Genotoxicity of the some selective estrogen receptor modulators: a review

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Abstract The objective of this article is to review genotoxicological profile of the major selective estrogen receptor modulators, including clomiphene, tamoxifen, toremifene, raloxifene. These drugs have been used for infertility treatment and breast cancer prevention in high risk-women. However, some studies reported that especially tamoxifen is a genotoxic agent and is related with endometrial cancer. Our review indicate that clomiphene and tamoxifen were found as genotoxic agent in majority of the tests. However published reports showed that toremifene is a weakly genotoxic agent. The genotoxic effects of raloxifene are still poorly known. Further genotoxicity studies should be conducted especially for raloxifene.

Keywords Genotoxicity · Clomiphene · Tamoxifen · Toremifene · Raloxifene · Review

Introduction

Estrogens are a class of sex steroid hormones that are synthesized from cholesterol and are secreted primarily

by the ovaries, with contributions from placenta, adipose tissue, and adrenal glands (Lewis and Craig Jordan 2005). They have a number of important physiological effects on hormone-dependent reproductive tissues (Robertson 2004). They control the growth of breast epithelial tissue, regulate the menstrual cycle by promoting endometrial proliferation in the uterus, and regulate circulating cholesterol levels, thereby providing a protective effect on the cardiovascular system (Robertson 2004; Jordan 1996). The biological role of estrogen is mediated through high-affinity binding to the estrogen receptor (ER) (Robertson 2004; Cavailles 2002). Exposure to high level of estrogens, especially due to in vitro fertilization, can cause different tumours. For example, breast tumours that express the ER are stimulated by estrogen (Lippman et al. 1977) and anti-estrogen therapy has thus become an established treatment for ER-positive breast cancer (Robertson 2004). Estrogens in general showed their genotoxicity by different mechanisms; (a) formation of apurinic sites in DNA (Cavalieri et al. 2002), (b) generation of reactive oxygen species (Roy and Liehr 1999), (c) other undetermined, especially epigenetic mechanisms (Dipple et al. 1984).

Selective estrogen receptor modulators (SERMs) are compounds that act as estrogen agonists on selected targets while being estrogen antagonists on others. The main roles of SERMs are estrogen agonist activity on bone metabolism, several functions of the cardiovascular system and estrogen antagonism in the breast and uterus (Halbreich and Kahn 2000). Several

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Fig. 1 Structural formula of the clomiphene

compounds that exhibit a SERM property are currently available for clinical use. Among them clomiphene, tamoxifen, toremifene, raloxifene are used most frequently (Goldstein et al. 2000). Tamoxifen is the first approved drug by the international authorities (FDA, WHO, etc.) to prevent malign breast carcinoma. However, studies have been conducted that long-term use of tamoxifen may result in a two to sevenfold increase in the risk of developing endometrial cancer (Singh et al. 2007).

The aim of the present review was to evaluate genotoxicological profile of these SERMs. Genotoxicity is a broader term that refers to the ability to interact with DNA and/or the cellular apparatus that regulates the accuracy of the genome, such as the spindle apparatus and topoisomerase enzymes (Maurici et al.). In general genotoxic agents can lead to cancer. Hence, evaluation of the possible genotoxicity of a chemical is an important issue.

Selective estrogen receptor modulators (SERMs)

Clomiphene

Clomiphene is a non steroidal, ovulation stimulant (Csemiczky et al. 2002) and SERM (Fig. 1). It is a synthetic hormone that deceives the hypothalamus into perception that body's estrogen level is too low. In return, the hypothalamus releases GnRH (gonadotropin-releasing hormone), which activates the pituitary gland to release a sufficient amount of follicle stimulating hormone (FSH). Then an increased amount of FSH stimulates development of follicles, ultimately resulting in ovulation (Randal and Templeton 1991;

Martikainen et al. 2006; Chaman and Asmatullah 2011). Some authors have reported that treatment with clomiphene is related with various malignancies including endometrial and ovarian cancers (Derman and Adashi 1994; Althuis et al. 2005; Lerner-Geva et al. 2006; Calderon-Margalit et al. 2009; Jensen et al. 2009).

Genotoxicity

Genotoxic effects of clomiphene have been reported in different test systems by several authors. Zambrano et al. (1982) reported that clomiphene showed no increase in frequency of either chromosomal aberrations or polychromatic erythrocytes in mouse bone marrow. Ohnishi et al. (1986) reported that clomid which contains clomiphene, induced DNA-strand breaks and SOS responses such as mutation and expression of the *umuC+* gene in *E. coli* (Ohnishi et al. 1986). Styles et al. (1997) investigated the clastogenic and aneugenic effects of clomiphene in human lymphoblastoid cell line (MCL5). Different authors have reported that there was an increase in micronuclei and chromosomal aberrations (aneuploidy) in cells treated with clomiphene. Arriaga-Alba et al. (1996) stated that clomiphene induced frame-shift mutations in the *Salmonella typhimurium* TA1538, TA97 and TA100 strains in vitro by using metabolic S9 activation. However, it did not induce base pair substitution mutations. Clomiphene induced genolethal DNA damages in *Escherichia coli* PolA⁻/PolA⁺ in the pre-incubation method with S9 and in the disk diffusion method without S9 (Arriaga-Alba et al. 1996). London et al. (2000) reported that clomiphene decreased the number of ovulated oocytes and significantly ($P < .05$) increased the hyperploidy at 100 mg/kg in vivo. Similarly, in vitro 5.0 µg/mL concentration of clomiphene significantly ($P < .05$) increased the level of hyperploidy and reduced the proportion of metaphase I oocytes in mouse in vitro (London et al. 2000). Duran et al. (2006) investigated the potential genotoxicity of clomiphene in rat reticulocytes by micronucleus assay. 0.16, 0.32 and 0.64 mg/kg concentrations of clomiphene were administrated to rats intraperitoneally for 5 days. Authors have observed that clomiphene showed a dose-dependent increase in genomic instability and micronucleus frequency in bone marrow stem cells of rats (Duran et al. 2006). Hastwell et al. (2009) reported

Table 1 Summary of the genotoxicity of the clomiphene

Test material	Genotoxicity end-point	Results	Reference
<i>E. coli</i>	DNA strand break, SOS response	+	Ohnishi et al. (1986)
<i>S. typhimurium</i> TA97, TA100, TA1538	Frame-shift mutation/base pair substitution	+/-	Arriaga-Alba et al. (1996)
<i>Escherichia coli</i> PolA- / PolA+	Genolethal DNA damage with and without S9	+	Arriaga-Alba et al. (1996)
Mouse oocyte	Hyperploidy/cytotoxicity	+	London et al. (2000)
Rat reticulocytes	Micronucleus	+	Duran et al. (2006)
Human lymphoblastoid TK6 cell	GreenScreen HC	–	Hastwell et al. (2009)
Human lymphoblastoid cell line (MCL5)	Micronuclei and chromosomal numerical aberrations	+	Styles et al. (1997)
Mouse bone marrow cells	Chromosomal aberrations, micronucleus,	–	Zambrano et al. (1982)
Human lymphocytes	Chromosomal aberrations, micronucleus, comet	+	Yilmaz et al. (2014)
<i>S. typhimurium</i> TA97, TA98	Frame-shift mutation/base pair substitution	–	Yilmaz et al. (2014)
Human lymphocytes	Sister chromatid exchanges	+	Yilmaz et al. (unpublished)

+: Positive, -: Negative

that clomiphene has no genotoxic affect on the human lymphoblastoid TK6 cell line using the GADD45a-GFP “Green Screen HC” genotoxicity assay. Yilmaz et al. (2014) reported that 0.4, 0.8, 1.6 and 3.2 µg/mL concentrations of clomiphene caused significant increase in chromosomal aberrations, micronucleus frequency and DNA damage (comet assay) in human lymphocytes. It also caused cell cycle delay by decreasing the mitotic index. In this work authors have also reported that clomiphene did cause neither base-pair substitutions nor frame-shift mutations in *S. typhimurium* TA98 and TA100 strains (Yilmaz et al. 2014). In our laboratory we have also worked on the SCE induction of clomiphene in human lymphocytes (Unpublished report). Our results support most of the previous findings since clomiphene caused a significant increase in sister chromatid exchanges in human lymphocytes (Table 1).

Tamoxifen

Tamoxifen is a nonsteroidal antiestrogenic agent used for the breast cancer therapy for over 30 years (Fig. 2). It is a SERM and primarily binds to ERs to inhibit the growth of malignant cell (development of malignancy). Some authors have reported that tamoxifen increases the risk for endometrial cancer (Magriples et al. 1993; Seoud et al. 1993; Fisher et al. 1994; van Leeuwen et al. 1994; Cook et al. 1995; Cuenca et al. 1996; Curtis et al. 1996) while it is a clearly

Fig. 2 Structural formula of the tamoxifen

beneficial agent for the breast cancer prevention. Furthermore, tamoxifen is a potent hepatocarcinogen in rats (Greaves et al. 1993; White 1999). Tamoxifen-DNA adducts (Philips et al. 1994; Phillips 2001; Beland et al. 1999; Gamboa da Costa et al. 2001; Carthew et al. 2001) can cause hepatic tumors. It has also been demonstrated that rat and human liver microsomal fractions metabolize tamoxifen to reactive intermediates that bind covalently protein and DNA (Mani and Kupfer 1991; Pathak and Bodell 1994; Carmichael et al. 1996).

Genotoxicity

Genotoxicity of tamoxifen has been investigated by different test systems. Tucker et al. (1984) observed that tamoxifen is not mutagenic in the AMES test. Tamoxifen induced a significant increase in micronucleus

formation in a dose dependent manner in cultures of MCL-5 cells, a human cell line that expresses five different human cytochrome P450 isoenzymes, as well as epoxide hydrolase. Exposure of rat hepatocytes to tamoxifen in vitro, resulted in induction of unscheduled DNA synthesis, when preparations from rats which had been pretreated with tamoxifen in vivo were used (White et al. 1992). Wilson et al. (1995) reported that 50 μ M tamoxifen decreased human lymphocyte proliferation but it did not exert a genotoxic activity. Tamoxifen induced aneuploidy was observed by Sargent et al. (1994, 1996) in Sprague–Dawley rats. Vijayalaxmi and Rai (1996) studied the genotoxic effects of tamoxifen citrate using a mouse bone marrow chromosome aberration assay and micronucleus test. 0.1, 0.2, 0.4 and 0.8 mg/kg/day dose were administered to the mice for 10 days. A statistically significant chromosomal aberration and MN induced by tamoxifen revealed its clastogenic potency. Carmichael et al. (1996) reported that tamoxifen did not cause DNA adducts in human endometrial cells. The authors concluded also that genotoxic events observed with tamoxifen in the rat may not apply to the human endometrium. The clastogenicity of tamoxifen was tested in six human lymphoblastoid cell lines each expressing increased monooxygenase activity associated with a specific transfected human cytochrome P450 cDNA (CYP1A1, CYP1A2, CYP2D6, CYP2E1 or CYP3A4). Dose-related increases in micronuclei were observed when cells expressing 2E1, 3A4, 2D6 or MCL-5 cells were exposed to tamoxifen (Styles et al. 1997). The cytotoxicity of the tamoxifen has been investigated in the RPE cell line D407 and pig RPE cell culture using the WST-1 test, which is an assay of cell proliferation and viability (Mannerström et al. 2002). Tamoxifen was found cytotoxic in this test system. Chronic treatments with tamoxifen citrate were performed with 3-day-old larvae of the standard and high bioactivation crosses of the wing spot test in *Drosophila* at concentrations of 0.66, 1.66 and 3.33 mM by Heres-Pulido et al. (2004). Somatic spots on normal wings from marker heterozygous flies and on serrate wings from balancer heterozygous flies were scored to determine mutation and recombination events in somatic cells. The results showed genotoxic effects of tamoxifen at 1.66 and 3.33 mM in the ST cross only and without a clear dose–response effect. This suggests a weak genotoxicity of this anti-estrogen. Al-Jassabi et al. (2011) investigated the genotoxicity of drug tamoxifen

Table 2 Summary of the genotoxicity of the tamoxifen

Test material	Genotoxicity end-point	Results	Reference
AMES	Frame-shift mutation/base pair substitution	–	Tucker et al. (1984)
Mouse bone marrow cells	Chromosomal aberrations, micronucleus,	+	Vijayalaxmi and Rai (1996)
Human endometrial cells	DNA adduct formation	–	Carmichael et al. (1996)
<i>Drosophila melanogaster</i>	Wing spot test	+	Heres-Pulido et al. (2004)
Male Balb/c mice	DNA damage/ 8-OH-dG	+	Al-Jassabi et al. (2011)
Human lymphoblastoid cells	Micronuclei	+	Styles et al. (1997)
Human MCL-5 cells	Micronuclei	+	White et al. (1992)
Human lymphocytes	Cytotoxicity/ genotoxicity	+/-	Wilson et al. (1995)
Sprague–Dawley rats	Aneuploidy	+	Sargent et al. (1994, 1996)
Rat hepatocytes	Unscheduled DNA synthesis	+	Philips et al. (1994)
Wistar-Hannover rats	Micronucleus	–	Rodrigues et al. (2012)
RPE cell line D407, pig RPE cell culture	WST-1 test	+	Mannerström et al. (2002)

+: Positive, -: Negative

in Balb/c male mice to induce oxidative DNA damage through the formation of 8-hydroxydeoxyguanosine using HPLC with electrochemical detection. Authors have reported that tamoxifen initiates reactive oxygen species formation followed by damage of DNA and other cellular compartments. Rodrigues et al. (2012) studied the tamoxifen induced micronuclei in female Wistar-Hannover rats. The results indicate that tamoxifen reduced formation of micronucleated polychromatic erythrocytes (Table 2).

Toremifene

Toremifene is a nonsteroidal estrogen antagonist and has been used for over 20 years (Fig. 3). The drug is

cytotoxic to breast cancer cells at high concentrations ($>10^{-6}$ mol/L) and cytostatic at lower concentrations, blocking cells in the G0–G1 phase of the cell cycle (Wiseman and Goa 1997). Tamoxifen's antitumor activity is generally postulated also for toremifene. Stimulation of the transforming growth factor β secretion, inhibition of TGF- α and epidermal growth factor secretion, interaction with antiestrogen binding sites, inhibition of protein kinase C activity and binding to calmodulin may be the possible mechanisms of antitumour activity of this drug (Wiseman and Goa 1997; Buckley and Goa 1989; Jordan 1993). Toremifene has not been linked to an increase in endometrial cancer (Holli et al. 1998) and is available as an alternative to tamoxifen for the treatment of metastatic breast cancer (Buzdar and Hortobagyi 1998).

Fig. 3 Structural formula of the toremifene

Genotoxicity

Styles et al. (1997) investigated the clastogenic and aneugenic effects of toremifene and 4-hydroxytoremifene in the human lymphoblastoid cell line (MCL5). With MCL-5 cells there was an increase in micronuclei formation with 4-hydroxytoremifene. The incidence of cells with numerical chromosomal aberrations (aneuploidy) and structural abnormalities (excluding gaps) was increased in cells treated with toremifene. Williams et al. (1998) evaluated toremifene genotoxicity in three in vitro assays: reversion of bacterial point mutations, unscheduled DNA synthesis in cultured hepatocytes from two rat strains and cytogenetics of human lymphocytes and in one in vivo assay: the mouse erythrocyte micronucleus assay. 100–250 mg/mL concentrations were used depending on the system. To test for chromosome damage in vivo, mice were administered up to 2 g/kg toremifene once by gavage, for 3 days. Toremifene lacked genotoxicity or myelotoxicity detectable by any of the above assays. Okuboa et al. (1998) reported that toremifene and *N*-desmethyltoremifene did not cause damage in pUC18 plasmid DNA. The cytotoxicity of toremifene has been investigated in RPE cell line D407 and the pig RPE cell culture using the WST-1 test (Mannerström et al. 2002). Toremifene was found cytotoxic in these test systems. Umemoto et al. (2006) studied the capability of forming DNA adducts by toremifene in the leukocytes of patients. Leukocytes were collected from 27 breast cancer patients taking toremifene (40 mg/day for 25 patients,

Table 3 Summary of the genotoxicity of the toremifene

Test material	Genotoxicity end-point	Results	Reference
Human lymphoblastoid cells (MCL5)	Micronuclei/chromosomal aberrations	+	Styles et al. (1997)
<i>S. typhimurium</i>	Point mutations	–	Williams et al. (1998)
Cultured rat hepatocytes	Unscheduled DNA synthesis	–	Williams et al. (1998)
Human lymphocytes	Chromosomal aberrations	–	Williams et al. (1998)
Mouse erythrocyte	Erythrocyte micronucleus assay	–	Williams et al. (1998)
Human leukocytes	DNA adduct formation	–	Umemoto et al. (2006)
Rat liver, mice liver and uterus	DNA adduct formation	–	Williams, and Jeffrey (1997), White (1999), Rajaniemi et al. (1998a, b), Hellmann-Blumberg et al. (2000)
pUC18 plasmid DNA	DNA damage	–	Okuboa et al. (1998)
RPE cell line D407, pig RPE cell culture	WST-1 test	+	Mannerström et al. (2002)

+: Positive, –: Negative

Fig. 4 Structural formula of the raloxifene

Table 4 Summary of the genotoxicity of the raloxifene

Test material	Genotoxicity end-point	Results	Reference
Wistar-Hannover rat peripheral blood	Micronucleus	—	Rodrigues et al. (2012)
Rat liver	DNA adduct formation	—	Rajaniemi et al. (1998a)

+: Positive, —: Negative

80 mg/day for one patient, and 120 mg/day for one patient) and 20 untreated breast cancer patients. No DNA adducts were detected in the leukocytes of either toremifene-treated or non-treated patients by ^{32}P -postlabeling/high-performance liquid chromatography. Furthermore, DNA adduct formation was low or negative in the experiments of Williams and Jeffrey (1997), White (1999), Rajaniemi et al. (1998a, b), Hellmann-Blumberg et al. (2000) (Table 3).

Raloxifene

Raloxifene, was initially approved for the treatment and prevention of osteoporosis in postmenopausal woman (Fig. 4). Raloxifene inhibits the estrogen-induced breast tissue proliferation and seems to prevent breast cancer in high-risk women (Liu et al. 2003). Unlike tamoxifen, it is not reacting with DNA due to absence of ethyl moiety (Cummings et al. 1999). Goldstein et al. (2000) reported that raloxifene was not increasing the incidence of endometrial cancer. Martino et al. (2005) studied the safety of raloxifene over 8 years in a clinical trial setting. The authors reported that 8-year data support the known clinical safety profile of raloxifene.

Genotoxicity

The genotoxicity of the raloxifene is poorly investigated. Only a few studies have been conducted.

Rajaniemi et al. (1998a, b) reported that raloxifene did not cause DNA adducts in rat liver. Rodrigues et al. (2012) studied the micronucleus induction by raloxifene in female Wistar-Hannover rats (Table 4). The authors have reported that raloxifene did not cause micronuclei induction, furthermore it decreased the micronucleated polychromatic erythrocytes in rats.

Conclusion

Selective estrogen receptor modulators are very effective drugs for the treatment and prevention of the breast cancer, and also treatment of infertility. Tamoxifen is an important drug for the prevention of breast cancer, however long term use can cause side effects especially endometrial cancer. Clomiphene is the leading drug for the treatment of infertility. But genotoxic profiles of clomiphene should be taken into consideration. Toremifene is an estrogen antagonist and unlike for tamoxifen experimental data showed that it did not cause endometrial cancer. Raloxifene is currently used for the treatment and prevention of post-menopausal osteoporosis and is also used for breast cancer prevention in high-risk women. Information on the genotoxicity of raloxifene is scanty, so detailed works should be conducted using different test systems. Our review indicates that tamoxifen and clomiphene are more genotoxic agents than raloxifene and toremifene.

References

- Al-Jassabi S, Saad A, Al-Omari A (2011) Toxic effects of tamoxifen and the protective role of silymarin and zizyphus. *Middle East J Sci Res* 9:110–114
- Althuis MD, Moghissi KS, Westhoff CL, Scoccia B, Lamb EJ, Lubin JH, Brinton LA (2005) Uterine cancer after use of clomiphene citrate to induce ovulation. *Am J Epidemiol* 161:607–615
- Arriaga-Alba M, Flores-Paz R, Díaz-Hernández R, González-Patiño ME (1996) Assessment of genetic toxicity of the exposure to clomiphene citrate, with various bacterial test systems. *Ginecol Obstet Mex* 64:490–497
- Beland FA, McDaniel LP, Marques MM (1999) Comparison of the DNA adducts formed by tamoxifen in vivo. *Carcinogenesis* 20:471–477
- Buckley MT, Goa KL (1989) Tamoxifen: a reappraisal of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* 37:451–490
- Buzdar AU, Hortobagyi GN (1998) Tamoxifen and toremifene in breast cancer: comparison of safety and efficacy. *J Clin Oncol* 16:348–353

- Calderon-Margalit R, Friedlander Y, Yanetz R, Kleinhaus K, Perrin MC, Manor O, Harlap S, Paltiel O (2009) Cancer risk after exposure to treatments for ovulation induction. *Am J Epidemiol* 169:365–375
- Carmichael PL, Ugwumadu AH, Neven P, Hewer AJ, Poon GK, Phillips DH (1996) Lack of genotoxicity of tamoxifen in human endometrium. *Cancer Res* 56:1475–1479
- Carthew P, Lee PN, Edwards RE, Heydon RE, Nolan BM, Martin EA (2001) Cumulative exposure to tamoxifen: DNA adducts and liver cancer in rats. *Arch Toxicol* 75:375–380
- Cavaillès V (2002) Estrogens and receptors: an evolving concept. *Climacteric* 5:20–26
- Cavalieri EL, Devanesan P, Bosland MC, Badawi AF, Rogan EG (2002) Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. *Carcinogenesis* 23:329–333
- Chaman A, Asmatullah (2011) Teratogenic and embryotoxic effects of clomiphene citrate in developing mice. *Asian-Aust J Anim Sci* 24:1053–1059
- Cook LS, Weiss NS, Schwartz SM, White E, McKnight B, Moore DE, Daling JR (1995) Population-based study of tamoxifen therapy and subsequent ovarian, endometrial, and breast cancers. *J Natl Cancer Inst* 87:1359–1364
- Csemiczky G, Harlin J, Fried G (2002) Predictive power of clomiphene citrate challenge test for failure of in vitro fertilization treatment. *Acta Obstet Gynecol Scand* 81:954–961
- Cuenca RE, Giachino J, Arrendondo MA, Hempling R, Edge SB (1996) Endometrial carcinoma associated with breast carcinoma: low incidence with tamoxifen use. *Cancer* 77:2058–2063
- Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, Norton L, Jordan VC (1999) The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple outcomes of raloxifene evaluation. *J Am Med Assoc* 281:2189–2197
- Curtis RE, Boice JD, Shriner DA Jr, Hankey BF, Fraumeni JF Jr (1996) Second cancers after adjuvant tamoxifen therapy for breast cancer. *J Natl Cancer Inst* 88:832–834
- Derman SG, Adashi EY (1994) Adverse effects of fertility drugs. *Drug Saf* 11:408–421
- Dipple A, Pigott MA, Anderson LM (1984) 7,12-Dimethylbenzanthracene-DNA adducts in cultured cells from mouse fetuses of different gestational ages. *Cancer Lett* 21:285–292
- Duran B, Özdemir İ, Demirel Y, Özdemir Ö, Çetin A, Güven A (2006) In vivo evaluation of the genotoxic effects of clomiphene citrate on rat reticulocytes: a micronucleus genotoxicity. *Gynecol Obstet Invest* 61:228–231
- Fisher B, Costantino JP, Redmond CK, Fisher ER, Wickerham DL, Cronin WM (1994) Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J Natl Cancer Inst* 86:527–537
- Gamboa da Costa GG, McDaniel-Hamilton LP, Heflich RH, Marques MM, Beland FA (2001) DNA adduct formation and mutant induction in Sprague-Dawley rats treated with tamoxifen and its derivatives. *Carcinogenesis* 22:1307–1315
- Goldstein SR, Scheele WH, Rajagopalan SK, Wilkie JL, Walsh BW, Parsons AK (2000) A 12-month comparative study of raloxifene, estrogen, and placebo on the postmenopausal endometrium. *Obstet Gynecol* 95:95–103
- Greaves P, Goonetilleke R, Nunn G, Topham J, Orton T (1993) Two-year carcinogenicity study of tamoxifen in Alderly Park Wistar-derived rats. *Cancer Res* 53:3919–3924
- Halbreich U, Kahn LS (2000) Selective oestrogen receptor modulators—current and future brain and behaviour applications. *Expert Opin Pharmacother* 1:1385–1398
- Hastwell PW, Webster TW, Billinton MTN, Lynch AM, Harvey JS, Rees RW, Walmsley RM (2009) Analysis of 75 marketed pharmaceuticals using the GADD45a-GFP ‘Green-Screen HC’ genotoxicity assay. *Mutagenesis* 24:455–463
- Hellmann-Blumberg U, Taras TL, Wurz GT, DeGregorio MW (2000) Genotoxic effects of the novel mixed antiestrogen FC-1271a in comparison to tamoxifen and toremifene. *Breast Cancer Res Treat* 60:63–70
- Heres-Pulido ME, Duenas-García I, Castaneda-Partida L, Sanchez-García A, Contreras-Sousa M, Duran-Dóaz AA, Graf U (2004) Genotoxicity of tamoxifen citrate and 4-nitroquinoline-1-oxide in the wing spot test of *Drosophila melanogaster*. *Mutagenesis* 19:187–193
- Holli K, Joensuu H, Valavaara R, Blanco G, Kataja V, Pukkala E (1998) The Finnish Breast Cancer Group, Tampere University Hospital. Interim results of the Finnish toremifene vs. tamoxifen adjuvant trial (abstract 332). In: Proceedings of 21st annual San Antonio Breast Cancer symposium, San Antonio (TX)
- Jensen A, Sharif H, Kjaer SK (2009) Use of fertility drugs and risk of uterine cancer: results from a large Danish population based cohort study. *Am J Epidemiol* 170:1408–1414
- Jordan VC (1993) A current view of tamoxifen for the treatment and prevention of breast cancer. *Br J Pharmacol* 110:507–517
- Jordan VC (1996) Why tamoxifen? In: Jordan VC (ed) Tamoxifen: a guide for clinicians and patients. Huntington, New York, pp 15–23
- Lerner-Geva L, Keinan-Boker L, Blumstein T, Boyko V, Olmar L, Mashiach S, Rabinovici J, Potashnik G, Lunenfeld E, Schenker JG, Shushan A, Fishman A, Cohen I, Vagman I, Lunenfeld B (2006) Infertility, ovulation induction treatments and the incidence of breast cancer—a historical prospective cohort of Israeli women. *Breast Cancer Res Treat* 100:201–212
- Lewis JS, Craig Jordan V (2005) Selective estrogen receptor modulators (SERMs): mechanisms of anticarcinogenesis and drug resistance. *Mutat Res* 591:247–263
- Lippman M, Monaco ME, Bolan G (1977) Effects of estrone, estradiol, and estriol on hormone-responsive human breast cancer in long-term tissue culture. *Cancer Res* 37:1901–1907
- Liu H, Lee ES, Gajdos C, Pearce ST, Chen B, Osipo C, Loweth J, McKian K, De Los Reyes A, Wing L, Jordan VC (2003) Apoptotic action of 17beta-estradiol in raloxifene resistant MCF-7 cells in vitro and in vivo. *J Natl Cancer Inst* 95:1586–1597
- London SN, Young D, Caldito G, Mailhes JB (2000) Clomiphene citrate-induced perturbations during meiotic

- maturation and cytogenetic abnormalities in mouse oocytes in vivo and in vitro. *Fertil Steril* 73:620–626
- Magriples U, Naftolin F, Schwartz P, Carcangiu ML (1993) High grade endometrial carcinoma in tamoxifen-treated breast cancer patients. *J Clin Oncol* 11:485–490
- Mani C, Kupfer D (1991) Cytochrome P-450-mediated activation and irreversible binding of the antiestrogen tamoxifen to proteins in rat and human liver: possible involvement of flavin-containing monooxygenases in tamoxifen activation. *Cancer Res* 51:6052–6058
- Mannerström M, Zorn-Kruppa M, Diehl H, Engelke M, Toimela T, Mäenpää Huhtala H, Uusitalo AH, Salminen L, Pappas P, Marselos M, Mäntylä M, Mäntylä E, Tähti H (2002) Evaluation of the cytotoxicity of selected systemic and intravitreally dosed drugs in the cultures of human retinal pigment epithelial cell line and of pig primary retinal pigment epithelial cells. *Toxicol In Vitro* 16:193–200
- Martikainen H, Ronnberg L, Roukonen A, Kauppila A (2006) Effect of clomiphene citrate on prolactin and gonadotropin release during GnRH-analog treatment. *Theriogenology* 66:1560–1567
- Martino S, Disch D, Dowsett SA, Keech CA, Merhsion JL (2005) Safety assessment of raloxifene over eight years in a clinical trial setting. *Curr Med Res Opin* 21:1441–1452
- Maurici D, Aardema M, Corvi R, Kleber M, Krul C, Laurent C, Loprieno N, Pasanen M, Pfuhrer S, Phillips B, Sabbioni E, Sanner T, Vanparys P. Establishment of timetables for the phasing out of animal experiments for Cosmetics. [http://ec.europa.eu/consumers/sectors/cosmetics/files/doc/antest/\(5\)_chapter_3/7_genotox-mutagen_en.pdf](http://ec.europa.eu/consumers/sectors/cosmetics/files/doc/antest/(5)_chapter_3/7_genotox-mutagen_en.pdf)
- Ohnishi T, Ohashi Y, Amano I, Nozu K (1986) An ovulation inducing agent containing clomiphene citrate causes DNA-strand breaks without SOS responses in *Escherichia coli*. *Mutat Res* 165:57–61
- Okuboa T, Nagaia F, Ushiyama K, Yokoyama Y, Ozawac S, Kanoc K, Tomitad S, Kubod H, Kanoa I (1998) DNA cleavage and 8-hydroxydeoxyguanosine formation caused by tamoxifen derivatives in vitro. *Cancer Lett* 122:9–15
- Pathak DN, Bodell WJ (1994) DNA adduct formation by tamoxifen with rat and human liver microsomal activation systems. *Carcinogenesis* 15:529–532
- Philips DH, Carmichael PL, Hower A, Cole KJ, Poon GK (1994) Alpha-hydroxytamoxifen, a metabolite of tamoxifen with exceptionally high DNA-binding activity in rat hepatocytes. *Cancer Res* 54:5518–5522
- Phillips DH (2001) Understanding the genotoxicity of tamoxifen? *Carcinogenesis* 22:839–849
- Rajaniemi H, Koskinen M, Mantyla E, Hemminki K (1998a) DNA binding of tamoxifen and its analogues: identification of the tamoxifen-DNA adducts in rat liver. *Toxicol Lett* 102–103:453–457
- Rajaniemi H, Mantyla E, Hemminki K (1998b) DNA adduct formation by tamoxifen and structurally-related compounds in rat liver. *Chem Biol Interact* 113:145–159
- Randal JM, Templeton A (1991) The effects of clomiphene citrate upon ovulation and endocrinology when administered to patients with unexplained infertility. *Hum Reprod* 6:659–664
- Robertson JFR (2004) Selective oestrogen receptor modulators/new antioestrogens: a clinical perspective. *Cancer Treat Rev* 30:695–706
- Rodrigues DM Jr, dos Santos AR, Cavalcante AA, Alencar AP, Lopes-Costa PV, Silva BB (2012) Assessment of Micro-nucleus frequency in the peripheral blood of female rats in persistent estrus treated with selective estrogen receptor modulators. *Environ Mol Mutagen* 53:51–54
- Roy D, Liehr JG (1999) Estrogen, DNA damage and mutations. *Mutat Res* 424:107–115
- Sargent LM, Dragan YP, Bahnub N, Wiley JE, Sattler CA, Schroeder P, Sattler GL, Jordan VC, Pitot HC (1994) Tamoxifen induces hepatic aneuploidy and mitotic spindle disruption after a single in vivo administration to female Sprague-Dawley rats. *Cancer Res* 54:3357–3360
- Sargent LM, Dragan YP, Sattler C, Bahnub N, Sattler G, Martin P, Cisneros A, Mann J, Thorgerisson S, Jordan VC, Pitot HC (1996) Induction of hepatic aneuploidy in vivo tamoxifen, toremifene and idoxifene in female Sprague-Dawley rats. *Carcinogenesis* 17:1051–1056
- Seoud MAF, Johnson J, Weed JC Jr (1993) Gynecologic tumors in tamoxifen-treated women with breast cancer. *Obstet Gynecol* 82:165–169
- Singh MN, Stringfellow HF, Paraskevaidis E, Hirsch PL, Martin FL (2007) Tamoxifen: important considerations of a multi-functional compound with organ-specific properties. *Cancer Treat Rev* 33:91–100
- Styles JA, Davies A, Davies R, White INH, Smith LL (1997) Clastogenic and aneugenic effects of tamoxifen and some of its analogues in hepatocytes from dosed rats and in human lymphoblastoid cells transfected with human P450 cDNAs (MCL-5 cells). *Carcinogenesis* 18:303–313
- Tucker MJ, Adam HK, Patterson JS (1984) Tamoxifen. In: Laurence DR, McLean AEM, Weatherall M (eds) *Safety testing of new drugs*. Academic Press, New York, pp 125–161
- Umamoto A, Lin CX, Ueyama Y, Komaki K, Santosh Laxmi YR, Shibutani S (2006) Absence of DNA adduct in the leukocytes from breast cancer patients treated with toremifene. *Chem Res Toxicol* 19:421–425
- van Leeuwen FE, Benraadt J, Coebergh JW, Kiemeny LA, Gimbrère CH, Otter R, Schouten LJ, Damhuis RA, Bontenbal M, Diepenhorst FW (1994) Risk of endometrial cancer after tamoxifen treatment of breast cancer. *Lancet* 343:448–452
- Vijayalaxmi KK, Rai SP (1996) Studies on the genotoxicity of tamoxifen citrate in mouse bone marrow cells. *Mutat Res* 368:109–114
- White INH (1999) The tamoxifen dilemma. *Carcinogenesis* 20:1153–1160
- White INH, De Matteis F, Davies A, Smith LL, Crofton-Sleigh C, Venitt S, Hower A, Phillips DH (1992) Genotoxic potential of tamoxifen and analogues in female Fischer F344/n rats, DBA/2 and C57BL/6 mice and in human MCL-5 cells. *Carcinogenesis* 13:2197–2203
- Williams GM, Jeffrey AM (1997) Safety assessment of tamoxifen and toremifene. *Oncology* 4:41–47
- Williams GM, Ross PM, Jeffrey AM, Karlsson S (1998) Genotoxicity studies with the antiestrogen toremifene. *Drug Chem Toxicol* 21:449–476
- Wilson AS, Tingle MD, Kelly MD, Park BK (1995) Evaluation of the generation of genotoxic and cytotoxic metabolites of benzo[a]pyrene, aflatoxin B1, naphthalene and tamoxifen using human liver microsomes and human lymphocytes. *Hum Exp Toxicol* 14:507–515

- Wiseman LR, Goa KL (1997) Toremifene, a review of its pharmacological properties and clinical efficacy in the management of advanced breast cancer. *Drugs* 54:141–160
- Yılmaz S, Ünal F, Yılmaz E, Yüzbaşıoğlu D, Erkal İlhan S (2014) Evaluation of the genotoxicity of clomiphene citrate. *Mutat Res* 759:21–27
- Zambrano MA, Targa HJ, Nazareth Rabello-Gay M (1982) Evaluation of the action of clomiphene citrate on Mouse chromosomes by the metaphase and micronucleus tests. *Rev Brasil Genet* 2:339–344