한국수의병리학회지 . Korean J. Vet. Pathol. 3(2):83~85(1999)

Effects of 7,12-dimethylbenz[A]-anthracene(DMBA) on the Spleen in Syrian Golden Hamsters after Subcutaneous Injections

W. C. Son[1] and K. Kamino[2]

[1]*Biotech Research Institute, LG Chem, Korea*
[2]*Institute of cell and molecular pathology, Hannover Medical School, Germany*

Abstract: Weekly subcutaneous injection of 7,12-dimethylbenz[a]anthracene(DMBA) at a dose level of 0.25 mg/kg body weight induced proliferative lesions in the spleen of syrian golden hamsters. In addition, subcutaneous tumors at injection sites were observed. The splenic lesions included stromal hyperplasia and haemangioma-like lesion.

Key words : 7,12-dimethylbenz[A]-anthracene(DMBA), syrian golden hamster, spleen, stromal hyperplasia, hemangioma-like lesion

Introduction

7,12-dimethylbenz[a]anthraecene (DMBA) is a well-known carcinogen for skin and mammary gland,[3,6] a mutagenl and a well characterized immunosuppressant[2,10] in rodents.

To the best of our knowledge, histopathologic changes of the spleen after DMBA treatmemt have not previously been reported.

Materials and Methods

Thirty male and 30 female 8-week-old syrian golden hamsters (F_1D Alexander Hybrids; Bio-Research Consultants Inc., Cambridge, USA) were distributed into two experimental groups and kept individually in Macrolon cages type III (E Beckerand Co., GmbH, Castrop-Rauxel, Germmny) under standard laboratory conditions(room temperature: 22±1°C; relative humidity: 55±5%; air changes: 8 times/h; 12 h/12 h light/dark cycle). They were given free access to a pelleted diet (RMH-TMB, Hope Farms, Woorden, The Netherlands) and water. DMBA was dissolved in olive oil and inje cted subcutanously once a week to 15 female and 15 male in the treat ment group at a dose of 0.25 mg/kg body weight for the duration of their lives. Fifteen males and 15 females in the control group received a were obsered until spontaneous death or killed when moribund. After complete autopsy all organ were fixed in 10% buffered formalin.

Histological sections from all organs were stained with haematoxylin and eosin. Immunohistochemical examination of the spleen was performed with antibodies (DAKO, Hamburg, Germany) to factor-VIII-related antigen and ulex europaeus-lectin,type 1 (UEA-1) to determine the endothelial cells.

Table 1. Mean survival (weeks)°

	Treatment	
Sex	Control	DMBA
males	76±16	31±6
females	59±24	27±4

°mean±S.D.

Table 2. Tumour incidence in syrian golden hamsters treated with subcutaneous injections of 7,12-dimethylbenz[a]anthracene (DMBA) for life

Treatment sex	Control		DMBA	
	m	f	m	f
no. of animals	15	15	15	15
lymphoreticular tissue: malignant lymphoma	4	4	2	4
thyroid: c-cell adenoma	–	1	–	–
pharynx: squamous cell papilloma	–	1	–	–
thoracic cavity: malignant hibernoma	1	–	–	–
lung: bronchiolo-alveolar adenocarcinoma	–	–	–	1
adrenal: cortical adenoma	1	–	–	–
uterus: adenoma	–	–	–	1
adenocarcinoma	–	1	–	–
skin: fibrosarcoma	–	–	15	15
lipoma	–	1	–	–
squamous cell carcinoma	1	–	–	–

Results

The average survival rate of the treated animals was very short compared with that of the control ainmals(Table 1). Within the control group the femals showed a shorter survival than the males.

The tumor incidences are given in Table 2.

Fibrosarcoma was induced at the injection site in all DMBA-treatd hamsters. All other tumours in different organs occurred sporadically or in both experimental group.

In addition, all animals in the treatment group, both males and females, developed splenic stromal hyperplasia(Table 3). This lesion arose focally or multifocally in the red pulp, around the white pulp (Fig. 1) and/or subcapsular and consisted of cells with a moderate amount of poorly circumscribed eosinophilic cytoplasm and round to oval nuclei (Fig. 2). It frequently contained multifocal atrophic lymphoid structures and showed a sinusoidal congestion. Occasionally the hyperplatic area exhibited a round nodular

Table 3. Splenic lesion in syrian golden hamsters treatd with subcutaneeous injections of 7,12-demethylbenz[a]anthracene (DMBA) for life

treatment	control		DMBA	
sex	m	f	m	f
no. of animals	15	15	15	15
stromal hyperplasia	–	–	15	15
haemangioma-like lesion	–	–	4	3
capsular fibrosis	–	–	–	1

Request reprints from Woo-Chan Son, Biotech Research Institute, LG Chem Research Park, 104-1 Munji-dong, Yusong-gu, Taejon, Korea 305-380 Tel. 042) 866-2302, Fax. 042) 862-0333, E-mail: wcson@lgchem.co.kr

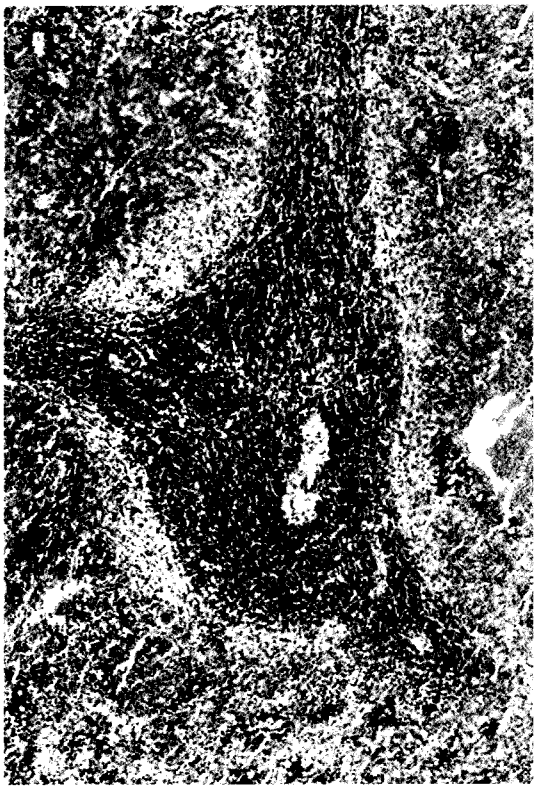

Fig. 1. Slight stromal hyperplasia around the white pulp. H&E, ×40

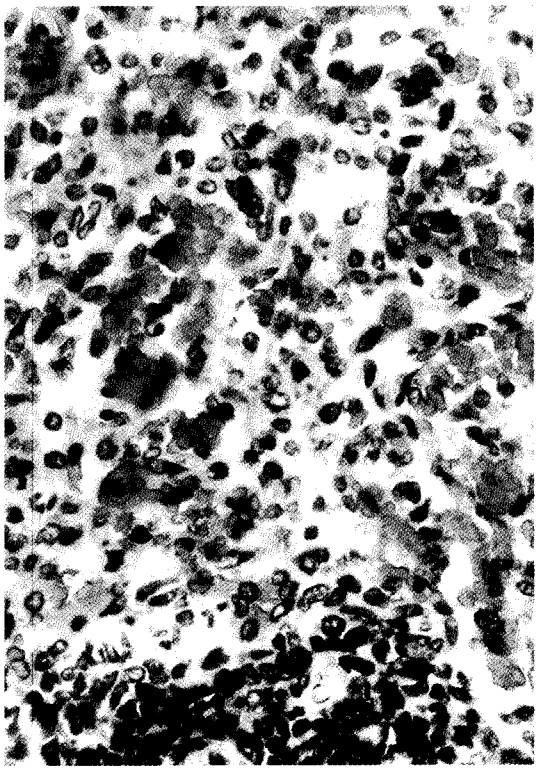

Fig. 2. High magnification of splenic stromal hyperplasia. note the reticulo-endothelial cells with round to ovalnuclei and the blood-filled sinusoids. H&E, ×100.

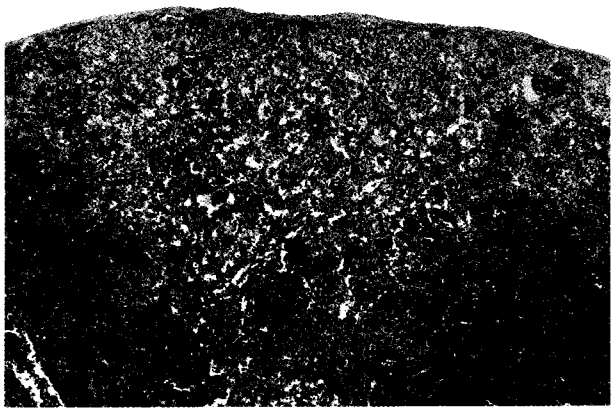

Fig. 3. Haemangioma-like lesion with poorly demarcated borders and enlarged blood-filled sinusoids. H&E, ×16.

appearence with poorly demarcated borders and the sinusoids were enlarged and filled with blood (Fig. 3), resembling a splenic haemangioma. The immunohistochemistry, using antibodies to factor-VIII-related antigen and UEA-1, obtained negative results. No stromal hyperplasia was found in the control animals.

Discussion

Splenic stromal hyperplasia is a proliferation of cells that cells that appear to be associated with the splenic trabecular and marginal zone.[8] This lesion seems to be the seem type of lesion which has elsewhere been termed reticulo-endothlial hyperplasia of the splenic stroma with respect to its morphologial feature.[7] Spontaneous occurrence of this lesion was reported to be found in less than 1% of old syrian golden hamsters[7] and is an uncommon finding in fischer rats.[8] The pathogenesis and biological behaviour of this lesion are not known; it is uncertain whether it represents a preneoplastic change.[8] In the present study this uncommon lesion was induced through subcutaneous injections of DMBA. The conventional light-microscopic examination showed that the proliferating cells were reticulo-endothelial cells. We obtained negative results in the immumo-histochemical examination. However, a negitive result does not mean that the cells being analyzed are not endothlial cells since the expression of detectable factor-VIII-related antigen in benign vascular neoplasms and in non-neoplastic vascular proliferations is variable.[9] Furthermore, most investigators agree that endothlial in lymphatic tissue lack immuno-reactivity.[4,5]

The haemangioma-like lesion is not considered to be a true haemangioma but to be a severe focal stromal hyperplasia because of its histomorphological appearance. These haeman-gioma-like lesions often occurred multifocally associat-ed with stromal hyperplasia, and the blood-filled spaces were mostly small, while the true haemangiomas generally develop singly in the otherwise rugular area and include large lagoon-like blood-filled spaces.

Further work should be carried out to clarify whether these lesions are reversible or progress to true haemangiomas and whether the known inmumosuppressing effect and lympho-toxicity of DMBA[2,10] are related to the splenic stromal hyperplasia.

Acknowledgements

The authors gratefully acknowledge the technical assistance given by their technologist colleagues in Hannover medical school.

References

1. Digiovanni G, Juchau MR Biotransformation and bioactivation of 7,12-dimethylben[a]anthracene. Drug Mehab Rev, **11**:61-101, 1980.
2. Ha JR, Jeong TC, Yang KH suppression of the in vitro immune response by 7,12-dimethylbenz[a]anthracene in mouse splenocytes co-cultured with rat hepatocytes. Biochem Mol Biol Int, **29**(2):387-393, 1993.
3. Howell J Skin tumors in the rat produced by 9,10-dimehtyl-1,2-benzanthracene and mehtyl cholanthrene. Br J Cancer, **16**:101-109, 1962.
4. McComb RD, Jones JR,Pizzo SV, Bigner DD Localization of factor 8/von willebrand factor and glial fibrillary acidic protein in the hemangiolastoma: Implications for stromal cell histogenesis. Acta Neuropathol, **56**:207-213, 1982.
5. Mukai K, Rosai J, Burgdorf WHC Localisation of factor-VIII-related antigen in vascular endothelial cells using an immunoperoxidase methods. Am J Surg Pathol, **4**:273-276, 1980.
6. RussoJ,Saby J, Isenberg WM Pathogenesis of mammary carcinomas induced in rats by 7,12-dimethylbenz[a]anthracene. JNCI **59**:435-445, 1977.
7. Schmidt RE, Eason RL, Hubbard GB, Young JT, Eisenbrandt DL Patholgy of aging Syrian hamsters. CRC Press, Inc., Boca Raton, Florida, pp. 121-139, 1983.
8. Stefanski SA, Atromberg PC, Elwell MR Spleen, Lymph Nodes, and Thymus. In: Boorman GA, Eustis SL, Elwell MR, Montgomery, Jr. CA, MacKenzie WF (eds): Pathology of the Fischer Rat.Academic Press, Inc., San Diego, pp. 369-393, 1990.
9. True LD: Vascular antigens. *In*: True LD (ED) Atlas of diagnostic immunohistopathology, Chapter nine. J.B. Lippincott Company, Philadelphia, pp. 9.1-9. **19**, 1990.
10. Ward EC, Murray MJ, Dean JH: Immunotoxicity of non halogenated polycyclic aromatic hydrocarbons. *In*: Dean JH, Luster MI, Munson AE, Amos H (eds):Immunotoxicology and Immunopharmacology. Raven Press, New York, pp. 291-303, 1983.

Address for correspondence: Woo Chan Son, Biotech Research Institute, LG Chem. Research Park, 104-1 Munji-dong, Yusong-gu, Taejon, Korea 305-380 Tel. 042) 866-2302, Fax.: 042) 862-0333, E-mail: wcson@lgchem.co.kr.