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Induction of Apoptosis in the Testes of SD Rats After Exposure to 2-Bromopropane

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ABSTRACT *: Exposure to 2-Bromopropane has been known to cause degeneration of male germ cells. However, the mechanism underlying this process is poorly understood. The objective of this study was to determine whether or not the exposure of male Sprague-Dawley rats to 2-BP induces apoptosis in male germ cells. Male rats (N=3 or 4 in each group) were orally administered either with the corn oil vehicle (10 ml/kg body weight) or with 2-BP (3,500 mg/kg) once a day for 3 days. The presence of apoptosis was determined by TUNEL detection in situ and by an increase in DNA fragmentation. A low spontaneous incidence of apoptosis was observed in vehicle control animals, especially in pre-meiotic germ cells of stages I-VI and stages XII-XIV of the seminiferous tubules. In 2-BP exposure rats, the incidence of apoptosis markedly increased at 4 h, reached a peak at 8 h (about 7-fold over control), and then decreased rapidly to control levels by 48 h after the last administration. Although apoptosis induced by 2-BP occurred in all stages of germ cells, it was most pronounced in spermatogonia and early spermatocytes in stages I-VI and stages XII-XIV. Taken together, our results suggest that apoptosis is involved in the toxicity of testicular germ cells resulting in oligospermia or azoospermia after exposure to 2-BP.*

Key Words *: 2-Bromopropane, Apoptosis, Seminiferous tubules, TUNEL, DNA ladder*

I. INTRODUCTION

2-Bromopropane (2-BP) has been commercially used as an alternative to freon, and industrial exposure has been associated with possible reproductive toxicity, resulting in severe anemia accompanied by amenorrhea among female workers, and azoospermia or oligospermia among male workers (Ichihara *et al.*, 1999). To date, testicular and hematopoietic toxicities of 2-BP have been noted in the clinical history of patients. In animals, 2-BP has been known to cause male reproductive toxicity including testicular atrophy with necrosis of germ cells in seminiferous tubules as determined by a 28-day repeated dose toxicity test (Yu *et al.*, 1997). Hematopoietic toxicity of 2-BP also has been confirmed in another 28-day repeated dose toxicity test (Lee *et al.*, 1998). Previous studies from our laboratory showed that 2-BP caused a specific toxicity to spermatogonia (Son *et al.*, 1997; Son *et al.*, 1998; Son *et al.*, 1999). Others also reported similar results (Omura *et al.*, 1999). But little is known about the mechanism of testicular toxicity induced by 2-BP, which warrants further investigation.

Apoptosis is a way for the body to remove damaged or unnecessary cells (Kerr *et al.*, 1972; Thompson, 1995). Cell death through apoptosis is distinct from pathological cell death or necrosis (Farber, 1994). Apoptosis is characterized morphologically by chromatin condensation, cytoplasmic shrinkage, and membrane blebbing, while necrosis involves disruption of membrane integrity, subsequent cellular swelling, and lysis (Saraste, 1999). Spontaneous cell death of spermatogenic germ cells appears to be a constant feature of normal spermatogenesis in variety of mammalian species. Male germ cell death has been found in spermatogonia spontaneously (Blanco-Rodriguez and Martinez-Garcia, 1996; Kerr, 1992) as well as in response to chemicals such as endocrine disrupters and various en-

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vironmental agents, but the mechanisms by which these induce apoptosis have not been elucidated completely. It has been previously reported that toxic chemicals such as mono-(2-ethylhexyl)phthalate, 2,5-hexanedione, ethane dimethane sulfonate, hydroxyurea, mitomycin C, cyclophosphamide, etoposide, adriamycin, 1,3-dinitrobenzene, methoxyacetic acid and phosphamidon induce apoptosis in the seminiferous tubules.

The objectives of this study were to examine whether 2-BP induces apoptosis in the male germ cell, and if so, whether the apoptosis was correlated with any particular stage of germ cell differentiation. Apoptosis was assessed by electrophoresis demonstrating a DNA ladder and by terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL) staining, an *in situ* end labeling method. This study demonstrated that 2-BP induced testicular germ cell apoptosis in a time and stage-specific pattern, and that spermatogonia and early spermatocytes were the main target cells which most susceptible to testicular toxicants.

II. MATERIALS AND METHODS

1. Animals and treatment

Male Sprague-Dawley rats, 10 weeks old, were obtained from Screening and Toxicology Research Center, Korea Research Institute of Chemical Technology, Korea. Rats were randomized by body weight and housed 2 or 3 per cage in wire cages (410×220×200 mm) with filter tops at 23±1°C, 55±5% humidity and a 12-h light/dark cycle with 150 to 300 lux. Rats received rodent chow (Jeil Feed Co., Daejeon, Korea) and water *ad libitum*. Rats were orally treated with 3,500 mg/kg/d of 2-Bromopropane[isopropyl bromide, $(CH_3)_2CHBr$: Aldrich Chemical Co., Milwankee, WI, USA] for 3 consecutive days at a total volume of 10 ml/kg body weight, and vehicle control rats received an equivalent volume of corn oil. One testis was decapsulated, snap frozen in liquid nitrogen and stored at -80°C for low molecular weight DNA isolation. The control lateral testis was fixed in Bouin's solution and embedded in paraffin, Five-micron sections were used for histopathology and quantitation of apoptotic cells using the *in situ* detection protocol described below.

2. *In situ* detection of apoptosis

The fixed testes were dehydrated through a graded series of ethanol and embedded in paraffin according to standard procedures. Paraffin sections (5 μm thick) were placed on slides pretreated with 3-aminopropyltriethoxysilane and stored at room temperature until further processing. TUNEL staining was performed for the direct immunoperoxidase detection of digoxigenine-labeled genomic DNA in Bouin's solution fixed paraffin-embedded testicular sections using an Apoptag-peroxidase kit (Intergen, Purchase, NY, USA). In brief, after deparaffinization and rehydration, tissue sections were incubated with proteinase-K (20 μg/ml) for 15 min at room temperature, washed in distilled water, and then treated with 2% hydrogen peroxide in tris buffer solution (TBS) for 10 min at room temperature to quench endogenous peroxidase activity. Subsequently, the sections were incubated in a solution of terminal deoxynucleotidyl transferase (TdT) and digoxigenin-dUTP in a humidified chamber at 37°C for 1 h and then treated with antidigoxigenin-peroxidase for 30 min at room temperature. The sections were exposed to diaminobenzidine, washed with distilled water and TBS, then counterstained with Mayer's hematoxylin (Sigma chemical Co., St. Louis, MO, USA) for 20 sec, dehydrated in 95% and 100% ethanol, cleared in xylene, and mounted with permount (Fish Scientific Co., Fair Lawn, NJ, USA). Negative staining was processed in an identical manner, except that the TdT enzyme was substituted by the same volume of distilled water. The sections were DNase-digested before treatment with TdT enzyme as positive control. To determine the stage specific of TUNEL staining in the seminiferous epithelium, the seminiferous tubules were divided into four groups, i.e. stages I-VI, VII-VIII, IX-XI and XII-XIV, according to the standards described by Russell *et al.* (Russell *et al.*, 1990). The TUNEL-stained germ cells were identified as spermatogonia and spermatocytes or as spermatids by their location in the seminiferous epithelium and by the features of neighboring cells.

3. DNA fragmentation assay

For the DNA fragmentation assay, low molecular weight DNA was isolated as described Blanchard *et*

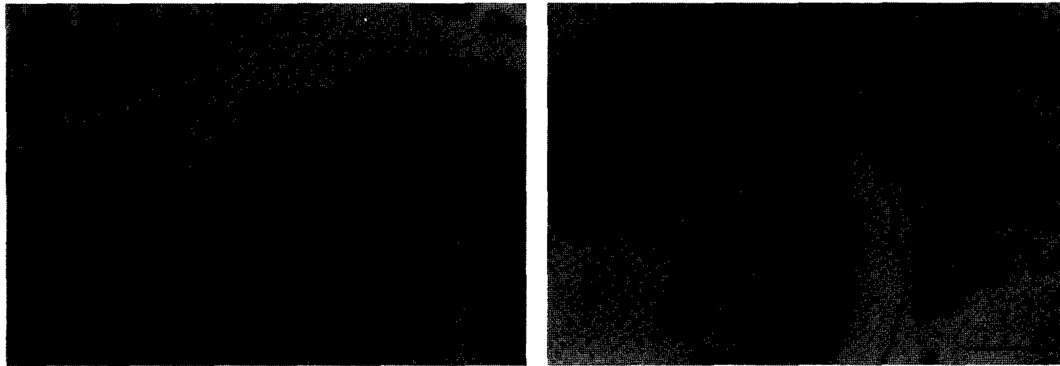

Fig. 1. Seminiferous tubules of stages I-VI (A) and XII-XIV (B) have degenerating spermatogonia and early spermatocytes 8 h after treatment in rat. H&E stain. Arrowhead: degenerating spermatogonia, arrow: degenerating spermaticytes. Bar = 50 μm.

al. (Blanchard *et al.*, 1996). After thawing the testes, homogenization buffer (20 mM EDTA, 0.5% Triton X-100, 5 mM Tris-HCl [pH 8.0]) was added. The testes were homogenized with 50 strokes in a homogenizer. To each homogenate, 12 μ*l* of 10% SDS was added and then incubated for 30 min at 65°C. Following the addition of 35 μ*l* of 8 M potassium acetate to each sample for precipitation, the collected supernatants were extracted with phenol:chloroform:isoamylethanol (25:24:1) and then chloroform: isoamyalcohol (24:1), precipitated with ethanol, and resuspended. RNA in each sample was digested with 1 μ*l* (0.5 mg/ m*l*) DNase-free RNase for 60 min at 60°C. The samples were again extracted, precipitated with ethanol, and dried. Extracted DNA electrophoresed on a 1.5% agarose gel for 30 min at 50 V. DNA was stained with ethidium bromide and visualized with an ultraviolet transilluminator. The size of the resulting DNA bands was estimated on the base of a 100-bp DNA ladder standard markers (Pharmacia, NJ, USA).

4. Statistics

The quantification of TUNEL labeled germ cells was assessed on 500 cross sectioned seminiferous tubules from each rat and expressed as numbers of TUNEL labeled germ cells in each group of stages. Statistical analyses were performed with one way analysis of variance (ANOVA) using Statistical analysis Systems and Dunnett's multiple comparisons. When only two groups were compared, the data were analyzed statistically by uncompared Students *t*-test. All results were presented as means±SD and a level of significance was taken as $P<0.05$, $P<0.01$ compared with the respective control.

III. RESULTS

1. Histopathology

Histopathology was evaluated using cross sections of paraffin-embedded testes stained with H&E stain and PAS stain. No remarkable change was evident in testes from rats treated with 3,500 mg/kg/d of 2-BP throughout this study. But only a subtle changes were observed in the seminiferous tubules of 2-BP treated rats. In stages I-VI and stages XII-XIV seminiferous tubules 8 h after the last administration with 2-BP, germ cell degeneration was most pronounced in spermatogonia and spermatocytes, showing pyknotic nuclei and eosinophilic cytoplasm (Fig. 1).

2. *In situ* detection of apoptosis

Figure 2 shows apoptotic cells in the rat testes as assessed by the TUNEL method. Apoptotic cells in the seminiferous tubules were increased in 2-BP treated animals compared with controls. The kinetics of apoptosis following 2-BP exposure are shown in Table 1. 2-BP induced a striking increase in the relative prevalence of apoptotic cells to the total germ cells in seminiferous tubules of 7 fold over the control levels. 2-BP induction of apoptosis reached a maximum level at 8 h, and then slowly declined to the control levels by 48 h. Table 2 shows that the number of seminiferous tubules containing apoptotic germ cells increased with time until 8 h and then decreased gradually to the control levels by 48 h after the last

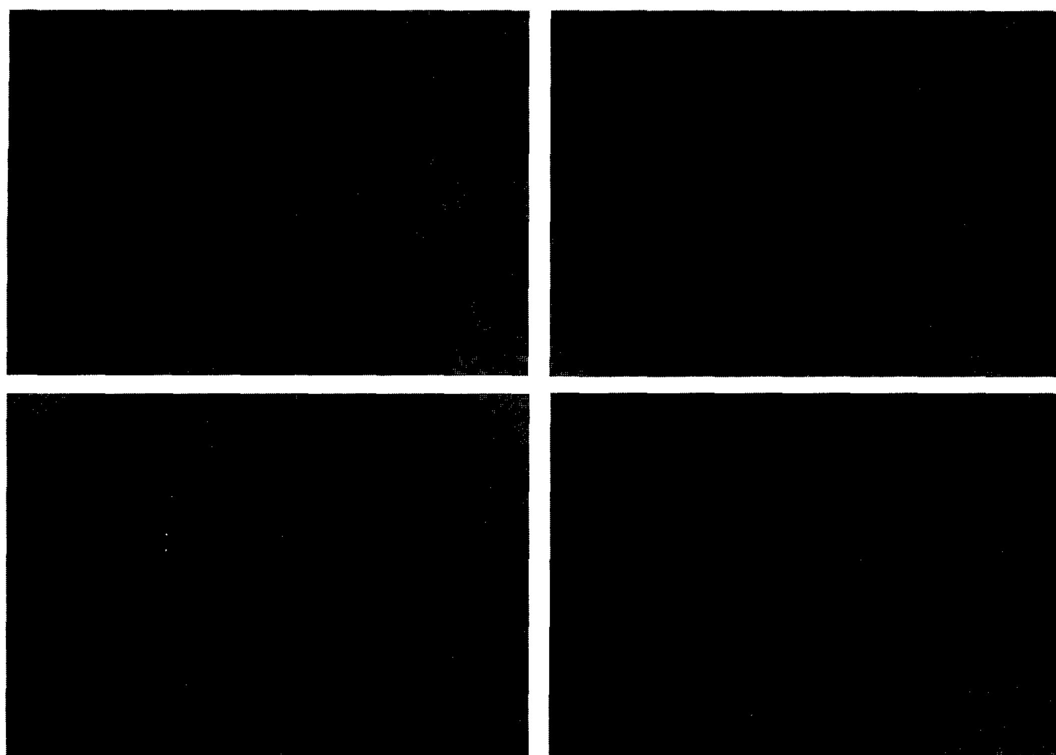

Fig. 2. Apoptotic cells in the seminiferous tubules of the rat testis 8 h after 2-BP treatment. Seminiferous tubules of stages I-VI, VII-VIII, IX-XI, and XII-XIV respectively, in 2-BP treated male rat (A-D). TUNEL stain. Arrowhead: apoptotic spermatogonia, arrow: apoptotic spermatocytes. Bar = 50 μm.

treatment. The stage and cell specificity of 2-BP induced apoptosis in spermatogonia and spermatocytes, and in spermatids in different stages of the seminiferous tubules, are shown in Table 2, Table 3 and Table 4. An increased prevalence of apoptotic cells was observed in stages I-VI and stages XII-XIV; these stages coincide with those in which apoptosis was observed to occur spontaneously in the control animals (Fig. 3).

3. DNA fragmentation analysis

Apoptosis is also characterized by low molecular weight DNA fragments that give a laddering pattern in gel electrophoresis. Therefore, to confirm the finding of *in situ* TUNEL analysis, we examined the DNA frag-

Table 1. Number of total apoptotic cells per 500 seminiferous tubules at different stage in male rats given 2-BP orally for 3 days; various hours after the last administration

Hour	Dose (mg/kg/d)	Stage			
		I-VI	VII-VIII	IX-XI	XII-XIV
4	0	17.8±7.2	0.6±0.9	0.4±0.9	37.6±20.3
	3500	195±75.5**	0.3±0.5	0.5±0.6	81.8±32.2*
8	0	15.9±6.8	0.5±0.5	1.0±0.8	35.6±18.5
	3500	289±113.2**	1.0±0.8	0.8±0.95	114.3±5.6**
12	0	17.2±8.0	0.6±0.9	0.5±0.6	36.5±15.8
	3500	110.8±68.9**	0.5±0.5	1.0±0.8	59.3±22.6*
24	0	18.2±9.5	0.0±0.0	0.6±0.5	38.2±21.2
	3500	98.8±9.5**	0.0±0.0	1.0±1.4	48.5±9.4
48	0	17.5±7.3	1.0±0.5	1.0±0.8	37.2±21.5
	3500	48.5±13.9	0.5±0.5	2.5±2.3	10.8±5.5

Values are means±SD.
* and * * indicate significant difference at $P<0.05$ and $P<0.01$ levels, respectively, when compared with the control group.

Table 2. Number of apoptotic tubule per 500 seminiferous tubules at different stage in male SD rats given 2-BP orally for 3days; various hours after the last administration

Hour	Dose (mg/kg/d)	Stage			
		I-VI	VII-VIII	IX-XI	XII-XIV
4	0	10.8±4.2	0.4±0.6	0.4±0.6	23.0±8.9
	3500	56.3±6.5**	0.3±0.5	0.5±0.6	33.3±6.5
8	0	11.3±4.6	0.3±0.5	0.5±0.6	22.0±9.9
	3500	70.3±12.4**	0.8±1.0	0.5±0.6	40.3±2.2*
12	0	9.2±2.6	0.5±0.6	0.5±0.6	21.3±9.2
	3500	40.3±14.1*	0.3±0.5	0.5±0.6	24.5±8.7
24	0	11.8±4.1	0.5±0.6	0.3±0.5	25.6±7.4
	3500	36.3±5.9*	0.0±0.0	0.3±0.5	22.5±5.1
48	0	11.5±4.4	0.3±0.5	0.5±0.6	25.0±8.8
	3500	22.5±6.8	0.3±0.5	1.5±1.3	6.5±1.7

Values are means±SD.
* and ** indicate significant difference at $P<0.05$ and $P<0.01$ levels, respectively, when compared with the control group.

Table 3. Number of apoptotic cells in different time in male rats given 2-BP orally for 3 days; various hours after the last administration

Germ cell	Termination time after the last administration				
	4 hrs	8 hrs	12 hrs	24 hrs	48 hrs
Sg&Sc[a]	273.5±53.1**	393.1±80.9**	164.2±31.4**	142.3±21.1**	50.3±6.7
St[b]	4.0±4.2	6.3±5.6	4.5±4.9	4.6±4.3	8.6±7.6
Total	277.5±57.1	399.4±85.9	168.7±36.1	146.9±25.4	58.9±13.3

Values are expressed as the apoptotic cells/500 seminiferous tubules in each group and represent the means±SD, N = 4.
[a]Sg, Spermatogonium; Sc, Spermatocyte.
[b]St, Spermatid.
* and ** indicate significant difference at $P<0.05$ and $P<0.01$ levels, respectively, when compared with the control group.

Table 4. Number of apoptotic cells in the seminiferous tubules at various stages of male SD rats administere orally with corn oil for 3 days*

Germ cell	Stages			
	I-VI	VII-VIII	IX-XI	XII-XIV
Sg&Sc[a]	16.8±6.9	0.6±0.6	0.2±0.4	37.4±18.1
St[b]	0.5±0.8	0.2±0.1	0.1±0.1	0.2±0.3
Total	17.3±7.7	0.8±0.7	0.3±0.5	37.6±18.4

Values are expressed as the apoptotic cells/500 seminiferous tubules in each group and represent the means±SD, N = 15.
[a]Sg, Spermatogonium; Sc, Spermatocyte.
[b]St, Spermatid.

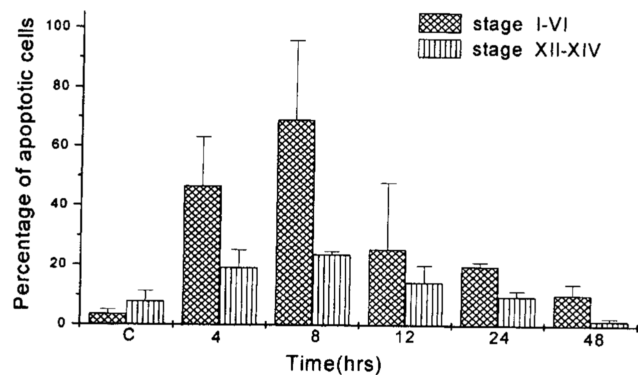

Fig. 3. Time course of the occurrence of apoptosis at stages I-VI and XII-XIV.

mentation pattern in testes of the control and 2-BP treated rats (Fig. 4). DNA ladders with an ordered pattern of multiple bands on agarose gels were evident in testes from rats euthanized 4 h, 8 h, 12 h after the last administration.

IV. DISCUSSION

A number of reports have indicated that 2-BP causes severe anemia, azoospermia or oligospermia. 2-BP induced a base-pair substitution type mutagenicity in the reverse mutation assays using *Salmonella typhimurium* TA100, whereas proving negative for both micronucleus test using experimental animal and the chromosome aberration test using chinese hamster lung cells cultured in vitro (Maeng and Yu, 1997). The present study is to investigate whether 2-BP induce the apoptosis to express its toxicity in male rat germ cells. Germ cell apoptosis was demonstrated *in situ*

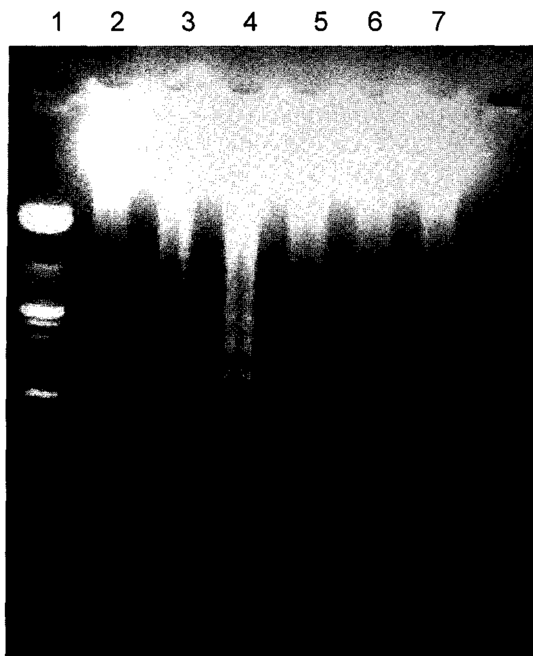

Fig. 4. Agarose gel electrophoresis (1.5%) of low molecular weight DNA isolated from male rats administration orally with 2-BP for 3 days. Lanes 1, 2, 3, 4, 5, 6and 7 represent the DNA standard, Control, 4 h, 8 h, 12 h, 24 h and 48 h after 2-BP administration respectively.

by the TUNEL method and confirmed by the appearance of low-molecular-weight DNA ladder patterns. Germ cell apoptosis increased in incidence and severity with time after 2-BP administration reaching a maximum at 8 h and then gradually declined to control levels by 48 h. 2-BP increased the frequency of TUNEL positive cells and also the number of seminiferous tubules containing affected germ cells. On the TUNEL methods, germ cells at stages I-VI appeared to be most sensitive to 2-BP induced apoptosis, and spermatogonia and early spermatocytes were the primary cell population undergoing apoptosis. A DNA fragmentation assay also demonstrated that germ cell apoptosis induced by 2-BP reaches a maximum peak at 8 h after treatment. On the gel electrophoresis for DNA ladders, a significant increase in DNA fragmentation was observed at 8 h after 2-BP treatment when TUNEL positive cells increased at after 2-BP treatment. Germ cell apoptosis has been reported to occur in response to other testicular toxicants, hormonal manipulation (Kiess and Gallaher, 1998; Brinkworth *et al.*, 1995; Woolveridge et al., 1998), cryptorchidism (Henriksens *et al.*, 1995a), radiation (Henriksens *et al.*, 1996), and heat (Lue *et al.*, 1999). Mono-(2-ethylhexyl)phthalate (Richburg and Boekelheide, 1996) and 2,5-hexanedione (Blanchard *et al.*, 1996; Richburg *et al.*, 1994; Allard and Boekeheide, 1996) is known to induce apoptosis in Sertoli cells.

Treatment with ethane dimethane sulfonate increased apoptosis, predominantly in spermatocytes and spermatids at stages VII and VIII (Nandi *et al.*, 1999). After 1, 3-nitrobenzene treatment, extensive damages of seminiferous tubules were caused by degeneration of pachytene spermatocytes at stages VII through VIII (Strandgaard and Miller, 1998). Methoxyacetic acid induced apoptosis mainly in spermatogonia preparing for mitotic divisions and spermatocytes during or after S-phase of the cell cycle (Krishnamurthy *et al.*, 1998). Etoposide caused a significant inhibition of stage-specific DNA synthesis, especially at the stages corresponding to the DNA synthesis of intermediate spermatogonia and type B spermatogonia (stages II-VI) (Hakovirta *et al.*, 1993; Sjoblom *et al.*, 1998). In this study, the number of apoptotic cells induced by 2-BP was markedly increased in spermatogonia and spermatoytes at stages I-VI. The results showed the consistency with the results reported previously concerning apoptosis induced by various chemicals. For example, treatment with hydroxyurea increased germ cell apoptosis in apoptotic tubules, and the apoptotic cells were identified as spermatogonia and spermatocytes in the region near the basement membrane of the tubules (Shin *et al.*, 1999). In cyclophosphamide treated rats, a high prevalence of apoptotic cells was found synchronously in the seminiferous tubules (Cai *et al.*, 1997). One study showed that there were at least two pathways for the induction of apoptosis in male germ cells, i. e., testosterone-dependent and testosterone-independent pathway (Henriksen *et al.*, 1995b). Testosterone is known to preferentially affect the cells at stages VII-VIII of seminiferous epithelium and to have an important effect on the conversion of step 7-8 spermatids (O'Donnell *et al.*, 1994). Therefore, it is unlikely that hormonal changes can a cause of apoptosis in testicular germ cells which was occurred after the treatment of 2-BP. 2-BP greatly increases apoptosis in rapidly proliferating tissue. Azoospermia or oligospermia may result from the apoptosis induced by 2-BP on spermatogonia and early spermatocytes which are active in DNA proliferation.

This study demonstrated that 2-BP induced testicu-

lar germ cell apoptosis in a time and stage-specific pattern, and that spermatogonia and early spermatocytes were the main target cells which most susceptible to testicular toxicants. In conclusion, our results suggest that apoptotic germ cell death is required for elimination of the affected cells, resulting in the prevention of fertility disturbance and the protection of the next generation from the transmission of damaged DNA.

REFERENCES

Allard, E.K. and Boekeheide, K. (1996): Fate of germ cell in 2,5-hexanedione induced testicular injury: II. Atrophy persists due to a reduced stem cell mass and ongoing apoptosis. *Toxicol. Appl. Pharmacol.*, **137**, 149-156.

Blanchard, K.T., Allard, E.K. and Boerkelheide, K. (1996): Fate of germ cell in 2,5-hexandione induced testicular injury: I. Apoptosis is the mechanism of germ cell death. *Toxicol. Appl. Pharmacol.*, **137**, 141-144.

Blanco-Rodriguez, J. and Martinez-Garcia, C. (1996): Spontaneous germ cell death in the testis of the adult rat takes the form of apoptosis: re-evaluation of cell types that exhibit the ability to die during spermatogenesis. *Cell Prolif.*, **29**, 13-31.

Brinkworth, M.H., Weinbauer, G.F., Schlatt, S. and Nieschlag, E. (1995): Identification of male germ cells undergoing apoptosis in adult rats. *J. Reprod Fertility*, **105**, 25-33.

Cai, L., Hales, B.F. and Robaire, B. (1997): Induction of apoptosis in the germ cells of adult male rats after exposure to cyclophosphamide. *Biol. Reprod.*, **56**, 1490-1497.

Farber, E. (1994): Programmed cell death: Necrosis versus Apoptosis. *Modern Pathol.*, **7**, 605-609.

Hakovirta, H., Parvinen, M. and Lahdetie, J. (1993): Effects of etoposide on stage-specific DNA synthesis during rat spermatogenesis. *Mutat Res.*, **301**, 189-193.

Henriksens, K., Hakovirta, H. and Parvinen, M. (1995a): *In situ* quantification of stage specific apoptosis in the rat seminiferous epithelium: effects of short-term experimental cryptorchidism. *Int. J. Androl.*, **18**, 256-262.

Henriksen, K., Hakovirta, H. and Parvinen, M. (1995b): Testosterone inhibits and induces apoptosis in rat seminiferous tubules in a stage-specific manner: *in situ* quantification in squash preparation after administration of ethane sulfonate. *Endocrinology*, **136**, 3285-3291.

Henriksens, K., Kulmala, J., Toppari, J., Mehrotra, K. and Parvinen, M. (1996): Stage specific apoptosis in the rat seminiferous epithelium: quantification of irradiation effects. *J. Androl.*, **17**, 394-401.

Ichihara, G., Ding, X., Yu, X., Wu, X., Kamijima, M., Peng, S., Jiang, X. and Takeuchi, Y. (1999): Occupational health survey on workers exposed to 2-bromopropane at low concentrations. *Am. J. Ind. Med.*, **35**, 523-531.

Kerr, J.F., Wyllie, A.H. and Currie, A.R. (1972): Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer.*, **26**, 239-257.

Kerr, J.B. (1992): Spontaneous degeneration of germ cells in rat testis: Assessment of cell types and frequency during the spermatogenic cycle. *J. Reprod Fertil.*, **95**, 825-830

Kiess, W. and Gallaher, B. (1998): Hormonal control of programmed cell death/apoptosis. *Eur. J. Endocrinol.*, **138**, 482-491

Krishnamurthy, H., Weinbauer, G.F., Aslam, H., Yeung, C.H. and Nieschlag, E. (1998): Quantification of apoptotic testicular germ cells in normal and methoxyacetic acid treated mice as determined by flow cytometry. *J. Androl.*, **19**, 710-717.

Lee, H.S., Kang, B.H., Son, H.Y., Kim, H.Y., Cho, Y.C. and Roh J.K. (1998): A 4-weeks oral toxicity study of 2-bromopropane in Sprague-Dawley male rats. *J. Toxicol. Pub. Health*, **14**, 129-141.

Lue, Y.H., Hikim, A.P.S., Swerdloff, R.S., Im, P., Taing, H.S., Bui, T., Leung, A. and Wang, C. (1999): Single exposure to heat induces stage specific germ cell apoptosis in rats: Role of intratesticular testosterone on stage specificity. *Endocrinology*, **140**, 1709-1717.

Maeng, S.H. and Yu, I.J. (1997): Mutagenicity of 2-bromopropane. *Ind. Health*, **35**, 87-95.

Nandi, S., Banerjee, P.P. and Zirkin, B.R. (1999): Germ cell apoptosis in the testes of Sprague-Dawley rats following testosterone withdrawal by ethane 1, 2-dimethanesulfonate administration: relationship to Fas?. *Biol. Reprod.*, **61**, 70-75.

O'Donnell, L., McLachlan, R.I., Wreford, N.G. and Robertson, D.M. (1994): Testosterone promotes the conversion of round spermatids between stages VII and VIII of the rat spermatogenic cycle. *Endocrinology*, **135**, 2608-2614.

Omura, M., Romero, Y., Zhao, M. and Inoue N. (1999): Histopathological evidence that spermatogonia are the target cells of 2-bromopropane. *Toxicol. Lett.*, **104**, 19-26.

Richburg, J.H. and Boekelheide, K. (1996): Mono-(2-ethylhexyl)phthalate rapidly alters both Sertoli cell vimentin filaments and germ cell apoptosis in young rat testes. *Toxicol. Appl. Pharmacol.*, **137**, 42-50.

Richburg, J.H., Redenbach, D.M. and Boerkelheide, K. (1994): Seminiferous tubule fluid secretion is a Sertoli cell microtubule dependent process inhibited by 2,5-hexanedione exposure. *Toxicol. Appl. Pharmacol.*, **128**, 302-309.

Russell, L.D., Ettlin, R.A., Sinha hikim, A.P. and Clegg, E.D. (1990): Histological and histopathological evaluation of the testis. Cache River Press: Clearwater Fl UAS.

Son, H.Y., Cho, S.W., Kim, Y.B., Ha, C.S. and Kang, B.H. (1997): Testicular lesion in the Sprague-Dawley rats treated with high dose of 2-bromopropane. *Kor. J. Vet. Pathol.*, **1**, 97-105.

Son, H.Y., Kang, B.H., Cho, S.W., Cha, S.W. and Roh, J.K. (1998): Histopathological observation and flow cytometry analysis of testicular atrophy induced by 2-bromopropane on the Sprague-Dawley rat. *J. Toxicol. Pub. Health*, **14**, 143-149.

Son, H.Y., Kim, Y.B., Kang, B.H., Cho, S.W., Ha, C.S. and Roh, J.K. (1999): Effect of 2-bromopropane on spermatogenesis in Sprague-Dawley rats. *Reprod Toxicol.*, **13,** 179-187.

Saraste, A. (1999): Morphologic criteria and detection of apoptosis. *Herz.*, **24**, 189-195.

Shin, J.H., Mori, C. and Shiota K. (1999): Involvement of germ cell apoptosis in the induction of testicular toxicity following hydroxyurea treatment. *Toxicol. Appl. Pharmacol.*, **155**, 139-149.

Sjoblom, T., West, A. and Lohdetie, J. (1998): Apoptotic response of spermatogenic cells to the germ cell mutagens etoposide, adriamycin, and diepoxybutane. *Environ Mol. Mutagen*, **31**, 133-148.

Strandgaard, C. and Miller, M.G. (1998): Germ cell apoptosis in rat testis after administration of 1,3-dinitrobenzene. *Reprod Toxicol.*, **12**, 97-103.

Thompson, C.B. (1995): Apoptosis in the pathogenesis and treatment of disease. *Science*, **267**, 1456-1462.

Woolveridge, I., Bryden, A.A.G., Taylor, M.F., George, N.J.R., Wu, F.C.W. and Morris, I.D. (1998): Apoptosis and expression of apoptotic regulators in the human testis following short and long term anti-androgen treatment. *Mol. Hum. Reprod.*, **4**, 701-707.

Yu, I.J., Chung, Y.H., Lim, C.H., Maeng, S.H., Lee, J.Y., Kim, H.Y., Lee, S.J., Kim, C.H., Kim, T.K., Lim, C.H., Park, J.S. and Moon YH. (1997): Reproductive toxicity of 2-bromopropane. *Scand J. Work Environ. Health*, **23**, 281-288.