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# DNA Damage Caused by Bisphenol A and Estradiol through Estrogenic Activity

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**Evidence exists that raises concern about genotoxic effects induced by estrogen: oxidative stress caused by estrogen-derived oxidants, DNA adducts formed by estrogen metabolites and estrogen-induced chromosomal aberration. Estrogen receptors (ER) participate in some of these genotoxic effects by estrogen. In this study, we showed the effects of bisphenol A (BPA), an endocrine-disrupting chemical eliciting weak estrogenic activity, and of 17$\beta$-estradiol (E2), on DNA damage in ER-positive MCF-7 cells by Comet assay. Higher concentrations of BPA, more than 1000 times of E2, were needed to induce the same levels of effects by E2. Immunofluorescence microscopy showed that $\gamma$H2AX, an early marker of DNA breaks, increased after treatment with E2 or BPA in MCF-7 cells. $\gamma$H2AX foci colocalized with Bloom helicase, which is considered to be responsible for the repair of DNA damage after treatment with E2 or BPA. Interestingly, DNA damage was not as severe in ER-negative MDA-MB-231 cells as in MCF-7 cells. The ER antagonist ICI182780 blocked E2 and BPA genotoxic effects on MCF-7 cells. These results together suggest that BPA causes genotoxicity ER dependently in the same way as E2.**

**Key words** bisphenol A; 17$\beta$-estradiol; endocrine-disrupting chemical; DNA damage; Bloom helicase

Epidemiological studies and animal experiments have shown carcinogenic properties of estrogen. Studies to clarify the molecular mechanisms of carcinogenesis by estrogen suggest that estrogen causes carcinogenic effects by combined genotoxicity and stimulation of cell proliferation.[1—3] Estrogen causes DNA damage by estrogen-derived oxidants,[4,5] DNA adducts formed by estrogen metabolites[5,6] and formation of micronuclei.[7,8] Recent studies strongly suggest that DNA damage induced by estrogen is dependent on estrogen receptors (ER): the ER antagonist tamoxifen inhibits E2 effects in ER-positive MCF-7 cells, but not in ER-negative MDA-MB-231 cells.[4,9] Detoxifying enzyme activity markedly decreases by treatment with 17$\beta$-estradiol (E2) in MCF-7 cells, leading to increased susceptibility of cells to DNA damage, but E2 has no effect on detoxifying enzyme activity in MDA-MB-231 cells.[4]

Bisphenol A (BPA) was first shown to be estrogenic in 1938 in ovariectomized rats[10] and later in MCF-7 human breast cancer cell culture assay.[11] BPA is an endocrine-disrupting chemical and has a weak affinity for ER, estimated at about 1/1000 of E2,[12] and its additional estrogenic effects on the hormonal homeostatic system has recently received much attention.

We also studied proteins involved in the repair of DNA damage induced by E2 and BPA. Histone H2AX (H2AFX) is responsible for maintaining genomic stability by recognizing DNA double-strand breaks.[13] At the sites of stalled replication forks, H2AX is phosphorylated to $\gamma$H2AX, which forms foci[14] that appear immediately after DNA damage and recruit proteins responsible for repair of DNA damage, including Bloom helicase (BLM),[14,15] the product of BLM that is the causative gene of Bloom syndrome, an autosomal recessive genetic disorder.[16,17] Clinical features of patients having Bloom syndrome include growth retardation, immunodeficiency, male infertility but not female infertility, and a high incidence of cancers. Cells from Bloom syndrome patients show a high frequency of sister chromatid exchange.[18,19] BLM responds to DNA damage and accumulates at the site of DNA double-strand breaks and physically interacts with $\gamma$H2AX.[15] In this study we investigated the effects of E2 and BPA on DNA damage in ER-positive MCF-7 and ER-negative MDA-MB-231 cells, both of which are derived from adenocarcinomas; these effects were assessed by alkaline single cell electrophoresis (Comet assay). We also investigated colocalization of $\gamma$H2AX and BLM at sites of DNA damage.

## MATERIALS AND METHODS

**Chemicals** E2 and BPA were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka). Estrogen receptor antagonist ICI182780 was obtained from TOCRIS (Ellisville, MO, U.S.A.).

**Cells, Cell Culture and Chemical Treatment** MCF-7 cells and MDA-MB-231 cells were obtained from the American Type Cell Culture (Bethesda, MA, U.S.A.). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 50 $\mu$g/ml gentamycin (both from Sigma-Aldrich, St. Louis, MO, U.S.A.) in a humidified atmosphere under 5% $CO_2$ at 37 °C. For all experiments cells were transferred to phenol red-free DMEM supplemented with 10% charcoal-dextran-stripped FBS (Hyclone, Logan, UT, U.S.A.) for 48 h before use to avoid hormonal effects, including by estrogen in FBS. Chemical treatments continued for 1, 3 or 24 h at the indicated concentrations. Pre-treatment with ICI182780 was done for 1 h and then by E2 or BPA treatments. Chemicals were solubilized in ethanol and the final concentration of ethanol in the culture was 0.1%. A control culture was exposed to a culture medium containing 0.1% ethanol.

**Colorimetric Assay of Cell Number by WST-8 Method** The WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt)

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method was used for assessment of cell number. According to the manufacturer's protocol (Nacalai Tesque, Kyoto), WST-8 solution was added to the culture, and then cells were incubated for another 4 h. The absorbance was measured at 450 nm by using a spectrophotometer (ARVO MX, PerkinElmer, Boston, MA, U.S.A.) with a reference wavelengh of 620 nm.

**Comet Assay** To detect DNA double-strand breaks in a single cell by using Comet assay, alkaline lysis and then alkaline gel electrophoresis were used.[20] Briefly, cells were incubated with various concentrations of E2 or BPA up to 24 h. The cells were treated with trypsin to detach cells from the dish and from each other, and then they were suspended in phosphate-buffered saline (PBS) and were mixed with a 10-fold volume of 1% low-melting-point agarose (FMC Bioproducts, Rockland, ME, U.S.A.). Aliquots (75 $\mu$l) of the cell suspension were layered on a fully frosted glass slide (Matsunami, Osaka) pre-coated with 1% agarose. The gel was covered with a cover slip and was incubated at 4 °C for 20 min. The cover slips were removed and the slides were immersed in a lysis solution containing 2.5 M NaCl, 100 mM ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA) at pH 10, 10 mM Tris, 1% lauryl sarcosinate and 1% Triton X-100 for 1 h at 4 °C. The slides were transferred to an alkali solution (300 mM NaOH, 1 mM EDTA, pH >13) at 4 °C for an additional 25 min, and then electrophoresis was done in the fresh alkali electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH >13) at 20 V for 25 min at 4 °C. The cells were neutralized with 400 mM Tris–HCl at pH 7.5 and were fixed in 70% ethanol for 10 min at room temperature. The gel was dried, and the DNA was stained with SYBR Green (Trevigrn, Gaitherburg, MD, U.S.A.). All processes were done under dimmed light to avoid damage by UV. Comet formation of cells was observed at ×400 magnification by using a fluorescence microscopy (Fluoview, Olympus, Tokyo) and the Comet tail length (CTL) was measured for 30 cells (10 cells from each of three slides). Statistical analysis of the CTL values between treated and control groups was done by using Dunnett's test.

**Immunofluorescence Microscopy** Subnuclear localization of $\gamma$H2AX and BLM proteins was investigated by using confocal immunofluorescence microscopy (Fluoview, Olympus, Tokyo). Cells were grown to subconfluence on a chamber slide (BD Falcon, Bedford, MA, U.S.A.) in the presence or absence of E2 or BPA. They were fixed with 4% formaldehyde for 10 min at room temperature and were washed three times with PBS containing 0.05% Tween 20. The cells were permeabilized with 0.1% Triton X-100 in PBS for 5 min, and then were blocked with 3% skim milk in PBS for 30 min at room temperature. The following primary antibodies were used: mouse anti-$\gamma$H2AX monoclonal antibody (Upstate, Charlottesville, VA, U.S.A.) and rabbit anti-BLM polyclonal antibody (Abcam, Cambridge, U.K.). Anti-mouse IgG labeled with Alexa Fluor 594 and anti-rabbit IgG labeled with Alexa Fluor 488 (both from Molecular Probes, Eugene, OR) were used as secondary antibodies. Cells were mounted under cover slips on glass slides in DAKO fluorescent mounting medium (DAKO, Glostrup, Denmark). The detailed procedure was described previously.[21]

## RESULTS

**Genotoxic Effect of E2 and BPA** DNA damage in MCF-7 cells was assessed by measuring the CTL. E2 was added at concentrations from $10^{-9}$ to $10^{-7}$ M, which induced Comet formation after 3 h, after which the CTL increased dose dependently (Table 1). Because physiological concentrations of E2 in the blood are between pg/ml and ng/ml ($10^{-10}$ to $10^{-8}$ M),[22] the significant effective dose of E2 that induced Comet formation is assumed to be within physiological concentrations. A similar effect to induce Comet formation was observed with BPA, but the concentrations needed to induce similar levels of CTL were much higher (Table 1). BPA is generally used in the manufacture of polycarbonate, and elicits weak estrogenic activity[23]: the activity of BPA at concentration $10^{-6}$ M is almost equivalent to the activity of E2 at concentration $10^{-8}$ M.[11,24] Thus, the observation of the effective concentrations of BPA ($10^{-6}$ to $10^{-4}$ M) is consistent with the difference in ER affinity previously reported.[11,24] Notably, effective concentrations of BPA that induce genotoxicity did not affect the viability of MCF-7 cells, indicating that genotoxicity was not due to cytotoxicity of BPA. Figure 1 shows typical Comet formations elicited by E2 and BPA. MCF-7 cells without treatment did not show Comet tail. The Comet assay was done at 1, 3 and 24 h after treatment with $10^{-7}$ M E2 or $10^{-4}$ M BPA, and a significant increase in CTL was detectable at 3 h after treatment with E2 and at 1 h after treatment with BPA (Table 2). The increased levels of CTL by treatment with E2 or BPA were remained after the 24-h treatment (Table 2). However, the ER-negative MDA-MB-231 cells were less sensitive to DNA damage by E2 or BPA: $10^{-7}$ M E2 slightly increased CTL in MDA-MB-231 cells at 3 h, but did not affect CTL at 24 h after treatment (Table 3), nor at ten times higher concentration of E2 at 24 h after treatment (data not shown). BPA at concentration $10^{-4}$ M slightly increased CTL in MDA-MB-231 cells at 3 and 24 h after treatment, but its effect was much weaker compared with MCF-7 cells (Table 2). These results indicate that ER-positive MCF-7 cells have much higher susceptibility than ER-negative MDA-MB-231 cells and support the idea that ER participates in genotoxicity by E2 or BPA.

**Further Evidence of ER Participation in Genotoxicity by E2 or BPA** To investigate further if ER participate in DNA damage by E2 or BPA, the effect of ER antagonist ICI182780 on the effect of E2 or BPA to induce genotoxicity was studied. MCF-7 cells were pre-treated with $10^{-6}$ M

Table 1. Effect of E2 and BPA on Comet Formation in MCF-7 Cells after 3 h Treatment

| Treatment | Concentration (M) | Cell number (%)[a] | Comet tail length ($\mu$m)[b] |
|---|---|---|---|
| Control (0.1% ethanol) | | 100 | 12.80±2.00 |
| E2 | $10^{-11}$ | 105 | 15.15±3.76 |
| | $10^{-9}$ | 103 | 22.61±9.89** |
| | $10^{-7}$ | 101 | 28.21±9.70** |
| Control (0.1% ethanol) | | 100 | 11.54±2.78 |
| BPA | $10^{-8}$ | 104 | 15.94±6.95 |
| | $10^{-6}$ | 93 | 20.73±8.16** |
| | $10^{-4}$ | 96 | 29.47±11.69** |

*a*) Percentage of control as assessed by WST-8 assay. *b*) Mean±S.D., 30 cells. ** $p<0.01$.

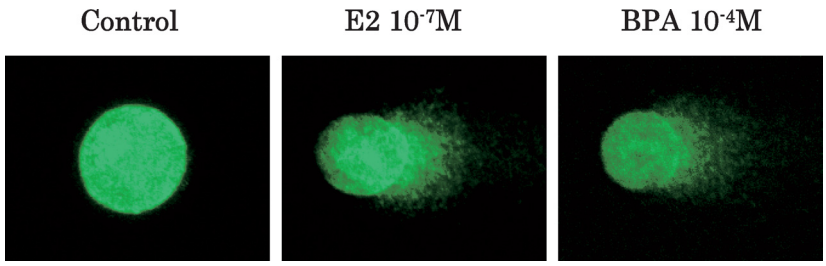


Fig. 1. Comet Formation Induced by E2 and BPA

MCF-7 cells treated with $10^{-7}$ M E2 or $10^{-4}$ M BPA for 3 h show typical tails.

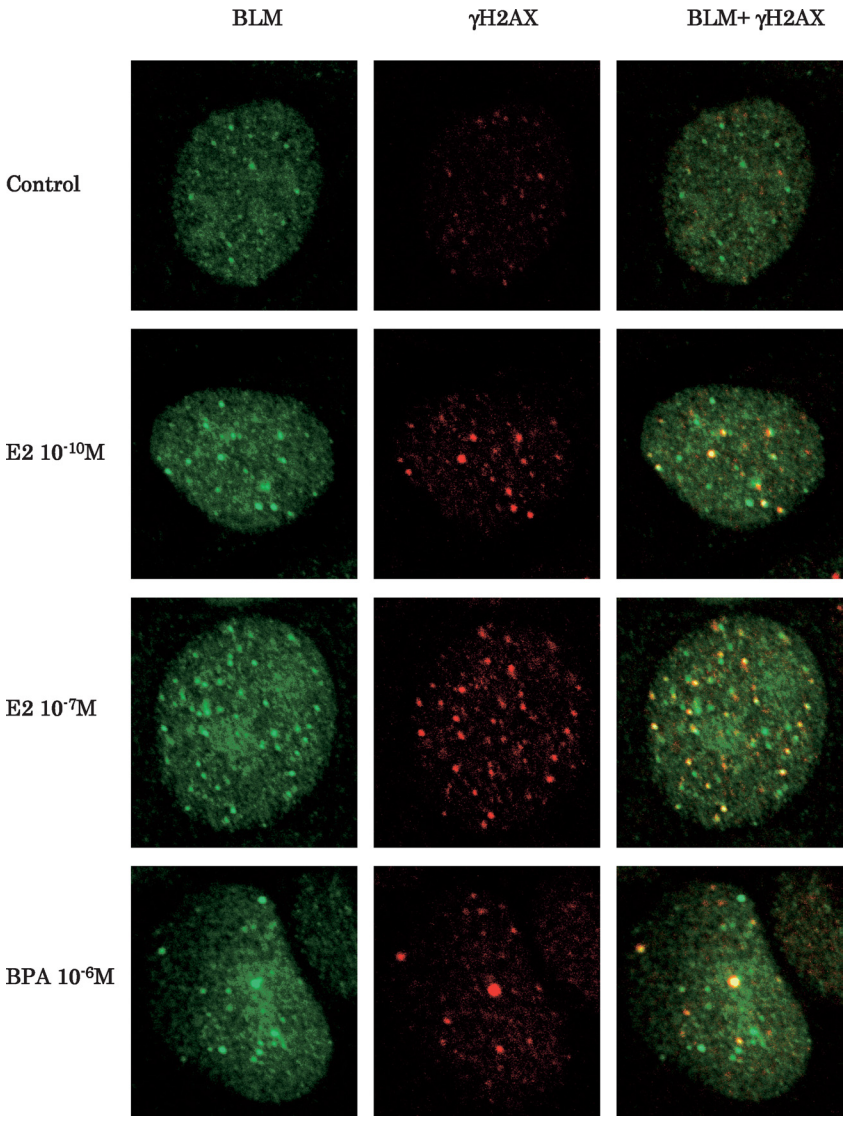


Fig. 2. Localization of BLM with γH2AX after DNA Damage Induced by E2 and BPA

MCF-7 cells treated with $10^{-10}$ and $10^{-7}$ M E2 or $10^{-6}$ M BPA for 3 h, were simultaneously stained with anti-BLM and anti-γH2AX antibodies. BLM and γH2AX were stained green and red, respectively. Colocalization of BLM and γH2AX were stained yellow.

ICI182780 for 1 h and then were treated with $10^{-7}$ M E2 or $10^{-6}$ and $10^{-4}$ M BPA for 3 h. The pre-treatment with ICI182780 antagonized the genotoxic effect by E2 or BPA, and an increase in CTL by E2 or BPA was not observed in the presence of ICI182780 (Table 4). These results also strongly support the idea that ER participate in the genotoxic effect by E2 or BPA.

**Replication Stress after Treatment with E2 or BPA** Histone H2AX has been implicated in the maintenance of genomic stability by participating in the repair of DNA damage.[14,15] H2AX is phosphorylated to γH2AX, which then forms foci in response to DNA double-strand breaks resulting in replication arrest in cells.[13] γH2AX foci are formed rapidly in response to DNA damage.[14] In this study, 10—20 γH2AX foci appeared in the MCF-7 nucleus at 3 h after treatment with E2 or BPA (Fig. 2). Fluorescence signals of

Table 2. Time-Course Analysis of DNA Damage Induced by E2 and BPA in MCF-7 Cells

| Treatment | Concentration (M) | 1 h | | 3 h | | 24 h | |
|---|---|---|---|---|---|---|---|
| | | Cell number (%)[a] | Comet tail length ($\mu$m)[b] | Cell number (%)[a] | Comet tail length ($\mu$m)[b] | Cell number (%)[a] | Comet tail length ($\mu$m)[b] |
| Control (0.1% ethanol) | | 100 | 14.70±7.32 | 100 | 11.30±2.95 | 100 | 12.99±2.33 |
| E2 | $10^{-7}$ | 100 | 17.69±6.60 | 101 | 41.79±14.40** | 106 | 37.33±12.87** |
| BPA | $10^{-4}$ | 97 | 29.77±34.46* | 96 | 37.67±11.31** | 109 | 43.91±19.16** |

*a*) Percentage of control as assessed by WST-8 assay. *b*) Mean±S.D., 30 cells. * $p<0.05$, ** $p<0.01$.

Table 3. Effect of E2 and BPA on Comet Formation in ER-Negative MDA-MB-231 Cells

| Treatment | Concentration (M) | 3 h | | 24 h | |
|---|---|---|---|---|---|
| | | Cell number (%)[a] | Comet tail length ($\mu$m)[b] | Cell number (%)[a] | Comet tail length ($\mu$m)[b] |
| Control (0.1% ethanol) | | 100 | 7.03±2.49 | 100 | 9.15±2.15 |
| E2 | $10^{-7}$ | 99 | 9.34±2.44* | 92 | 9.99±2.26 |
| BPA | $10^{-4}$ | 96 | 8.97±3.69* | 94 | 10.63±2.22* |

*a*) Percentage of control as assessed by WST-8 assay. *b*) Mean±S.D., 30 cells. * $p<0.05$.

Table 4. DNA Damage Induced by E2 and BPA in the Presence of ICI182780 in MCF-7 Cells

| Treatment | Concentration (M) | Cell number (%)[a] | Comet tail length ($\mu$m)[b] |
|---|---|---|---|
| Control (0.1% ethanol) | | 100 | 3.26±3.10 |
| E2 | $10^{-7}$ | 101 | 25.77±12.84** |
| ICI | $10^{-6}$ | 100 | 6.21±2.39 |
| ICI/E2 | $10^{-6}/10^{-7}$ | 90 | 6.52±2.51 |
| Control (0.1% ethanol) | | 100 | 9.48±2.51 |
| BPA | $10^{-6}$ | 101 | 26.41±6.62** |
| ICI | $10^{-6}$ | 96 | 10.33±2.57 |
| ICI/BPA | $10^{-6}/10^{-6}$ | 102 | 11.13±3.19 |
| Control (0.1% ethanol) | | 100 | 11.12±3.31 |
| BPA | $10^{-4}$ | 96 | 27.42±6.74** |
| ICI | $10^{-6}$ | 90 | 12.83±4.66 |
| ICI/BPA | $10^{-6}/10^{-4}$ | 92 | 11.46±5.58 |

*a*) Percentage of control as assessed by WST-8 assay. *b*) Mean±S.D., 30 cells. ICI/E2: pre-treatment with ICI182780 followed by E2 treatment. ICI/BPA: pre-treatment with ICI182780 followed by BPA treatment. ** $p<0.01$.

$\gamma$H2AX foci intensified as the concentration of E2 increased, but the foci remained indistinct in untreated MCF-7 cells. These results are consistent with the results by Comet assay that E2 and BPA induced DNA double-strand breaks. $\gamma$H2AX foci were indistinct in ER-negative MDA-MB-231 cells treated with E2 or BPA (data not shown). By using anti-BLM antibody BLM partially colocalized with $\gamma$H2AX foci, suggesting that part of BLM was associated with damaged DNA sites. BPA treatment produced a similar result. Notably, large foci colocalized with BLM and $\gamma$H2AX, which were shown by yellow staining and were obvious in cells treated with E2 or BPA but not in untreated control cells. These results suggest that DNA double-strand breaks caused by E2 or BPA stimulate formation of $\gamma$H2AX foci and accumulate BLM in the foci.

## DISCUSSION

Although genotoxic effects of E2 are ER-dependent, the sensitivity of ER-negative cells to E2 effects is inconsistent: E2 metabolites are genotoxic in ER-negative MDA-MB-231 cells[5] and physiological doses of E2 induce oxidative DNA damage in MDA-MB-231 cells.[9] However, E2 induces micronuclei formation in ER-positive tumor cells from breast and ovary, but not in ER-negative cells.[8,25] In this study, we showed: 1) E2 or BPA produced statistically significant genotoxic effects in ER-positive MCF-7 cells, but much less genotoxic effects, if any, in MDA-MB-231 cells, 2) ER antagonist ICI182780 weakened E2 and BPA genotoxic effects in MCF-7 cells and 3) E2 stimulated formation of $\gamma$H2AX foci colocalized with BLM. These results strongly support the idea that genotoxic effects of E2 are mediated by ER. Our results and conclusion are supported by the evidence that estrogen-induced DNA damage is inhibited by the estrogen receptor antagonist tamoxifen.[8,9,25] Also, E2 downregulates detoxifying enzyme activity ER dependently.[4] And, Fischer *et al.*[8] and Stopper *et al.*[25] suggest DNA damage may be due to an overriding checkpoint under ER-dependent cell proliferation induced by hormone.

We conclusively showed, we believe for the first time, that BPA had essentially similar effects as E2 to cause DNA damage depending on ER in MCF-7 cells, although higher concentrations of BPA were needed. Lee *et al.*[26] observed genotoxicity of BPA by using Comet assay in mouse lymphoma cells, but they concluded that the effect was false positive due to cell death, because effective doses of $4\times10^{-6}$—$4\times10^{-4}$ M BPA were cytotoxic. In our study, BPA at doses of $10^{-6}$—$10^{-4}$ M were genotoxic in MCF-7 cells but were not cytotoxic, excluding the possibility that genotoxicity was due to cytotoxicity. Interestingly, BPA administration reduces the activity of detoxifying enzymes, including superoxide dismutase, glutathine peroxidase and catalase, in mouse tissue,[27] consistent with E2 markedly suppressing enzymes to metabolize oxidative products in MCF-7 cells.[4] To cause DNA damage, BPA, an endocrine-disrupting chemical, needed higher concentrations than the levels of BPA detected in various kinds of human biological fluids contaminated with this compound.[28] Studies of the biological fate of BPA by using animal tests have shown that most radioactivity is

recovered in urine and feces at about 7 d after administration of $^{14}$C- BPA in rats.[29—31] These observations suggest BPA is not likely to accumulate in the body. Overall, genotoxicity of BPA may not be serious.

H2AX is rapidly phosphorylated to γH2AX, which forms foci at the sites of DNA double-strand breaks. BLM at γH2AX sites and is considered to interact with the DNA damage response protein 53BP1 and to participate in DNA repair processes.[15] Our preliminary study (Iso *et al.*, unpublished data) showed that damage induced by E2 and BPA was restored reversibly in MCF-7 cells: when cells were cultured for 24 h in the absence of E2 or BPA after 24 h-treatment of cells with E2 or BPA, few Comet forming cells were observed. Thus, the integrity of DNA structure may be recovered, probably by a system to stabilize the genome, including DNA repair enzymes such as BLM.

To sum up, our findings contribute to show genotoxicity of estrogenic agents, including BPA, ER dependently, and whether genomic instability induced by estrogenic agents can be overcome in a DNA repair system will be of further interest.

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## REFERENCES

1) Bhat H. K., Calaf G., Hei T. K., Loya T., Vadgama J. V., *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 3913—3918 (2003).
2) Liehr J. G., *Endocr. Rev.*, **21**, 40—54 (2000).
3) Cavalieri E., Frenkel K., Liehr J. G., Rogan E., Roy D., *J. Natl. Cancer Inst. Monogr.*, **27**, 75—93 (2000).
4) Mobley J. A., Brueggemeier R. W., *Carcinogenesis*, **25**, 3—9 (2004).
5) Chen Y., Liu X., Pisha E., Constantinou A. I., Hua Y., Shen L., van Breemen R. B., Elguindi E. C., Blond S. Y., Zhang F., Bolton J. L., *Chem. Res. Toxicol.*, **13**, 342—350 (2000).
6) Cavalieri E. L., Li K.-M., Balu N., Saeed M., Devanesan P., Higginbotham S., Zhao J., Gross M. L., Rogan E. G., *Carcinogenesis*, **23**, 1071—1077 (2002).
7) Yared E., McMillan T. J., Martin F. L., *Mutagenesis*, **17**, 345—352 (2002).
8) Fischer W. H., Keiwan A., Schmitt E., Stopper H., *Mutagenesis*, **16**, 209—212 (2001).
9) Bianco N. R., Perry G., Smith M. A., Templeton D. J., Montano M. M., *Mol. Endocrinol.*, **17**, 1344—1355 (2003).
10) Dodds E. C., Lawson W., *Proc. R. Soc. Biol.*, **125**, 222—232 (1938).
11) Krishnan A. V., Stathis P., Permuth S. F., Tokes L., Feldman D., *Endocrinology*, **132**, 2279—2286 (1993).
12) Gould J. C., Leonard L. S., Maness S. C., Wagner B. L., Conner K., Zacharewski T., Safe S., McDonnell D. P., Gaido K. W., *Mol. Cell. Endocrinol.*, **142**, 203—214 (1998).
13) Ward I. M., Chen J., *J. Biol. Chem.*, **276**, 47759—47762 (2001).
14) Davalos A. R., Campisi J., *J. Cell Biol.*, **162**, 1197—1209 (2003).
15) Sengupta S., Robles A. I., Linke S. P., Sinogeeva N. I., Zhang R., Pedeux R., Ward I. M., Celeste A., Nussenzweig A., Chen J., Halazonetis T. D., Harris C. C., *J. Cell Biol.*, **166**, 801—813 (2004).
16) Ellis N. A., Groden J., Ye T.-Z., Straughen J., Lennon D. J., Ciocci S., Proytcheva M., German J., *Cell*, **83**, 655—666 (1995).
17) Karow J. K., Chakraverty R. K., Hickson I. D., *J. Biol. Chem.*, **272**, 30611—30614 (1997).
18) Hickson I. D., Davies S. L., Li J.-L., Levitt N. C., Mohaghegh P., North P. S., Wu L., *Biochem. Soc. Trans.*, **29**, 201—204 (2001).
19) Nicotera T. M., *Cancer Genet. Cytogenet.*, **53**, 1—13 (1991).
20) Miyamae Y., Zaizen K., Ohara K., Mine Y., Sasaki Y. F., *Mutat. Res.*, **415**, 229—235 (1998).
21) Shiratori M., Suzuki T., Itoh C., Goto M., Furuichi Y., Matsumoto T., *Oncogene*, **21**, 2447—2454 (2002).
22) Zeisler H., Jirecek S., Hohlagschwandtner M., Knofler M., Tempfer C., Livingston J. C., *Wien. Klin. Wochenschr.*, **114**, 458—461 (2002).
23) Roy D., Palangat M., Chen C.-W., Thomas R. D., Colerangle J., Atkinson A., Yan Z.-J., *J. Toxicol. Environ. Health*, **50**, 1—29 (1997).
24) Hiroi H., Tsutsumi O., Momoeda M., Takai Y., Osuga Y., Taketani Y., *Endocr. J.*, **46**, 773—778 (1999).
25) Stopper H., Schmitt E., Gregor C., Mueller S. O., Fischer W. H., *Mutagenesis*, **18**, 243—247 (2003).
26) Lee M., Kwon J., Chung M.-K., *Mutat. Res.*, **541**, 9—19 (2003).
27) Kabuto H., Hasuike S., Minagawa N., Shishibori T., *Environ. Res.*, **93**, 31—35 (2003).
28) Ikezuki Y., Tsutsumi O., Takai Y., Kamei Y., Taketani Y., *Hum. Reprod.*, **17**, 2839—2841 (2002).
29) Kurebayashi H., Betsui H., Ohno Y., *Toxicol. Sci.*, **73**, 17—25 (2003).
30) Snyder R. W., Maness S. C., Gaido K. W., Welsch F., Sumner S. C. J., Fennell T. R., *Toxicol. Appl. Pharmacol.*, **168**, 225—234 (2000).
31) Pottenger L. H., Domoradzki J. Y., Markham D. A., Hansen S. C., Cagen S. Z., Waechter Jr. J. M., *Toxicol. Sci.*, **54**, 3—18 (2000).