. Venous blood (10 ml) was taken before the administration of the drug and at the time of surgery. Tissue samples were taken as described above, including from 5 women who had not received tamoxifen, which was used as controls.

Determination of Radioactivity in Urine, Blood, and Whole Tissues. Radioactivity in pooled 24 h urine (1 ml aliquot) and plasma from whole blood (10 ml) were measured by liquid scintillation counting. Tissues (100 mg) were solubilized in Soluene (1 ml) overnight at 37°C and radioactivity measured by liquid scintillation counting or AMS. In some cases, both methods were used and values were averaged.

Determination of Covalent Binding of [^{14}C]Tamoxifen to DNA and Protein. Tissues were finely minced and digested in lysis buffer [800 mM guanidine-HCl, 30 mM EDTA, 30 mM Tris-HCl, 5% Tween 20, 0.5% Triton X-100 (pH 8.0)], containing proteinase K (1 mg/ml) for 18 h at 37°C. Undigested tissue was removed by centrifugation, and the supernatant was treated for 1 h at room temperature with RNase A, 0.19 mg/ml lysis buffer (14). DNA was extracted using Qiagen column chromatography (Qiagen Ltd., Crawley, United Kingdom) according to the manufacturers instructions. DNA purity was determined by the $A_{260\ nm}/A_{280\ nm}$ ratio. Only DNA with a ratio of 1.6–1.9 was used. Using this DNA extraction method when tissue lysates are spiked with [^{14}C]tamoxifen (226 fmol/mg tissue) at a concentration equivalent to tissue levels detected in this study, all ^{14}C-radiolabel is efficiently extracted as demonstrated by there being no increase in ^{14}C-radiolabel over that present in control DNA (data not shown). Furthermore, we have previously shown using this extraction method, and after DNA digestion and HPLC separation, the remaining ^{14}C label coelutes on thin-layer chromatography plates with the tamoxifen-DNA adducts (15). Although it was not possible to carry out analogous studies with other known metabolites of tamoxifen because they are not available in radiolabeled form, they are all more polar than tamoxifen and therefore would be expected to be removed by Qiagen column chromatography more or as efficiently as tamoxifen itself. DNA samples (50–500 μg) were supplemented with a precise amount of carrier (2 mg of tributyrin) to provide sufficient carbon for optimal sample preparation and AMS analysis as described below. For protein isolation, tissues (50–100 mg) were homogenized in 20% (v/v) DMSO in methanol. The resulting protein pellets were exhaustively extracted with organic solvents as described previously (16). Proteins (2 mg) were dissolved in 0.1 M potassium hydroxide and submitted for analysis by AMS.

AMS Sample Preparation and Analysis. Coded DNA and protein samples were sent to the Lawrence Livermore National Laboratory for AMS analysis; however, it was not possible to analyze controls and treated samples blind because the level of ^{14}C in control samples is subtracted from that contained in the treated to determine the extent of drug binding. Protein and DNA samples were converted to elemental carbon using standard protocols, by combustion to CO_2, followed by reduction to filamentous graphite (17). The resulting graphite was then packed into individual sample holders and analyzed by AMS. AMS measures the concentration of a rare isotope, in this case ^{14}C, relative to a stable isotope, either ^{13}C or ^{12}C. From this ratio, the increase in ^{14}C above background for a particular sample is determined by subtracting contributions due to the natural abundance of ^{14}C in the sample and that due to sample processing (17, 18). The excess ^{14}C content, which is caused by the presence of radiolabeled tamoxifen or related derivatives, was then converted into an appropriate form (fmol tamoxifen equivalents/mg protein or DNA adducts/10^{12} nucleotides), taking into account the specific activity of tamoxifen. The average Fraction Modern value of DNA from tamoxifen-treated patients in study 2, after subtraction of the carrier contribution, was 2.10 ± 0.09 compared with the natural radiocarbon content measured in untreated control patients (1.45 ± 0.04 Fraction Modern). The limits of

Table 1 *Patient details*

Patient no.	Age at surgery (yrs)	Medical condition	Days since last menstrual cycle	Weight (kg)	Height (cm)	Cigarettes/day	Concurrent drugs
Study 1							
Uterine patients 1	37	Menorrhagia	NK[a]	70	165	Ex-smoker	None
2	36	Menorrhagia	NK	89	158	Smoker	Mirena coil, Becotide
3	36	P.M.T.[b]	NK	66	163	Nonsmoker	HRT, Zoladex, Prozac
4	35	Endometriosis	NK	53	150	Nonsmoker	Danazol
5	39	P.M.S.[c]	NK	78	173	Nonsmoker	Cetirizine, Evorel
6	44	Uterine polyp	NK	64	170	Nonsmoker	Ferrous sulphate
Breast patients 7	55	Breast cancer	NK	73	158	Nonsmoker	None
8	74	Breast cancer	NK	61	155	Smoker	Prozac, Loprazolam
9	63	Breast cancer	NK	83	163	Ex-smoker	None
10	52	Breast cancer	NK	61	168	Ex-smoker	None
11	43	Breast cancer	NK	86	165	Nonsmoker	None
Study 2							
Uterine patients 1	53	Menorrhagia	2	61	155	Smoker (15–20/day)	None
2	38	Menorrhagia	10	53	158	Nonsmoker	None
3	45	Menorrhagia	22	85	163	Nonsmoker	Thyroxine, diclofenac
4	45	Menorrhagia	13	68	168	Smoker (10/day)	None
5	39	Menorrhagia	5	58	160	Nonsmoker	Mefenamic acid
6	48	Menorrhagia	10	71	166	Nonsmoker	None
7	37	Menorrhagia	27	68	166	Nonsmoker	None
8	49	Menorrhagia	15	70	168	Nonsmoker	None
9	51	Menorrhagia	15	69	162	Nonsmoker	None
10	47	Menorrhagia	10	70	151	Nonsmoker	Flixotide, Bricanyl inhaler

[a] NK, not known.
[b] P.M.T., premenstrual tension.
[c] P.M.S., premenstrual syndrome.

detection were calculated based on the sum of the mean fraction modern for each set of control tissue samples and two times the SD of the mean.

Statistical Analysis. Results are given as the mean ± SE and were tested using ANOVA with Dunnett's test for significance at the 5% level. Each graphite sample was analyzed up to seven times for radiocarbon content by AMS or until the measurement variation was within ±5%.

RESULTS

Radiolabel Distribution and Excretion. In patients given a single dose of [^{14}C]tamoxifen (1.85 MBq), plasma levels of radiolabel taken at the time of surgery corresponded to 22 ± 3 ng tamoxifen equivalents/ml (n = 10; Table 2). This agrees well with the report that a single dose of 20 mg gives a peak plasma level of ~20 ng/ml after 24 h (19). The presence of radioactivity in tissues demonstrates that tamoxifen reached the uterus and breast, with 227 ± 36 fmol tamoxifen equivalents/mg (combined results from studies 1 and 2; n = 16) in uterine tissue and 335 ± 68 fmol tamoxifen equivalents/mg (n = 5) in breast tissue (Table 2). Similar levels of drug were detected in the uterine samples from studies 1 and 2, which was as expected because the same dose of tamoxifen was administered. Urinary excretion of radiolabel over the first 24 h after dosing with [^{14}C]tamoxifen in patients from study 1 was 7.2 ± 0.6% (n = 11) of the total dose administered. These results are consistent with earlier studies that showed the majority of [^{14}C]tamoxifen given to women was excreted in the feces (20).

Irreversible DNA and Protein Binding. Human DNA samples extracted from uterine tissues were analyzed for covalent binding of tamoxifen by AMS. In study 1, no DNA adducts were detected in the myometrial or endometrial DNA presumably because the dose of radiolabel was too low, meaning the adduct levels were below the limit of detection, which was in the order of 650 adducts/10^{12} nucleotides. In study 2, using the same dose of drug but a 5-fold greater concentration of radiolabel, DNA adducts were detected at low levels and were present in both myometrium (492 ± 112 adducts/10^{12} nucleotides, which is equivalent to 1.5 ± 0.3 fmol tamoxifen/mg DNA) and endometrium (237 ± 77 adducts/10^{12} nucleotides, equivalent to 0.7 ± 0.2 fmol tamoxifen/mg DNA; n = 10; Fig. 1 and Table 2). The typical limit of detection for samples analyzed from this study was ~130 adducts/10^{12} nucleotides, lower than that achieved in study 1, which is a reflection of the 5-fold higher dose of radioactivity administered to the second group of patients. The average level of myometrial adducts was ~2-fold higher than numbers of endometrial adducts, but this was not significant (P = 0.088). With breast tissue, sufficient DNA could not be extracted for AMS analysis, this was due partly to the small amount of tissue available and also because breast tissue is very fatty, which hinders the extraction process.

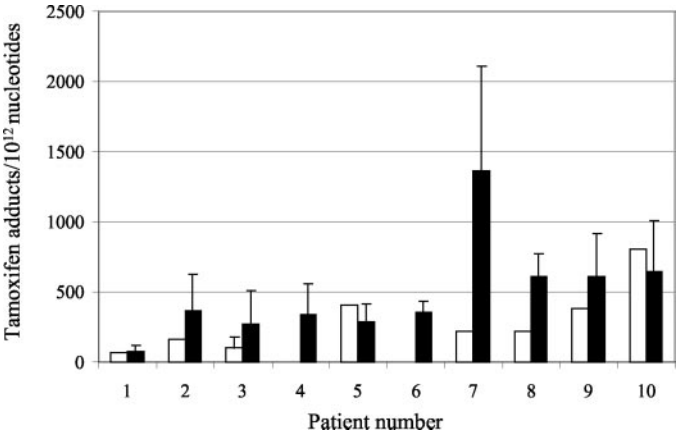

Fig. 1. Covalent binding of [^{14}C]tamoxifen to DNA (study 2). DNA adducts detected in human endometrial (□) and myometrial (■) DNA 18 h after the administration of 1.85 MBq of ^{14}C-radiolabeled tamoxifen. After tissue digestion using proteinase K and RNase A, DNA was extracted using Qiagen column chromatography according to the manufacturers instructions. DNA samples were converted to elemental carbon by combustion to CO_2, followed by reduction to filamentous graphite and analyzed by AMS to determine the concentration of ^{14}C relative to ^{13}C. From this measurement, the increase in ^{14}C content over naturally occurring background levels (*i.e.*, because of bound ^{14}C-radiolabel) could be calculated. For endometrium, where tissue was limited, results were based on a single DNA extraction for patients 4, 5, 6, 7, 9, and 10; two extractions for patients 1, 2, and 8; and four extractions for patient 3. For myometrial tissue, between three and six extractions were performed for each patient.

Table 2 *Levels of tamoxifen in tissue, plasma, and binding to protein and DNA in humans and rats*

	Tamoxifen equivalents fmol/mg (except*) (±SE)	Adducts/10^{12} nucleotides (±SE)
Human		
Plasma	22 ± 3*ng/ml	
Breast		
Tissue	335 ± 68	
Protein	358 ± 81	
DNA	ND[a]	ND
Uterine		
Tissue	227 ± 36	
Uterine		
Protein—myometrium	20 ± 4	
Protein—endometrium	10 ± 3	
Uterine		
DNA—myometrium	1.5 ± 0.3	492 ± 112
DNA—endometrium	0.7 ± 0.2	237 ± 77
Rat		
Liver		
DNA—Single dose (0.3 mg/kg)[b]	9	3,000
DNA—6-month chronic dietary dosing[c]	91,000	30,000,000
Uterine		
DNA—Single dose (0.3 mg/kg)[d]	0.5	160
DNA—6-month chronic dietary dosing[e]	Not detectable by ^{32}P-postlabeling	

[a] ND, not determined.
[b] Ref. 32.
[c] Ref. 6 (^{32}P-postlabeling results).
[d] Unpublished observations.
[e] Ref. 31 (^{32}P-postlabeling results).

The DNA isolation methods used here have previously been used in AMS studies measuring adduct formation by a variety of other chemicals such as benzene and MeIQx, and in each case, the extracted DNA has been shown to contain no detectable-free ^{14}C-labeled chemical or metabolite (21, 22). In this study, the human DNA samples did not contain sufficient [^{14}C]radiolabel to permit detection of individual adducts after hydrolysis and HPLC separation. However, we have previously demonstrated the presence of [^{14}C]radiolabeled nucleoside 3′-monophosphate adducts in rat liver DNA after dosing with high levels (37MBq) of [^{14}C]tamoxifen. The liver DNA was isolated using the same Qiagen column procedure as for the human samples, and in this instance, no unbound [^{14}C]tamoxifen could be detected in the HPLC chromatograms (15).

The extent of covalent binding of tamoxifen to breast and endometrial proteins was determined where sufficient material could be obtained. Protein binding was 10 ± 3 (n = 7) and 20 ± 4 (n = 9) fmol tamoxifen equivalents/mg protein for endometrium and myometrium, respectively (Fig. 2, Table 2). This is ~14-fold greater than binding to DNA extracted from the same tissue. Binding of tamoxifen to normal breast tissue protein (358 ± 81 fmol tamoxifen equivalents/mg; n = 4) was 24-fold greater than that detected in the uterus in study 2 (Table 2).

DISCUSSION

We have reported on an investigation of the binding of tamoxifen to human uterine DNA using AMS. Because of its extreme sensitivity,

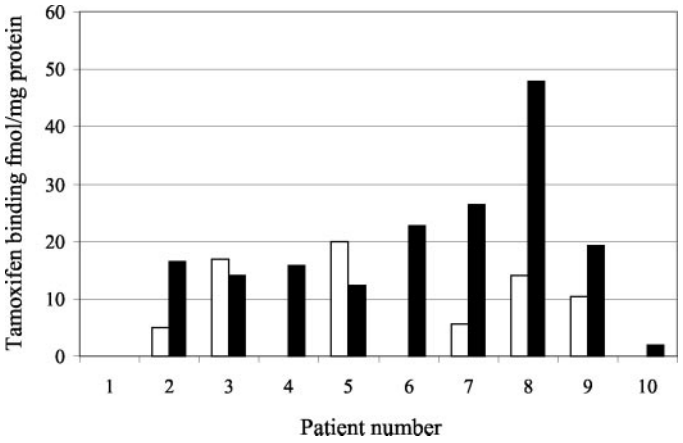

Fig. 2. Covalent binding of [^{14}C]tamoxifen to human uterine proteins (study 2). Protein binding in endometrial (□) and myometrial (■) tissue after administration of 1.85 MBq of ^{14}C-radiolabeled tamoxifen. Tissues (50–100 mg) were homogenized in 20% DMSO in methanol, and the resulting protein pellets were exhaustively extracted with organic solvents. Proteins were dissolved in 0.1 M potassium hydroxide and submitted for analysis by AMS. Protein was not available for analysis from patient 1 (endometrium and myometrium) and patients 4 and 6 (endometrium).

this technique, most commonly known for its use in radiocarbon dating of archaeological samples, is presently finding new applications in biomedicine (23, 24). Determining the extent, if any, of DNA adduct formation that occurs is important in understanding the mechanisms responsible for the increased incidence of endometrial cancers in both breast cancer patients and healthy women at high risk of breast cancer who are taking tamoxifen (2, 3).

Results from study 2 show that after this single dose procedure, only extremely low levels of uterine endometrial DNA adducts are detected in the order of 240 adducts/10^{12} nucleotides. Furthermore, there is no significant difference in DNA adduct levels between the endometrial and myometrial compartments. Although DNA adducts were not detectable in uterine tissue of patients recruited to study 1, increasing the dose of radioisotope in study 2 made it possible to detect the low levels of adducts formed. There need not be a linear correlation between DNA adduct level and the incidence of cancer, and therefore, low levels of DNA adducts do not necessarily equate to a low level of tumors or tumor risk; in fact, the presence of adducts can in some cases equate to no risk. For example, in rats, it has been shown that there is a threshold value of tamoxifen-induced DNA adducts (1,800,000 adducts/10^{12} nucleotides) required for subsequent induction of liver tumors (25).

The level of tamoxifen equivalents in plasma (22 ng/ml) was an order of magnitude lower than the steady-state values of tamoxifen and its metabolites measured during long-term therapy (26, 27). [^{14}C]Radiolabel measured in uterine tissues was ~6-fold higher than in plasma, however, after long-term exposure to tamoxifen, steady-state plasma levels are 10–60-fold higher than steady-state tissue levels (26, 27). After a single dose of tamoxifen, drug metabolism and disposition may differ quantitatively compared with long-term exposure. The question as to whether tamoxifen forms adducts in endometrial DNA in treated women has been controversial. Previously, using indirect methods such as ^{32}P-postlabeling, some groups report no ^{32}P-postlabeled DNA adducts (9), whereas others have found low levels, ranging from 15,000 to 130,000 adducts/10^{12} nucleotides (12). More recently, using HPLC coupled with electrospray ionization tandem mass spectrometry analysis, tamoxifen DNA adducts were detected in the livers of rats and monkeys given this drug but were not detectable in endometrial samples from two women who had received tamoxifen (20 mg) for >1 year, presumably because they were below the limit of detection of the assay, which is 500 adducts/10^{12} nucleotides (28, 29). The current study has shown that DNA adduct formation is possible in human endometrium and myometrium after a single dose of tamoxifen. If it were possible to conduct comparable studies in breast cancer patients receiving tamoxifen therapeutically, higher levels of uterine DNA adducts might be detected.

In rats given high doses (30 mg/kg), tamoxifen is a genotoxic liver carcinogen. ^{32}P-Postlabeled DNA adducts are present at levels in the order of 30,000,000/10^{12} nucleotides after 6 months exposure when neoplasia is first detected (6). This level of adducts is comparable with 27 other rat liver carcinogens where the level of DNA adducts that resulted in a 50% tumor incidence spanned from 500,000 to 21,000,000 adducts/10^{12} nucleotides (30). No ^{32}P-postlabeled uterine DNA adducts (31) or uterine tumors (5) have been reported in adult rats given tamoxifen in long-term studies. However, when rats are administered a single dose of [^{14}C]tamoxifen, comparable with the dose used to treat women in this study, DNA adducts measured by AMS are detected in both the liver (~3000 ± 300 adducts/10^{12} nucleotides; Ref. 32) and uterus (160 ± 80 adducts/10^{12} nucleotides; unpublished data). In rats, this level of uterine DNA adduct formation is similar to that detected in the uterus of women in the present study, and both are at least an order of magnitude lower than that found in the liver of rats after a single dose.

For risk assessment purposes, comparative metabolism studies carried out *in vitro* show there is good reason to suppose that women will be far less sensitive to tamoxifen than rats (33). For women, the risk of endometrial cancer increases with longer duration of tamoxifen use ($P < 0.001$), with relative risks of 2.0 for 2–5 years and 6.9 for at least 5 years compared with nonusers (34). One mechanistic hypothesis proposed to explain how these tumors develop is that they arise as a result of a genotoxic action, involving the formation of low levels of endometrial DNA adducts after tamoxifen treatment, as detected in this study. However, the fact that similar levels of DNA adducts were also detected in the myometrium, a tissue where tumors do not develop in women, highlights that tumor formation is a multistep process, and DNA adduct formation is only one of the initial steps. Furthermore, it has been shown that a threshold of tamoxifen DNA adducts exists before liver tumors develop in rats (25). Therefore, a more probable explanation in women is that uterine tumors develop as a result of the unopposed estrogenic action of this drug on cells of the endometrium, leading to increased mitotic activity of endometrial cells, increased number of DNA replication errors, and somatic mutations resulting in malignant phenotype (35). Although a number of other drugs of this class such as toremifene and raloxifene are currently being tested as breast cancer therapeutic agents, the development of two others, levormeloxifene and idoxifene, has been discontinued primarily because of endometrial concerns after trials reported a higher proportion of pelvic organ prolapse in treated *versus* untreated women (36).

In conclusion, tamoxifen forms adducts in human uterine DNA at very low levels compared with that seen in livers of rats treated with a single comparable dose or rats treated long term that develop liver tumors. This level of DNA adduct formation is unlikely to be mechanistically related to the development of endometrial cancer in women treated with tamoxifen.

REFERENCES

1. Powles, T. J. The case for clinical trials of tamoxifen for prevention of breast cancer. Lancet, *340:* 1145–1147, 1992.
2. Fisher, B., Costantino, J. P., Wickerham, D. L., Redmond, C. K., Kavanah, M., Cronin, W. M., Vogel, V., Robidoux, A., Dimitrov, N., Atkins, J., Daly, M., Wieand, S., Tan-Chiu, E., Ford, L., and Wolmark, N. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J. Natl. Cancer Inst. (Bethesda), *90:* 1371–1388, 1998.
3. Fornander, T., Cedermark, B., Mattsson, A., Skoog, L., Theve, T., Askergren, J., Rutqvist, L. E., Glas, U., Silfversward, C., Somell, A., Wilking, N., and Hjalmar, M.

Adjuvant tamoxifen in early breast cancer: occurrence of new primary cancers. Lancet, *1:* 117–119, 1989.
4. Anonymous. Tamoxifen. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, pp. 253–365. IARC Scientific Publ. No. 766. Lyon, France: IARC, 1996.
5. Greaves, P., Goonetilleke, R., Nunn, G., Topham, J., and Orton, T. Two-year carcinogenicity study of tamoxifen in Alderly Park Wistar-derived rats. Cancer Res., *53:* 3919–3924, 1993.
6. Carthew, P., Rich, K. J., Martin, E. A., De Matteis, F., Lim, C. K., Manson, M. M., Festing, M. F., White, I. N. H., and Smith, L. L. DNA damage as assessed by ^{32}P-postlabelling in three rat strains exposed to dietary tamoxifen: the relationship between cell proliferation and liver tumour formation. Carcinogenesis (Lond.), *16:* 1299–1304, 1995.
7. Martin, E. A., Carthew, P., White, I. N., Heydon, R. T., Gaskell, M., Mauthe, R. J., Turteltaub, K. W., and Smith, L. L. Investigation of the formation and accumulation of liver DNA adducts in mice chronically exposed to tamoxifen. Carcinogenesis (Lond.), *18:* 2209–2215, 1997.
8. Poirier, M. C., and Schild, L. J. The genotoxicity of tamoxifen: extent and consequences, Kona, Hawaii, January 23, 2003. Mutagenesis, *18:* 395–399, 2003.
9. Carmichael, P. L., Ugwumadu, A. H. N., Neven, P., Hewer, A. J., Poon, G. K., and Phillips, D. H. Lack of genotoxicity of tamoxifen in human endometrium. Cancer Res., *56:* 1475–1479, 1996.
10. Hemminki, K., Rajaniemi, H., Lindahl, B., and Moberger, B. Tamoxifen-induced DNA adducts in endometrial samples from breast cancer patients. Cancer Res., *56:* 4374–4377, 1996.
11. Shibutani, S., Dasaradhi, L., Terashima, I., Banoglu, E., and Duffel, M. W. α-Hydroxytamoxifen is a substrate of hydroxysteroid (alcohol) sulfotransferase, resulting in tamoxifen DNA adducts. Cancer Res., *58:* 647–653, 1998.
12. Shibutani, S., Suzuki, N., Terashima, I., Sugarman, S. M., Grollman, A. P., and Pearl, M. L. Tamoxifen-DNA adducts detected in the endometrium of women treated with tamoxifen. Chem. Res. Toxicol., *12:* 646–653, 1999.
13. Turteltaub, K. W., Vogel, J. S., Frantz, C. E., and Fultz, F. Studies on DNA adduction with heterocyclic amines by accelerator mass spectrometry: a new technique for tracing isotope-labelled DNA adduction. *In:* D. H. Phillips, M. Castegnaro, and H. Bartsch (eds.), Postlabeling Methods for Detection of DNA Adducts, pp. 293–301. Lyon: IARC, 1993.
14. Frantz, C. E., Bangerter, C., Fultz, E., Mayer, K. M., Vogel, J. S., and Turteltaub, K. W. Dose-response studies of MeIQx in rat liver and liver DNA at low doses. Carcinogenesis (Lond.), *16:* 367–373, 1995.
15. White, I. N. H., Martin, E. A., Vogel, J. S., Mauthe, R. J., Turteltaub, K. W., and Smith, L. L. Comparison of the binding of [^{14}C]radiolabelled tamoxifen or toremifene to rat DNA using accelerator mass spectrometry. Chem. Biol. Interact., *106:* 149–160, 1997.
16. Mani, C., and Kupfer, D. Cytochrome P450 mediated action and irreversible binding of the antiestrogen tamoxifen to proteins in rat and human liver: possible involvement of the flavin-containing monooxygenases in tamoxifen activation. Cancer Res., *51:* 6052–6058, 1991.
17. Vogel, J. S., Turteltaub, K. W., Finkel, R., and Nelson, D. E. Accelerator mass spectrometry. Anal. Chem., *67:* 353A–359A, 1995.
18. Turteltaub, K. W., Felton, J. S., Gledhill, B. L., Vogel, J. S., Southon, J. R., Caffee, M. W., Finkel, R. C., Nelson, D. E., Proctor, I. D., and Davis, J. C. Accelerator mass spectrometry in biomedical dosimetry: relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA. Proc. Natl. Acad. Sci. USA, *87:* 5288–5292, 1990.
19. Adam, H. K., Patterson, J. S., and Kemp, J. V. Studies on the metabolism and pharmacokinetics of tamoxifen in normal volunteers. Cancer Treat. Rep., *64:* 761–764, 1980.
20. Fromson, J. M., Pearson, S., and Bramah, S. Metabolism of tamoxifen (ICI 46,474) Part II: in female patients. Xenobiotica, *3:* 711–714, 1973.
21. Mani, C., Freeman, S., Nelson, D. O., Vogel, J. S., and Turteltaub, K. W. Species and strain comparisons in the macromolecular binding of extremely low doses of [^{14}C]benzene in rodents, using accelerator mass spectrometry. Toxicol. Appl. Pharmacol., *159:* 83–90, 1999.
22. Mauthe, R. J., Dingley, K. H., Leveson, S. H., Freeman, S. P., Turesky, R. J., Garner, R. C., and Turteltaub, K. W. Comparison of DNA-adduct and tissue-available dose levels of MeIQx in human and rodent colon following administration of a very low dose. Int. J. Cancer, *80:* 539–545, 1999.
23. Garner, R. C. Accelerator mass spectrometry in pharmaceutical research and development-a new ultrasensitive analytical method for isotope measurement. Curr. Drug. Metab., *1:* 205–213, 2000.
24. Turteltaub, K. W., and Vogel, J. S. Bioanalytical applications of accelerator mass spectrometry for pharmaceutical research. Curr. Pharm. Des, *6:* 991–1007, 2000.
25. Carthew, P., Lee, P. N., Edwards, R. E., Heydon, R. T., Nolan, B. M., and Martin, E. A. Cumulative exposure to tamoxifen: DNA adducts and liver cancer in the rat. Arch. Toxicol., *75:* 375–380, 2001.
26. Lien, E. A., Solheim, E., and Ueland, P. M. Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. Cancer Res., *51:* 4837–4844, 1991.
27. Peyrade, F., Frenay, M., Etienne, M. C., Ruch, F., Guillemare, C., Francois, E., Namer, M., Ferrero, J. M., and Milano, G. Age-related difference in tamoxifen disposition. Clin. Pharmacol. Ther., *59:* 401–410, 1996.
28. Da Costa, G. C., Marques, M. M., Beland, F. A., Freeman, J. P., Churchwell, M. I., and Doerge, D. R. Quantification of tamoxifen DNA adducts using on-line sample preparation and HPLC-electrospray ionization tandem mass spectrometry. Chem. Res. Toxicol., *16:* 357–366, 2003.
29. Beland, F. A., Chruchwell, M. I., Doerge, D. R., Malejka-Giganti, D., Zhang, X., Divi, R., Poirier, M. C., Gamboa da Costa, G., and Marques, M. M. High performance liquid chromatography electrospray ionization tandem mass spectrometry analysis of tamoxifen DNA adducts in rats, monkeys and humans. Proc. Am. Assoc. Cancer Res., *43:* 343, 2002.
30. Otteneder, M., and Lutz, W. K. Correlation of DNA adduct levels with tumor incidence: carcinogenic potency of DNA adducts. Mutat. Res., *424:* 237–247, 1999.
31. Carthew, P., Edwards, R. E., Nolan, B. M., Martin, E. A., and Smith, L. L. Tamoxifen associated uterine pathology in rodents: relevance to women. Carcinogenesis (Lond.), *17:* 1577–1582, 1996.
32. Martin, E. A., Gaskell, M., Boocock, D. J., Turteltaub, K. W., Al-Azzawi, F., White, I. N. H., and Brown, K. Detection of DNA and protein adducts in [^{14}C]tamoxifen treated women by accelerator mass spectrometry (AMS). Mutat. Res., *483* (Suppl. 1)*:* S146, 2001.
33. White, I. N. H. Tamoxifen. Is it safe? Comparisons of activation and detoxication mechanisms in rodents and in humans. Curr. Drug. Metab., *4:* 151–170, 2003.
34. Bergman, L., Beelen, M. L., Gallee, M. P., Hollema, H., Benraadt, J., and van Leeuwen, F. E. Risk and prognosis of endometrial cancer after tamoxifen for breast cancer. Comprehensive Cancer Centres' ALERT Group. Assessment of Liver and Endometrial cancer Risk following Tamoxifen. Lancet, *356:* 881–887, 2000.
35. Akhmedkhanov, A., Zeleniuch-Jacquotte, A., and Toniolo, P. Role of exogenous and endogenous hormones in endometrial cancer: review of the evidence and research perspectives. Human Fertility and Reproduction: the Oocyte, the Embryo, and the Uterus. Ann. N. Y. Acad. Sci., *943:* 296–315, 2001.
36. Hendrix, S. L., and McNeeley, S. G. Effect of selective estrogen receptor modulators on reproductive tissues other than endometrium. Ann. N. Y. Acad. Sci., *949:* 243–250, 2001.