ORIGINAL ARTICLES

Radiomimetic Toxicity of 1, 2-Dibromo-3-Chloropropane (DBCP)

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Abstract: Histopathological examination was carried out on male rats after single subcutaneous administration of 1, 2-dibromo-3-chloropropane (DBCP). DBCP produced injury in the kidney, testis, intestine, lympho-hematopoietic tissues, epididymis and liver. The renal injury consisted of proximal tubular necrosis in the outer medulla and degeneration of glomerular podocytes. Seminiferous epithelial desquamation and tubular atrophy were seen in testis. In lympho-hematopoietic tissues, there was depletion of lymphocytes as well as myeloid cells. Intestinal epithelial necrosis and rare mitosis occurred in the crypt of Lieberkühn. Hepatic and epididymal injuries were relatively mild. The lesions developed dose-dependently, the minimal effective doses appearing to be 30 mg/kg for kidney, 100 mg/kg for testis, intestine and lympho-hematopoietic tissues and 300 mg/kg for epididymis and liver. These results indicated that DBCP might be a radiomimetic toxicant.

Key words: DBCP—Histopathology—Target organs—Radiomimetic effect

INTRODUCTION

The pesticide 1,2-dibromo-3-chloropropane (DBCP) was at one time widely used as a soil fumigant. However, the use of DBCP was halted because of the incidence of infertility and testicular atrophy in pesticide workers.^{1,2,3)}

Experimental studies revealed development of stomach cancer after gastric intubation of DBCP⁴⁾ as well as nasal and lung cancer after its inhalation.^{5,6)} Acute toxicological studies showed that non-neoplastic lesions were produced in the kidney, testis, epididymis and liver of DBCP treated rats.^{7,8,9)} After inhalation of DBCP, the respiratory system was also shown to be involved.^{10,11)} On the other hand, intestinal toxicity has not been evaluated, though diarrhea was observed in rats after DBCP treatment.⁹⁾ Our previous study on rats exposed to DBCP-inhalation revealed splenic atrophy.¹¹⁾ Therefore, a detailed histopathological study on rats exposed to DBCP was performed to identify conclusively the primary target organs and to evaluate its toxic effects.

This study revealed that lympho-hematopoietic organs and intestine were also primary target organs of DBCP toxicity and that DBCP might be a radiomimetic toxicant.

MATERIALS AND METHODS

Animals: Male, 8-week-old Sprague-Dawley rats weighing 240 to 260 g were purchased from a commercial breeder (Japan Clea, Tokyo). Five or 6 animals were kept in wire-mesh cages and given pellets (Oriental Yeast, Tokyo) and tap water *ad libitum*. The animal room was controlled for temperature ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$) and light cycle (12 hr light, 12 hr dark).

DBCP treatment: Groups of rats were administered 0 (vehicle), 30, 100 or 300 mg/kg DBCP. DBCP supplied from Toyo Soda Co. was refined by distillation, collecting DBCP at 196°C , since the sample contained 10% toluene.¹¹⁾ Distilled DBCP was dissolved in olive oil (Wako Chemical, Tokyo) and injected subcutaneously (s.c.) to rats in the inguinal region at a volume of 1 ml/kg body weight.

Experimental design and pathology: Five or 6 animals from each group were sacrificed 1, 3, 7, 14 and 28 days after treatment, if not mentioned otherwise. The animals were deeply anesthetized with pentobarbital sodium (Abbot Laboratory, Illinois) and were bled from the carotid artery. Dead rats were autopsied as soon as possible. The main organs were weighed and sampled for histopathology. All tissue specimens were fixed in neutral buffered 10% formalin and embedded in paraffin. Sections were cut at $5\ \mu\text{m}$ thickness and stained with hematoxylin and eosin. Selected sections were also stained with periodic acid-Shiff (PAS), alcian blue-PAS or trichrome stain.

RESULTS

Rats treated with 300 mg/kg DBCP showed progressive body weight loss without recovery (Fig. 1), anorexia and excretion of watery stool with foul smell for 3 days after treatment. Four, 4 and 1 rats were dead 3, 5 and 7 days, respectively, after treatment. Observation of this group, therefore, ended on day 14 when 2 rats were sacrificed. Rats which received less than 100 mg/kg DBCP appeared clinically normal. Slight loss of body weight was seen 1 day after injection with 100 mg/kg, followed by considerable weight gain thereafter. No significant effect was observed on the body weight after treatment with 30 mg/kg.

In the group treated with 300 mg/kg DBCP, the kidney and adrenal gland increased in weight up to a maximum 7 days after treatment (Fig. 2). The relative liver weight increased 1 and 3 days after treatment, then it decreased on day 7 and 14 after treatment. In contrast, spleen and thymus were markedly decreased in weight, being at a minimum on day 3. Absolute testicular weight decreased progressively after DBCP treatment. No significant change in organ weight was

Fig. 1. Body weight gain after single exposure to DBCP.

Body weight at sacrifice is expressed relative to body weight immediately prior to administration of DBCP. Vehicle control (○), 30 mg/kg (△), 100 mg/kg (×) and 300 mg/kg (●).

observed after treatment with less than 100 mg/kg DBCP.

In rats treated with 300 mg/kg DBCP, histopathological findings were as follows.

Primary renal lesions appeared in the straight segment of proximal tubules in the outer medulla. On day 1, the proximal tubular epithelium of the *para recta* became necrotic and sloughed off forming granular casts in the lumen (Fig. 3A). The proximal tubular epithelium in the inner cortex had swollen cytoplasm and enlarged nuclei with margination of chromatin. Distal tubules in the outer medulla and cortex were dilated. By day 3, the proximal tubular lesions had increased in severity and extent. Focal mineralization occurred in the outer medulla. A number of eosinophilic hyaline casts were visible in most distal tubules in the inner medulla. The distal

Fig. 2. Changes in organ weight after single exposure to DBCP.

Absolute weight and that relative to body weight at sacrifice.
Vehicle control (○) and 300 mg/kg (●).

Fig. 3. Renal lesions after 300 mg/kg DBCP treatment.

(A) One day after exposure. Proximal tubular necrosis in the outer medulla. Bar=200 μ m. (B) Three days after exposure. Degeneration of glomerular podocytes with hyaline droplets (arrows). Bar=30 μ m. (C) Seven days after exposure. Bizarre giant cells (arrows) in dilated proximal tubules. Bar=50 μ m. (D) Seven days after exposure. Focal tubular dilation with sloughed epithelium. Bar=100 μ m.

tubular epithelium had enlarged basophilic cytoplasm containing PAS-positive fine granules. In addition to the tubular lesions, eosinophilic and PAS-positive hyaline droplets were detected in the cytoplasm of glomerular podocytes (Fig. 3B). Renal lesions in cases of death at 3 and 5 days after treatment were similar to those of rats sacrificed on day 3, both in quality and in quantity. Mineralization in the outer medulla was more prominent on day 5.

While considerable tubular regeneration occurred 7 days after treatment, focal tubular desquamation was still apparent with sloughed epithelial cells in the dilated lumen (Fig. 3D). Bizarre giant cells with large nuclei, 3 to 5 times normal size, appeared among the regenerative epithelium (Fig. 3C). Degenerated giant cells were frequently seen. Though less prominent, there were similar tubular lesions, focal mineralization in the outer medulla and a moderate amount of hyaline casts remaining in the papilla on day 14.

No morphological changes were apparent in the testis 1 day after treatment. By day 3, mild to severe degeneration and necrosis of seminiferous tubules appeared. Mitosis or meiosis of tubular epithelial cells was rare. Though all stages of germinal cells were involved, changes were most prominent in spermatids which showed chromatin margination. Degenerated spermatids often coalesced into multinucleated giant cells (Fig. 4B). Progressive necrosis of seminiferous tubules was prominent in 5 rats which died on day 5 or 7, with desquamation of germinal cells at all stages. In 3 out of 5 rats sacrificed on day 7, severe tubular atrophy was seen leaving a few normally appearing seminiferous tubules (Fig. 4C). Much more advanced atrophy was evident in one of the 2 cases sacrificed on day 14 (Fig. 4D).

The caput epididymis was moderately vacuolated in the epididymis of cases killed on days 1 and 3 as well as in 9 cases of death. A few necrotized cells were also seen in the degenerative epididymis. The epididymal duct contained sloughed epithelial cells with sparse sperms. Epididymal degeneration in the caput was still seen in 3 of 5 rats on day 7. The epididymis regained a normal appearance on day 14, while the tubules were moderately atrophic. Epididymal sperms were absent or low in density on day 7 or later.

In the thymus, lymphocyte necrosis in the cortex was apparent by day 1 (Fig. 5A). The pyknotic nuclei of the dead cells were phagocytized by macrophages (Fig. 5B). Lymphocyte necrosis was also observed in the medulla, while less severe.

On day 3, the thymus was extremely atrophic. Depletion of cortical thymocytes was so prominent that the corticomedullary boundary was unclear (Fig. 5C). Atrophy and severe depletion of lymphocytes were also conspicuous in cases of death on day 3 or 5. Thymic atrophy was still apparent on day 7, while considerable recovery of cortical thymocytes was observed (Fig. 5D). Macrophages with clear cytoplasm were scattered in the cortex, showing a "star in the sky" appearance (Fig. 5E). No lesions were detected in the thymus on day 14.

In the spleen, lymphocytes in the red pulp as well as in the atrophic white pulp decreased in number on day 1. Lymphocytic depletion in the spleen had progressed

Fig. 4. Testicular lesions after 300 mg/kg DBCP treatment.

(A) Vehicle control. Bar=200 μ m. (B) Three days after exposure. Severe degeneration and necrosis of tubular epithelium, forming the coalesced multinucleated giant cells. Bar=100 μ m. (C) Seven days after exposure. Tubular atrophy with a few normal-appearing tubules. Bar=200 μ m. (D) Fourteen days after exposure. Severe atrophy of tubules without germinal epithelium. Bar=200 μ m.

Fig. 5. Thymic lesions after 300 mg/kg treatment.

(A) One day after exposure. Lymphocytic necrosis in the cortex showing "moth-eaten" appearance. Bar=200 μ m. (B) Higher magnification of (A). Phagocytosis of pyknotic lymphocytes by macrophages in the cortex. Bar=40 μ m. (C) Three days after exposure. Deletion of cortical thymocytes. Corticomedullary boundary is unclear. Bar=200 μ m. (D) Seven days after exposure. "Star in the sky" appearance in the cortex. Bar=200 μ m. (E) Higher magnification of (D). Macrophages with foamy clear cytoplasm in the cortex. Bar=50 μ m.

Fig. 6. Changes in bone marrow after 300 mg/kg DBCP treatment.

(A) Vehicle control. Bar=100 μ m. (B) One day after exposure. Decrease in number of myeloid cells. Bar=100 μ m. (C) Three days after exposure. Severe deletion of myeloid cells resulting in pan-hypoplasia. Bar=100 μ m.

Fig. 7. Duodenal lesions after 300 mg/kg DBCP treatment.

(A) Vehicle control. Bar=200 μ m. (B) One day after exposure. Epithelial necrosis (arrow) in the crypt without mitotic figures. Bar=60 μ m. (C) Three days after exposure. Shortened and irregularly shaped villi with basophilic epithelium in the crypt. Bar=200 μ m. (D) Seven days after exposure. Slightly irregular but apparently normal villi. Bar=200 μ m.

by day 3. On day 7, considerable recovery was visible while atrophy of the white pulp was still apparent. Few changes were detected 14 days after treatment. Severe atrophy and lymphocytic depletion were prominent in all rats which died.

Lymphocytes were decreased in number in lymph nodes on day 1. A number of necrotized cells were present in the atrophic nodules. Depletion of lymphocytes was more severe in the atrophic nodes on day 3 as well as in all cases of death. On day 7 or later, recovery was remarkable, showing normal appearance.

In the bone marrow, myeloid cells decreased in number and mitotic figures were rare 1 day after treatment (Fig. 6B). Progressive depletion of myeloid cells resulted in panhypoplasia on day 3 and in all cases of death (Fig. 6C). Bone marrow myeloid cells showed remarkable recovery on day 7 or later, although delayed recovery was also seen in a few cases.

One day after treatment, epithelial necrosis was conspicuous in the proliferative zone of the crypt of Lieberkühn where mitosis had ceased (Fig. 7B). Such lesions were observed throughout the intestines, being most prominent in the duodenum. On day 3, irregularly shaped villi with vacuolated abnormal cells became shortened (Fig. 7C). There were active mitoses and a predominance of epithelial cells with basophilic cytoplasm in the proliferative zone of each crypt. No goblet cells could be detected. Dilatation of blood vessels and interstitial edema were apparent in the lamina propria. Ulceration of the duodenum was found in one case of death on day 5. Seven days after treatment, the intestine appeared normal with a small number of shortened or irregularly shaped villi (Fig. 7D).

Centrilobular eosinophilic necrosis or vacuolar degeneration were seen in the liver on day 1. While similar hepatic lesions were seen in all cases of death, hepatocellular changes were absent in cases sacrificed on day 3 or later.

Although no marked changes were found in the heart of sacrificed rats, interstitial edema was apparent in all dead rats. Focal myocarditis with mineralization was observed in 5 cases which died on day 5 or 7. The mineralization was more remarkable in the case of day 7 than in the 4 cases of day 5. The density of cardiac mineralization was correlated with the severity of renal mineralization in these cases.

The severity of lesions in tissues was graded from 0 to 3 according to the extent of necrosis and degeneration. As shown in Fig. 8, dose-dependent effects were apparent. In the case of acute, single-s.c. exposure, the minimal effective doses of DBCP appeared to be 30 mg/kg for kidney, 100 mg/kg for testis, lympho-hematopoietic tissues and intestine, and 300 mg/kg for liver and epididymis.

ORAGAN	GRADE OF LESION	DAYS AFTER DBCP EXPOSURE					
		1	3	5	7	14	28
KIDNEY	3	● ● ●	● ● ● ● ● ⊗ ⊗ ⊗ ⊗	⊗ ⊗ ⊗ ⊗	● ●		
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THYMUS	3	● ● ●	● ● ● ● ● ⊗ ⊗ ⊗ ⊗	⊗ ⊗ ⊗ ⊗	⊗		
	2	●			● ●	●	
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DUODENUM	3	● ● ●	● ● ⊗				
	2	● ●	● ● ⊗	⊗			
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Fig. 8. Severity of lesions in target organs at sacrifice or death.

Grades of lesions are; 0: no change, 1: slight, 2: moderate, 3: severe. Each symbol indicates an individual case. △; 30 mg/kg, ×; 100 mg/kg, ●; 300 mg/kg (sacrificed) and ⊗; 300 mg/kg (dead).

DISCUSSION

This study revealed that a single, high-dose DBCP treatment caused marked changes in lympho-hematopoietic tissues and intestine in addition to known target organs such as the kidney, testis, epididymis and liver. Among these target organs, the lympho-hematopoietic tissues, intestine and testis are known to be radiosensitive organs. High-dose DBCP treatment produced: 1) lymphocyte depletion in lymphatic organs, 2) suppression of mitosis and panhypoplasia in the bone marrow, 3) inhibition of mitosis and necrosis of immature epithelial cells in the crypt of Lieberkühn, resulting in villus-shortening, 4) necrosis of the germinal epithelium, following tubular atrophy in the testis. These changes were similar to those observed after acute irradiation.¹²⁾ Taken as a whole, one of the important toxic effects of DBCP might be radiomimetic.

The kidney is moderately radiosensitive. The renal lesions occurring after irradiation are primarily related to vascular injury, resulting in ischemia of nephrons with consequent atrophy of glomeruli as well as tubules, and interstitial fibrosis.¹²⁾ Since DBCP induced renal lesions primarily in the proximal tubular epithelium without vascular lesions, renal changes after DBCP treatment could not be attributed to the radiomimetic effects of this chemical, although the precise mechanism is unclear.

Renal lesions seen in this study were in agreement with the observations already reported.^{7,8,9,11)} In addition to known tubular lesions, degeneration of glomerular podocytes was also detected in this study. Involvement of glomerular podocytes might result in decline in the rate of glomerular filtration. Even if microscopic manifestation were not apparent, glomerular disfunction might be caused. In fact, BUN and serum creatinine concentrations were elevated in DBCP-treated rats without obvious glomerular changes.⁹⁾ On the other hand, decrease in glomerular filtration rate due to tubular disorders might have occurred secondarily, since proximal tubular necrosis was remarkable after intoxication.

While pathological lesions were produced in most target organs within 1 day after treatment, testicular lesions were not apparent on day 1 as reported already.^{8,9)} However, marked lesions appeared by day 3 when spermatids were involved most severely, suggesting that spermatids might be particularly susceptible to DBCP toxicity. High susceptibility of spermatids to DBCP was also indicated after dominant lethal mutation tests.¹³⁾ High-dose DBCP caused tubular atrophy and impaired tubular viability, indicating that DBCP might cause irreversible injury in all stages of germinal epithelium, although no pathological manifestation in spermatogonia was apparent at the earlier stage of intoxication.

Except for the kidney and testis, considerable recovery was found within 7 days after treatment (Fig. 8). Dilation of proximal tubules and focal tubular desquamation persisted in the kidney and adverse effects on seminiferous germinal cells with tubular atrophy were progressive in the testis, suggesting irreversible injury in the kidney and testis. Moreover, repeated exposure to subtoxic doses of DBCP resulted in severe injury to the kidney and testis (unpublished data). Kluwe⁸⁾ also reported

that four daily treatments with low-dose DBCP produced renal and testicular lesions resembling those produced after a single high-dose treatment. Even with a single subtoxic dose, repeated exposures might cause severe and cumulative injury in kidney and testis.

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