Effects of Tamoxifen on Endometrial Carcinogenesis in Mice

Kenji Niwa,1, 3 Shigeo Morishita,1 Midori Hashimoto,1 Tsuneo Itoh,1 Jiro Fujimoto,1 Hideki Mori2 and Teruhiko Tamaya1

Department of 1Obstetrics & Gynecology and 2Pathology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705

Two experiments were conducted to determine the effect of tamoxifen (TAM) in mouse endometrium in comparison with that of 17β-estradiol (E2). In a medium-term assay, TAM as well as E2 treatment semi-dose-dependently increased the levels of fos/jun mRNA and their oncoproteins (Fos/Jun). The long-term effect of TAM on mouse endometrial carcinogenesis was also examined in the following model. A total of 150 female ICR mice, 12–13 weeks of age, were used. Of these, 125 mice received an injection of N-methyl-N-nitrosourea (MNU) solution (1 mg/100 g body weight) into their left uterine tube and saline into the right. One week later, they were divided into four groups: groups 1 (35 mice) and 2 (30 mice) were given 25 ppm and 5 ppm E2-containing diet, respectively, while group 3 (30 mice) was fed 5 ppm TAM-containing diet. Group 5 (30 mice) was fed basal diet alone. The remaining 25 mice (group 4) received 5 ppm TAM-containing diet alone. At the termination of the experiment (30 weeks), endometrial carcinomas were confirmed to be present in the groups exposed to MNU. TAM increased the incidence of preneoplastic lesions of the endometrium, while E2 enhanced the occurrence of the carcinoma. No carcinomas were found in the group given TAM alone. In the ovaries, corpora lutea were lacking in most of the mice exposed to TAM, suggesting that the animals were not cycling. Such findings indicate that TAM has an enhancing effect on endometrial carcinogenesis in mice, probably via a mechanism involving overexpression of Fos/Jun proteins.

Key words: Tamoxifen — Endometrial carcinogenesis — Fos/Jun — Polymerase chain reaction — Mice

Tamoxifen (TAM) has been employed for adjuvant chemotherapy in advanced breast cancer patients.3 Recently, the use of this agent has been proposed for prophylactic therapy in disease-free women with an increased risk of breast cancers.2, 3 The main anti-tumor effects of TAM are suggested to be attributed to anti-estrogenic activities through the competitive blockade of estrogen receptors.8 Although TAM is an anti-estrogen, it also acts as a weak estrogen agonist, especially in response to estrogen deficiency in postmenopausal women.5, 6 TAM is reported to exert some estrogenic effects in human endometrial epithelium5 and to promote development of endometrial hyperplasia and polyps.5, 6 Several clinical reports have implied that long-term TAM treatment increases the risk of endometrial cancers.10, 11 Furthermore, TAM has been shown to possess carcinogenic activity in rat liver.10 It is known that TAM induces overexpression of c-fos mRNA in human endometrial carcinoma cells in nude mouse.13 though no studies on the effects of TAM on the endometrium have been done in rodents. In the present study, the effects of TAM on endometrial carcinogenesis in mice were examined by using a medium-term assay and a long-term assay with N-methyl-N-nitrosourea (MNU).

MATERIALS AND METHODS

Animals and chemicals Female ICR mice, 10 weeks of age, were purchased from Japan SLC Co. (Shizuoka). The basal diet (Oriental MF, Oriental Yeast Co., Tokyo) and distilled water were available ad libitum throughout the experiment. 17β-Estradiol (E2) and MNU were purchased from Sigma Chem. Co. (St. Louis, MO), TAM (trans-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethylamine) was also purchased from Aldrich Chem. Co. (Milwaukee, WI).

Experimental protocol for medium-term assay Ovariectomized ICR mice, 12–13 weeks of age, were divided into five experimental groups (6 mice in each). Groups 1 and 2 were given the diet containing E2 at the dose of 25 ppm or 5 ppm, respectively. The 5 ppm E2-containing diet was similar to that used in our previous study.14 Groups 3 and 4 were fed the diet containing TAM at the dose of 25 ppm or 5 ppm, respectively. The 5 ppm TAM-containing diet was selected so that the dose of TAM based on the mean intake of the diet by the mice would be similar to the clinical dose in humans 20 mg/50 kg daily. Group 5

1 To whom all correspondence should be addressed.
served as an untreated control. Two weeks later, resected uteri were cut in half longitudinally. One half was quickly frozen in liquid nitrogen, and the other was subjected to pathological examination.

**Reverse transcription-polymerase chain reaction (RT-PCR)** Total RNA was isolated from the frozen tissues by a guanidinium thiocyanate-phenol-chloroform extraction method. Total RNA (3 μg) was reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (MLV-RTase, 200 units, Gibco BRL, Gaithersburg, MO) in 20 μM Tris-HCl (pH 8.4), 50 μM KCl, 2.5 μM MgCl₂, 0.1 μg/ml bovine serum albumin, 10 μM dithiothreitol, and 0.5 μM deoxyoligonucleotides to generate cDNAs using random hexamers (50 ng, Gibco BRL) at 37°C for 60 min. The RT reaction mixture was heated at 94°C for 5 min to inactivate MLV-RTase. For c-fos or c-jun mRNA, forty cycles of PCR, consisting of 1 min at 94°C for denaturation, 1 min at 55°C for annealing, and 1 min at 72°C for extension, were carried out with reverse-transcribed cDNAs and 0.1 μM specific primers using an Iwaki TSR-300 thermal sequencer (Iwaki Glass, Tokyo) with Vent DNA polymerase (New England Biolabs, Beverly, MA) in 10 μM KCl, 20 μM Tris-HCl (pH 8.8), 10 μM (NH₄)₂SO₄, 2 μM MgSO₄, 0.1% Triton X-100, and 0.15 μM deoxynucleotide phosphates. Twenty cycles of PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH, a house-keeping gene) mRNA as an internal standard were performed similarly.

For quantitative PCR, a 50 μl reaction mixture, consisting of 2.5 μl of template, 25 pmol (0.25 μM) each of 3′- or 5′-primer, 2 μl of dNTPs (10 mM) and 2.5 IU of recombinant Taq DNA polymerase (Takara, Kyoto), was used. The following oligodeoxynucleotides were synthesized as specific primers according to the cited reports [cDNAs for c-fos,¹⁰ c-jun,¹¹ and GAPDH¹²]: sense for c-fos, 5′-CTTACGCGCAAGCGGAAATG-3′; anti-sense for c-fos, 5′-AAGCTTACCAGCACTTCAAC-3′; sense for c-jun, 5′-AGAGCTAGACCTTGAAC-3′; anti-sense for c-jun, 5′-CTGGGAACGCTTCTTGCTTCAAC-3′; sense for GAPDH, 5′-AGAGCTAGACCTTGAAC-3′; anti-sense for GAPDH, 5′-CTCCTTGGAGCCATGTAG-3′; antisense for GAPDH, 5′-CTCCTTGGAGCCATGTAG-3′.

**Quantitative analysis of c-fos/jun mRNA expressions** by Southern blot of PCR products The PCR products were applied to 1.2% agarose gel for electrophoresis at 50–100 V. PCR products were capillary-transferred to Immobilon transfer membrane (Millipore Corp., Bedford, MA) for 16 h. The membrane was dried at 80°C for 30 min, and UV-irradiated to fix the PCR products. The products on the membrane were prehybridized in 1 M NaCl, 50 mM Tris-HCl (pH 7.6) and 1% sodium dodecyl sulfate at 42°C for 1 h, and hybridized at 65°C overnight in the same solution with biotinylated oligodeoxynucleotide probes corresponding to sequences between the specific individual primers of c-fos or c-jun. Specific bands hybridized with biotinylated probes on the membranes were detected with a Bio image analyzer (Millipore Corp.). The intensity of specific bands was standardized with respect to that of GAPDH mRNA.

**Immunohistochemical expression of Fos/Jun** After having been fixed in 10% formalin, half portions of endometrial tissues were processed according to conventional methods. Briefly, staining with avidin-biotin-peroxidase complex was done using a Vectastain kit (Vector, Burlingame, CA). The primary antibodies used were directed against the proteins of c-fos and c-jun (anti-rabbit polyclonal, Oncogene Science, Inc., New York, NY), at 1:100 dilution. Staining intensity was assigned as follows: (+) positive; (++) minimally or randomly positive; (−) negative.

**Experimental protocol for long-term assay** One hundred and fifty female ICR mice, 12–13 weeks of age at the start of the experiment, were divided into five groups.

Mice in groups 1–3 and 5 underwent laparotomy under general anesthesia with diethyl ether and were injected with MNU solution (total volume: 0.1 ml) at a dose of 1 mg/100 g body weight into the left uterine tube and with normal saline into the right. One week after the MNU exposure, the animals were divided into four experimental groups. Group 1 (35 mice) was given 25 ppm E₂-containing diet, and group 2 (30 mice) was given 5 ppm TAM-containing diet. The level of E₂ in the diet was selected for the same reason as in the case of the medium-term assay. Group 3 (30 mice) and group 4 (25 mice) were fed with 5 ppm TAM-containing diet. The dose of TAM-containing diet was selected to match the clinically used dosage, as mentioned before. Group 5 (30 mice) served as a control, and was fed basal diet. The experiment was terminated 30 weeks after the MNU exposure. At the termination, all animals were killed and autopsied. All major organs, especially the reproductive organs, were grossly inspected. The uterus, ovaries, vagina and other lesions suspected of being neoplastic or hyperplastic were submitted to histological examination. Tissues were sectioned at 3 μm thickness and stained with hematoxylin and eosin (HE).

**Histology of the uterine lesions** According to the WHO criteria,¹³ uterine endometrial lesions were divided into four lesions: a) endometrial hyperplasia, simple; b) endometrial hyperplasia, complex; c) atypical endometrial hyperplasia; d) adenocarcinoma. Uterine cervical lesions were basically diagnosed according to the criteria of Munoz et al.²⁰

**Statistical analysis** Statistical analysis was done by using the χ² test or Student’s t test.
RESULTS

Medium-term experiment  The c-fos and c-jun mRNA levels are shown in Figs. 1 and 2, respectively. The levels of c-fos and c-jun mRNA in uteri of mice treated with TAM (25 ppm) were significantly higher than those of the control group (P<0.05). However, no significant differences were found between TAM (5 ppm)-treated groups and the control group. The levels 2 weeks after the start of feeding of E2- or TAM-containing diet were semi-dose-dependently increased as compared with the control.

The results of immunohistochemical detection of Fos/Jun oncoproteins after 2 weeks’ feeding of the diet containing E2 and TAM are summarized in Table I. Representative examples of Fos/Jun oncoprotein staining are shown in Figs. 3 and 4. The expression of Fos oncoprotein was prominent in the glandular cells in the groups treated with E2 (25 ppm) and TAM (25 ppm). The expression of Jun oncoprotein was also observed in glandular and luminal cells in the groups treated with TAM as well as E2.

Long-term experiment  Five mice in group 1, six in group 2, three in group 3, and four in group 5 died within 15 weeks. No pathological abnormalities other than pneumonia were found. The remaining animals survived until the termination of the experiment and were counted as effective animals. The mean body weights are summarized in Table II. No significant differences were obtained among the five experimental groups.

Adenocarcinomas were recognized in the bilateral uterine corpora in the groups treated with MNU. Histological features of the endometrial adenocarcinomas and hyperplasia in this study were the same as those in our previous

| Treatment | Fos | Jun |
|-----------|-----|-----|
| E2 (25 ppm) | (+) | (+) |
| E2 (5 ppm) | (+) | (+) |
| TAM (25 ppm) | (+) | (+) |
| TAM (5 ppm) | (+) | (+) |
| Control | (++) | (++) |

Table I. Immunohistochemical Analysis of Expression of Fos/Jun oncoproteins after 2 Weeks’ Feeding of Diet Containing E2 or TAM

Effect of Tamoxifen on Mouse Endometrium

Representative morphology of adenocarcinoma and atypical endometrial hyperplasia in this study is shown in Figs. 5 and 6. All adenocarcinomas arising in the endometria were well or moderately differentiated types. The incidence of preneoplastic and neoplastic lesions of the endometria is indicated in Fig. 7. While the incidence of carcinomas of the left (treated) uterine corpus in the groups given MNU and TAM was almost the same as in the group given MNU alone, that of preneoplastic endometrial lesions was higher. No carcinomas were found in the mice treated with TAM alone.

Pathological findings of ovary, oviduct and vagina in each group are summarized in Table III. Cystic ovaries were commonly seen in mice of groups 1–4. Corpora lutea were mostly absent in mice of groups 1, 3 and 4. No

Table II. Initial and Effective Numbers of Animals, and Mean Body Weights

| Groups (treatment) | Initial number of animals | Effective number of animals | Mean body weights (g) |
|--------------------|---------------------------|----------------------------|-----------------------|
| Group 1 [MNU/saline+E₂ (25 ppm)] | 35 | 30 | 39.9±2.6 |
| Group 2 [MNU/saline+E₂ (5 ppm)] | 30 | 24 | 40.4±5.0 |
| Group 3 [MNU/saline+TAM (5 ppm)] | 30 | 27 | 42.2±3.9 |
| Group 4 [TAM (5 ppm) alone] | 25 | 21 | 38.1±2.5 |
| Group 5 (MNU/saline alone) | 25 | 25 | 45.5±3.8 |

a) Animals that survived more than 15 weeks.
tumors were present in any of the groups. Marked epithelial hyperplasia of the oviduct, diagnosed as “progressive proliferative lesion,” was commonly observed in mice of groups 1–4 (Fig. 8). Papillary lesions were frequently seen in the vagina of mice treated with TAM (Fig. 9).

DISCUSSION

The transient expression of early genes, c-fos/jun, appears to be related to cellular proliferation and differentiation. Acute administration of E2 causes a transient increase in the expression of c-fos, c-jun, and c-myc. Among three natural estrogens (estrone, E2 and estriol), E2...
is considered to exert the most prominent enhancing effect on MNU-induced endometrial carcinogenesis in mice.14 Overexpression of c-fos/jun mRNA and their oncoproteins has been suggested to be related to the enhancing effects of the three natural estrogens.23 Expression of the c-fos gene is induced transiently by various factors, such as platelet-derived growth factor,26 epidermal growth factor,27 nerve growth factor,28 and even sex steroids,23 and does not require any de novo protein synthesis. Consistent overexpression of Fos oncoprotein leads to cell transformation.29 AP-1, a transcription regulation factor consisting of Jun-Jun or Fos-Jun protein dimers, binds to promoter sequences,30, 31 to activate the transcription of target genes. In the present study, the enhancing effects of TAM on c-fos/jun mRNA expression, though similar to those of E2, were weaker. The enhancing effects of TAM on c-fos mRNA expression are consistent with those found in a rat model.31 Thus, the effects of TAM on endometrial carcinogenesis induced by MNU in mice may be related to the overexpressions of Fos/Jun oncoproteins.

Enhancing effects of TAM and E2 on endometrial tumorigenesis in mice were seen in both uterine corpora. In this study, MNU was administered to the left uterine corpus, and normal saline to the right. It is possible that a small amount of the MNU solution flowed to the other uterine corpus from the injected side. This may be related to the different incidences of (pre)neoplastic lesions of the left and right uterine corpora. Such differences were also noted in our previous report.14

It is suggested that mutation of the rat p53 gene is related to the hepatocarcinogenicity of TAM.33 However, in our previous study, p53 or ras gene mutation was rarely detected in the tissues of MNU and E2-induced endometrial neoplasia or hyperplasia in mice.34 Thus, such gene mutation may not be involved in MNU- and TAM-induced endometrial carcinogenesis in mice. Nevertheless, it is speculated that the enhancing effect of TAM on the tumorigenesis is related to overexpression of Fos/ Jun oncoproteins via an estrogenic effect, even though the effect of TAM is weaker than that of E2.

In the present study, the enhancing effect of TAM was recognized in the precancerous stage of endometrial tumorigenesis initiated by MNU. Recently, induction of uterine adenocarcinoma in mice treated neonatally with TAM alone was reported.21 No adenocarcinomas, however, were found in the endometrium after treatment with TAM alone for 30 weeks in this study. The differences may be mainly due to the different ages at which TAM was administered. It is known that TAM is not hepatocarcinogenic in mice,35 but is in rats.36-37 This may be related to the lower level of DNA adducts induced by TAM in mice.36-37 Although E2 is carcinogenic to the mouse endometrium,38 TAM may be less estrogenic and less carcinogenic.

In the present study, the effect of TAM-exposure was seen in other tissues of the reproductive tract. The incidences of ovaries lacking corpora lutea in groups 1, 3 and 4 were significantly lower than in other groups. From the data in the present study, it is assumed that TAM affects ovarian functions more strongly than E2. Although ovarian tumors were not detected in any of the groups, cysts of the ovaries were commonly noted in all groups. Furthermore, hyperplastic epithelium in the oviducts21 was commonly observed in groups 1, 3 and 4, suggesting that TAM influences the oviducts more than E2 does. These findings are consistent with the results of studies on neonatal exposure to TAM.21
TAM has been used as an adjuvant chemotherapy for breast cancer and is considered to be beneficial for prevention of breast cancer. However, the results of this study indicate an enhancing effect of TAM on MNU-induced endometrial carcinogenesis in mice. Accordingly, it is suggested that a periodic check-up of the endometrium is necessary in patients taking TAM.

REFERENCES

1) Early Breast Cancer Trialists’ Collaborative Group. Systemic treatment of early breast cancer by hormonal, cytotoxic, or immune therapy. Lancet, 339, 1–15 (1992).
2) Powles, T. J. The case for clinical trials of tamoxifen for prevention of breast cancer. Lancet, 340, 1145–1147 (1992).
3) Stone, R. NIH fends off critics of tamoxifen study. Jpn. J. Cancer Res., 89, 951–955 (1993).
4) Jordan, V. C. Antitumor activity of antiestrogen ICI 46,474 (tamoxifen) in dimethylbenzanthracene (DMBA)-induced rat mammary carcinoma model. J. Steroid Biochem., 4, 354 (1974).
5) Lippman, M., Bolan, G. and Huff, K. Interactions of antiestrogens with human breast cancer in long-term tissue culture. Cancer Treat. Rep., 60, 1421–1429 (1976).
6) Lahti, E., Vuopala, S., Kauppila, A., Blanco, G., Ruokonen, A. and Laatikainen, T. Maturation of vaginal and endometrial epithelium in postmenopausal breast cancer patients receiving long-term tamoxifen. Gynecol. Oncol., 55, 410–414 (1994).
7) Neven, P., De Muylder, X., Vanderbeke, Y., Vanderick, G. and De Muylder, E. Hysteroscopic follow-up during tamoxifen treatment. Eur. J. Obstet. Gynecol. Reprod. Biol., 35, 235–238 (1990).
8) De Muylder, X., Neven, P., De Somer, M., Van Belle, Y., Vanderick, G. and De Muylder, E. Endometrial lesions in patients undergoing tamoxifen therapy. Int. J. Gynecol. Obstet., 36, 127–130 (1991).
9) Lahti, E., Blanco, G., Kauppila, A., Apaja-Sarkkinen, M., Taskinen, P. and Laatikainen, T. Endometrial changes in postmenopausal breast cancer patients receiving long-term tamoxifen. Obstet. Gynecol., 81, 660–664 (1993).
10) Le Bouedec, G. and Dauplat, J. Cancer of endometrium caused by antiestrogens. Rev. Fr. Gynecol. Obstet., 87, 345–348 (1992).
11) Fornander, T., Rutqvist, L. E., Cedermark, B., Glas, U., Mattson, A., Silfverswärd, C., Skoog, L., Somell, A., Theve, T., Wilking, N., Askgren, J. and Hjalmar, M. L. Adjuvant tamoxifen in early breast cancer. Occurrence of new primary cancers. Lancet, I, 117–119 (1989).
12) Williams, G. M., Iatropoulos, M. J., Djordjevic, M. V. and Kaltenberg, O. P. The triphenylethylen drug tamoxifen is a strong liver carcinogen in the rat. Carcinogenesis, 14, 315–317 (1993).
13) Sakakibara, K., Kan, N. C. and Satyaswaroopa, P. G. Both 17b-estradiol and tamoxifen induce c-fos messenger ribo-

nucleic acid expression in human endometrial carcinoma in nude mice. Am. J. Obstet. Gynecol., 166, 206–212 (1992).
14) Niwa, K., Murase, T., Furu, T., Morishita, S., Mori, H., Tanaka, T., Mori, H. and Tamaya, T. Enhancing effects of estrogens on endometrial carcinogenesis initiated by N-methyl-N-nitrosourea in ICR mice. Jpn. J. Cancer Res., 84, 951–955 (1993).
15) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. Anal. Biochem., 162, 156–159 (1987).
16) Van Beveren, C., Van Straaten, F., Curran, T., Muller, R. and Verma, I. M. Analysis of FBJ-MuSV provirus and c-fos (mouse) gene reveals that viral and cellular fos gene products have different carboxy termini. Cell, 32, 1241–1255 (1983).
17) Lamp, W. W., Wamsley, P., Sassone-Corsi, P. and Verma, I. M. Induction of protooncogene jun/AP-1 by serum and TPA. Nature, 334, 629–631 (1988).
18) Sabath, D., Broome, H. E. and Prystowsky, M. B. mRNA is a major interleukin-2-induced transcript in a cloned T-helper lymphocyte. Gene, 91, 185–191 (1990).
19) Scully, R. E., Bonfiglio, T. A., Kurman, R. J., Silverberg, S. G. and Wilkinson, E. J. Histological classification of tumours of the female genital tract. In “Histological Typing of Female Genital Tumours,” 2nd Ed., pp. 13–18 (1994). WHO, Geneva.
20) Muñoz, N., Dunn, T. B. and Turusov, V. S. Tumours of the vagina and uterus. In “Pathology of Tumours in Laboratory Animals. Vol. II. Tumours of the Mouse,” ed. V. S. Turusov, pp. 359–383 (1979). IARC, Lyon.
21) Newbold, R. R., Jefferson, W. N., Padilla-Burgos, E. and Bullock, B. C. Uterine carcinoma in mice treated neonatally with Tamoxifen. Carcinogenesis, 18, 2293–2298 (1997).
22) Adamson, E.D. Oncogenes in development. Development, 99, 449–471 (1987).
23) Weitz, A. and Bresciani, F. Estrogen induces expression of c-fos and c-myc protooncogenes in the rat uterus. Mol. Endocrinol., 2, 816–824 (1988).
24) Weitz, A., Cicatiello, L., Persiot, E., Scalona, M. and Bresciani, F. Estrogen stimulation of transcription of c-jun protooncogene. Mol. Endocrinol., 4, 1031–1050 (1990).
25) Morishita, S., Niwa, K., Ichigo, S., Hori, M., Murase, T., Fujimoto, J. and Tamaya, T. Overexpressions of c-fos/jun mRNA and their oncoproteins (Fos/Jun) in the mouse
Effect of Tamoxifen on Mouse Endometrium

uterus treated with three natural estrogens. *Cancer Lett.*, 97, 225–231 (1995).

26) Kruijer, W., Cooper, J. A., Hunter, T. and Verma, I. M. Platelet-derived growth factor induces rapid but transient expression of c-fos gene and protein. *Nature*, 312, 711–716 (1984).

27) Müller, R., Bravo, R. and Bruckhardt, J. Induction of c-fos gene and protein by growth factors precedes activation of c-myc. *Nature*, 312, 716–721 (1984).

28) Curren, T. and Mogan, J. I. Superinduction of c-fos by nerve growth factor in the presence of peripherally active benzodiazepines. *Science*, 229, 1265–1268 (1986).

29) Müller, R., Verma, I. M. and Adamson, E. D. Expression of c-oncogenes: c-fos observed in mouse and human tissues using an antibody to a synthetic peptide. *EMBO J.*, 4, 941–947 (1985).

30) Turner, R. and Tjian, R. Leucine repeats and adjacent DNA binding domain mediate the formation of functional c-Fos-c-Jun heterodimer. *Science*, 243, 1689–1697 (1989).

31) Bohmman, D., Admon, A., Turner, D. R. and Tjian, R. Transcriptional regulation by AP-1 family of enhancer binding proteins: a nuclear target for signal transduction. *Cold Spring Harbor Symp. Quant. Biol.*, 53, 695–700 (1989).

32) Nephew, K. P., Polek, T. C., Akcali, K. C. and Khan, S. A. The antiestrogen tamoxifen induces c-fos and Jun-B, but not c-jun or jun-D, protooncogenes in the rat uterus. *Endocrinology*, 133, 419–422 (1993).

33) Vancutsem, P. M., Lazarus, P. and Williams, G. M. Frequent and specific mutations of the rat p53 gene in hepatocarcinomas induced by tamoxifen. *Cancer Res.*, 54, 3864–3867 (1994).

34) Murase, T., Niwa, K., Morishita, S., Itoh, N., Mori, H., Tanaka, T. and Tamaya, T. Rare occurrence of p53 and ras gene mutations in preneoplastic and neoplastic mouse endometrial lesions induced by N-methyl-N-nitrosourea and 17β-estradiol. *Cancer Lett.*, 97, 223–227 (1995).

35) White, I. N. H., de Matteis, F., Davies, A., Smith, L. L., Crofton-Sleigh, C., Venitt, S., Hewer, A. and Phillips, D. H. Genotoxic potential of tamoxifen and analogues in female Fischer F344/N rats, DBA/2 and C57B1/6 mice and human MCL-5 cells. *Carcinogenesis (Lond.)*, 13, 2197–2203 (1992).

36) Greaves, P., Goonetilleke, R., Nunn, G., Topham, J. and Orton, T. Two-year carcinogenicity study of tamoxifen in Alderley Park Wistar-derived rats. *Cancer Res.*, 53, 3919–3924 (1993).

37) Hard, G. C., Atropoulos, M. J., Jordan, K., Radi, L., Kaltenberg, O. P., Imondi, A. R. and Williams, G. M. Major difference in the hepatocarcinogenicity and DNA adduct forming ability between toremifene and tamoxifen in female Crl:CD(BR) rats. *Cancer Res.*, 53, 4534–4541 (1993).

38) Niwa, K., Tanaka, K., Mori, H., Yokoyama, Y., Furui, T., Mori, H. and Tamaya, T. Rapid induction of endometrial carcinoma in ICR mice treated with N-methyl-N-nitrosourea and 17β-estradiol. *Jpn. J. Cancer Res.*, 82, 1391–1396 (1991).