

## Chemopreventive action of benzyliothiocyanate on epithelial changes induced in hamster cheek pouch with DMBA

Abdullah M. AlDosari, BDS, MSD, PhD

هدفت هذه الدراسة إلى تقييم مدى تأثيرات بينزيل سوثيوسينينات (BIT) على التغيرات البشروية المحدثة بمادة دي ميثيل بينزويك أسيد DMBA في الطية الخدية عند الهامستر. شملت الدراسة ثمانية من الهامستر السورية الذهبية ، والتي تم تقسيمها إلى أربعة مجموعات متساوية. تم في المجموعة الأولى طلاء الطية الخدية اليمنى بطلاء زيتي ثلاث مرات أسبوعياً. أما في المجموعة الثانية فاستخدم مادة BIT لطلاء الطية الخدية اليمنى. تلقت عناصر المجموعة الثالثة نفس علاج المجموعة الثانية، إضافة إلى طلاء DMBA مرتين أسبوعياً. أما المجموعة الرابعة فتضمنت استخدام طلاء DMBA مرتين أسبوعياً. أظهرت النتائج أن غالبية التغيرات الشائعة كانت عبارة عن تشكلات بوليبيبية حيث معدلها في الحيوان الواحد من المجموعة الرابعة ٧ مقارنة بـ ٣,٩ في المجموعة الثالثة. ٤٣٪ من الحيوانات أبدت تغيرات مجهرية. و ٤٢٪ من الحيوانات التي تلقت DMBA فقط أبدت غزوا سرطانياً وذلك بالمقارنة مع ٢٦٪ من الحيوانات التي عولجت بمادتي DMBA و BIT. لم يظهر التحليل الإحصائي أي اختلاف جوهري بين مجموعات الحيوانات. والقيمة العددية لهذه الدراسة تتوافق مع التطبيق الكيميائي الوقائي لمادة BIT ضد التأثير السرطاني لمادة DMBA في الطية الخدية عند الهامستر.

**OBJECTIVE:** The aim of this study was to assess the effects of benzyliothiocyanate [BIT] on epithelial changes induced by DMBA in the hamster cheek pouch. **MATERIALS and METHODS:** Eighty male Syrian golden hamsters were divided into four equal groups. Group I received painting of the right buccal pouch with mineral oil three times per week. Group II received painting of the right buccal pouch with BIT three times per week. Group III was treated as Group II and the treated site was painted with DMBA twice per week. Group IV received painting of the right buccal pouch with DMBA twice per week. **RESULTS:** The findings of this study showed that the most common gross epithelial changes were the formation of polyps. The average number of polyps among Group IV was 7 polyps per animal compared to 3.9 polyps in Group III. Forty-three percent of all the involved animals showed microscopic changes. Forty-two percent of the animals treated with DMBA alone showed invasive carcinoma compared to 26% in the animals treated with DMBA and BIT. **CONCLUSION:** Although the statistical analysis did not detect significant difference among the treated groups of animals, the numerical values of this study are in support for the chemopreventive action of BIT against the carcinogenicity of DMBA in the hamster cheek pouch.

### INTRODUCTION

Several compounds have been reported to demonstrate an ability to interfere with, delay, or retard neoplastic changes in experimental animals with a phenomenon that was referred to as a chemopreventive action. Benzyliothiocyanate [BIT] is one of the compounds that were reported to have such an effect.<sup>1-8</sup> Wattenberg<sup>9</sup> reported that the systemic administration of BIT to Sprague-Dawley rats which had been exposed to the carcinogen 7,12-dimethylbenz[a]anthracene [DMBA] resulted in a lower incidence of induced mammary neoplasm as well as a decrease in the average number of tumors per animal. In another study, the addition of

BIT to a diet containing benzo[a]pyrene inhibited carcinogenesis of the mouse's forestomach.<sup>10</sup> Hecht *et al.*<sup>11</sup> concluded from one of their studies that BIT was a strong inhibitor of lung tumorigenesis induced in mice by polycyclic aromatic hydrocarbons.

The aim of this study was to assess the effects of BIT on epithelial changes induced by DMBA in the hamster cheek pouch. BIT was selected due to the fact that it is a naturally-occurring substance reported to be present in abundant amount in cruciferous vegetables, such as cabbage, broccoli, brussel sprouts and cauliflowers.<sup>12,13</sup> It was reported to be released in significant amounts from cabbage, garden cress, Indian cress and

Received 3 October 2006  
Accepted 10 December 2006  
Consultant and Associate Professor  
Head, Division of Oral Diagnosis/Medicine  
College of Dentistry, King Saud University

Address reprint requests to  
Dr. Abdullah M. AlDosari  
P.O. Box 60169, Riyadh 11545, KSA  
Tel.: +966 1 4677423 - Fax: +966 1 4679018  
E-mail: amdosari@ksu.edu.sa

mustard spinach.<sup>14</sup> In 1981, BIT was reported to be isolated from chewing sticks obtained from the roots of *Salvadora persica* L. which is widely used in Saudi Arabia and some other Islamic countries as a tool for oral hygiene care.<sup>15</sup>

## MATERIALS AND METHODS

Eighty male Syrian golden hamsters, five weeks old, were used at the Research Center, College of Pharmacy, King Saud University. They were caged individually, weighed weekly, and fed rat chow and water ad libitum. They were divided into four equal groups (Table 1). Group I received painting of the right buccal pouch with mineral oil three times a week. Group II received painting of the right buccal pouch with BIT three times a week. Group III was treated as Group II and the treated site was painted with DMBA twice a week [the two treatments were done at different days with 24 hours interval]. Group IV received painting of the right buccal pouch with DMBA twice a week.

BIT\* and DMBA\*\* were dissolved in a heavy mineral oil. BIT was used at a

**Table 1.** Study design

Group	Duration in weeks	Agent	Treatment
I	24	Mineral oil	Painting of the right buccal pouch three times/ week.
II	24	BIT	Painting of the right pouch three times/week.
III	24	BIT & DMBA	Painting of the right buccal pouch. BIT three times/week and DMBA twice/ week with 24 hours interval.
IV	24	DMBA	Painting of the right buccal pouch, twice/ week.

BIT: benzylisothiocyanate

DMBA: 7,12-dimethylbenz[a]anthracene

\*Eastman Kodak Co., Rochester, NY, USA

\*\*Eastman Organic Chemicals, Rochester, NY, USA

concentration of 50 mg of BIT per 1 ml of mineral oil. DMBA was used as 0.5% solution. Both chemical agents were applied to the treated sites with a No. 4 Camel's hair brush. Animals were slightly sedated by ether inhalation before each treatment.

The buccal cheek pouches were observed throughout the experiment for gross changes and selected examples were photographed. After 12 weeks of the application of the first test agent, half of the animals in each group were sacrificed by CO<sub>2</sub> inhalation. Each animal was dissected, and cheek pouches were removed and prepared for light microscopic examination. The remaining animals were sacrificed after 24 weeks of treatments and the cheek pouches were dissected and processed as mentioned previously.

The histological sections were assessed under light microscope for the presence of disturbances in polarity, drop-shaped rete ridges, polymorphism, hyperchromatism, basal cell hyperplasia, alteration in the nuclear/cytoplasmic ratio, increased mitosis and intraepithelial keratinization.

The Chi-square and Mann-Whitney tests were used for data analysis, since the data were based on a nominal measurement level. A 0.05 significance level was chosen for hypothesis testing.

## RESULTS

The gross epithelial changes are shown in Table 2 and, Figures 1 and 2. Thirty-eight percent of the animals involved in this study showed such changes. The most commonly observed gross epithelial changes were the formation of polyps (26.9%) followed by white patches (7.7%) and ulcerations (3.8%). The average number of polyps among the animals treated with DMBA alone was 7 polyps per animal compared to 3.9 polyps among the animals treated with DMBA and BIT. Chi-

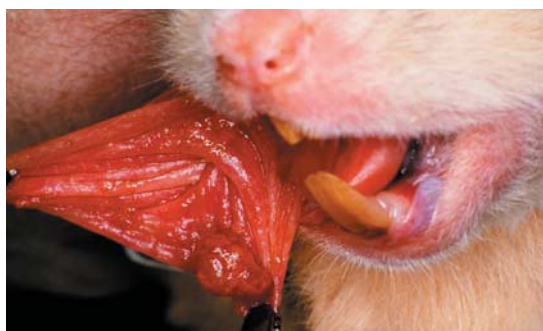
**Table 2.** Gross epithelial changes

Group	Normal		White Patches		Polyps		Ulcerations		Total*
	No	%	No.	%	No.	%	No.	%	
I	18	100%	0	0%	0	0%	0	0%	18
II	20	100%	0	0%	0	0%	0	0%	20
III	5	26.3%	2	10.5%	11	57.9%	1	5.3%	19
IV	5	23.8%	4	19%	10	47.6%	2	9.5%	21
Total*	48	62%	6	7.7%	21	26.9%	3	3.8%	78

\*Four animals died during the treatment period and two animals in Group IV showed both polyps formation and ulcerations.



**Fig. 1.** White patches developed on the hamster cheek pouch after 12 weeks of DMBA application.



**Fig. 2.** Different sizes of polyps developed on the hamster cheek pouch after 24 weeks of DMBA application.

square test did not detect any significant difference.

The microscopic changes are shown in Table 3, and Figures 3 and 4. None of the animals in control group showed any dysplastic microscopic changes. Forty-three percent of the involved animals showed microscopic changes. These changes included invasive carcinoma (17%), epithelial dysplasia (13%), carcinoma-in-situ (8%) and epithelial hyperatrophy (5%). Forty-two percent of the animals treated with DMBA (for both periods) alone showed invasive carcinoma compared to 26% in the animals treated with DMBA and BIT. Equal number of animals in these two groups showed epithelial dysplasia (30% after 12 weeks, and 22% after 24 weeks). Fifty-six percent of the animals treated with DMBA alone showed invasive carcinoma after 24 weeks of application compared to 33% among those treated with DMBA and BIT. Mann-Whitney test did not show a significant difference among these values.

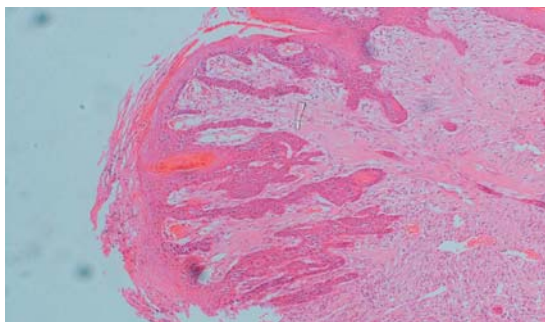
## DISCUSSION

The findings of this study showed a reduction of the DMBA-induced neoplastic changes in the hamster cheek pouch at both the gross and microscopic levels as a result of topical application of BIT. Chi-square and Mann-Whitney tests did not indicate a significant difference in

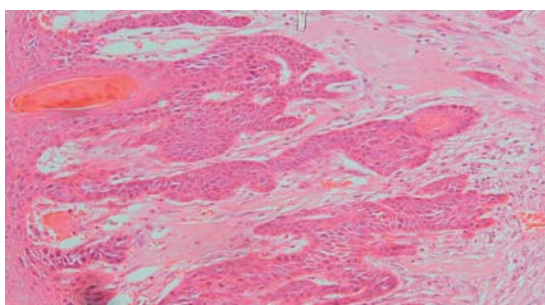
**Table 3.** Microscopic changes

Group	Duration [Wks]	Normal No.		Epithelial Hyperatrophy		Epihtelial Dysplasia		Carcinoma in-situ		Invasive Carcinoma		Total*
		No	%	No	%	No	%	No	%	No	%	
I	12	10	100%	0	0%	0	0%	0	0%	0	0%	10
	24	8	100%	0	0%	0	0%	0	0%	0	0%	8
II	12	9	90%	1	10%	0	0%	0	0%	0	0%	10
	24	9	90%	1	10%	0	0%	0	0%	0	0%	10
III	12	2	20%	1	10%	3	30%	2	20%	2	20%	10
	24	2	22%	0	0%	2	22%	2	22%	3	33%	9
IV	12	2	20%	1	10%	3	30%	1	10%	3	30%	10
	24	1	11%	0	0%	2	22%	1	11%	5	56%	9
Total*		43	57%	4	5%	10	13%	6	8%	13	17%	76

\* Four animals died during the treatment period.



**Fig. 3.** Histological section of hamster cheek pouch showing invasive carcinoma after 24 weeks of DMBA application (Original magnification x 20 Hematoxylin and eosin stain).



**Fig. 4.** Higher magnification of Figure 3 (Original magnification x 40 Hematoxylin and eosin stain).

the obtained values. This could be due to the fact that we are dealing with relatively few numbers of animals. This observation is similar to a previously reported effect of BIT in the hamster tongue.<sup>16</sup> In that study BIT was found to retard the development of neoplastic changes induced by trauma or trauma plus DMBA. These reports agreed with the findings in a substantial number of studies about the effectiveness of BIT as a good chemopreventive agent against neoplastic changes.<sup>17-19</sup>

The mechanism of action of chemopreventive agents, in general, is not fully understood. The concept of prevention of neoplastic changes goes beyond the mere protection of normal cellular structures to the broader approach of disturbing the whole process of different stages of neoplasm development. Several research findings support the concept that BIT and other

isothiocyanates [phenethyl isothiocyanate and sulforaphane] exert their chemoprotective action against neoplastic changes in tissues by more than one mean. They prevent the activation of carcinogenic agents into their ultimate active form through the inactivation of phase I enzymes [cytochromes P450].<sup>20-23</sup> They also help in detoxification of any formed ultimate active carcinogens through the inactivation of phase II enzymes [glutathione-s-transferases, UDP-glucuronosyl transferases and sulfotransferases].<sup>24-26</sup> Recent findings suggested a third way of chemopreventive action of BIT which is the activation of cell apoptosis.<sup>27-30</sup> The findings of the present study support such observation which was noticed through the reduction of number of tumours among the animals treated with both BIT and DMBA (3.9 polyps / animal) compared to animals treated with DMBA alone (7 polyps / animal).

The findings of this study beside the obtained information from working with BIT and other chemopreventive agents at different worldwide centers give a promising future to the war against cancer through chemoprevention. This is especially true for naturally occurring compounds that we can eat in our daily intake of food.

## REFERENCES

1. Wattenberg LW. Chemoprevention of Cancer. *Cancer Res* 1985; 45:1-8.
2. Fiala ES, Reddy BS, Weisburger JH. Naturally occurring anticarcinogenic substances in foodstuffs. *Annu Rev Nutr* 1985; 5:295-321.
3. DeVita VT Jr. A perspective on the war on cancer. *Cancer J* 2002; 8:352-356.
4. Taylor PR, Greenwald P. Nutritional interventions in cancer prevention. *J Clin Oncol* 2005; 23:333-345.
5. Greenwald P. Cancer chemoprevention. *Brit Med J* 2002; 324:714-718.

6. Bertram JS, Kolonel LN, Meyskens FL Jr. Rationale and strategies for chemoprevention of cancer in humans. *Cancer Res* 1987; 45:3012-3062.
7. Sabichi AL, Demierre MF, Hawk ET, Lerman CE, Lippman SM. Frontiers in cancer prevention research. *Cancer Res* 2003; 63:5649-5655.
8. Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, et al. Progress in cancer chemoprevention: Development of diet-derived chemopreventive agents. *J Nutr* 2000; 130:467S-471S.
9. Wattenberg LW. Inhibition of carcinogen-induced neoplasia by sodium cyanate, tert-butyl isothiocyanate and benzyl isothiocyanate administered subsequent to carcinogen exposure. *Cancer Res* 1981; 41:2991-2994.
10. Wattenberg LW. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *JNCI* 1977; 58:395-398.
11. Hecht SS, Kenney PMJ, Wang M, Upadhyaya P. Benzyl isothiocyanate: An effective inhibitor of polycyclic aromatic hydrocarbon tumorigenesis in A/J mouse lung. *Cancer Lett* 2002; 187: 87-94.
12. Fenwick GR, Heaney RK, Mawson, R. Glucosinolates. In: Toxicants of plant origin. Vol. II Glycosides. Cheeke PR (ed). Boca Raton, FL: CRC Press, 1989.
13. Tookey HL, Vanetten CH, Daxenbichler ME. Glucosinolates. In: Toxic constituent of plant stuffs. Liener IE (ed). New York: Academic Press, 1980.
14. Fenwick GR, Heaney RK, Mullin WJ. Glucosinolates and their breakdown products in food and food plants. *Crit Rev Food Sci Nutr* 1983; 18:123-201.
15. Ezmirly ST, El-Nasr MS. Isolation of glucotropaenolin from salvadora Persica L. *J Chem Soc Pak* 1981; 3:9-12.
16. Aldosari AM, Kafrawy AH, Standish SM. Effect of benzylisothiocyanate on epithelial changes induced by trauma and DMBA in the hamster tongue. *Saudi Dent J* 1992; 4:4-10.
17. Hecht SS. Chemoprevention of cancer by isothiocyanate, modifiers of carcinogen metabolism. *J Nutr* 1999; 129:768-774.
18. Sticha KRK, Kenney PMJ, Boysen G, Liang H, Su X, Wang M, et al. Effect of benzyl isothiocyanate and phenethyl isothiocyanate on DNA adducts formation by a mixture of benzo[a] pyrene and 4-C methylnitrosamino)-1-[3-pyridyl]-1-butanone in A/J mouse lung. *Carcinogenesis* 2002; 23:1433-1439.
19. Zhang Y. Role of glutathione in the accumulation of anticarcinogenic isothiocyanates and their glutathione by murine hepatoma cells. *Carcinogenesis* 2000; 21:1175-1182.
20. Zhang Y, Talalay P. Anticarcinogenic activities of organic isothiocyanates; Chemistry and mechanism. *Cancer Res* 1994; 54, 1976s-1981s.
21. Yang CS, Smith TJ, Hong JY. Cytochrome p. 450 enzyme as targets for chemoprevention against chemical carcinogenesis and toxicity: Opportunities and limitations. *Cancer Res* 1994; 54: 1982s-1986s.
22. Goosen TC, Kent UM, Brand L, Hollenbeng PF. Inactivation of cytochrome P450 281 by benzylisothiocyanate, a chemopreventive agent from cruciferous vegetables. *Chem Res Toxicol* 2000; 13: 1349-1359.
23. Tsao AS, Kim ES, Hong WK. Chemoprevention of Cancer. *CA Cancer J Clin* 2004; 54:150-180.
24. Fukushima S, Takada N, Hori T, Wanibuchi H. Cancer prevention by organosulfur compounds from garlic and onion. *J Cell Biochem* 1997; 27:100-105.
25. Prestera T, Holtzclaw WD, Zhang Y, Talalay P. Chemical and molecular regulation of enzymes that detoxify carcinogens. *Proc Nat Acad Sci* 1993; 90: 2965-2969.
26. Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res* 1992; 52: 2085s-2091s.

27. Abello PA, Fidler SA, Buchman TG. Thiol reducing agents modulates in porcine endothelial cells. *Shock* 1994; 2:79-83.
28. Slater AF, Stefan C, Nobel I, Van den Dobbelsteen DT, Orrenium S. Signalling mechanisms and oxidative stress in apoptosis. *Toxicol Lett* 1995; 83:149-153.
29. Buttke TM, Sandstrom PA. Oxidative stress as a mediator of apoptosis. *Immuno Today* 1994;15:7-10.
30. Evan G, Littlewood T. A matter of life and cell death. *Science* 1998; 281:1317-1322.