Pergamon

Oral Oncol, Eur J Cancer, Vol. 32B, No. 4, pp. 246–250, 1996

Printed in Great Britain
0964–1955/96 $15.00 + 0.00

0964–1955(95)00094–1

Hexamethylene Bisacetamide as a Chemopreventive Agent in Hamster Cheek Pouch Tumorigenesis

Y. Yura, H. Tsujimoto, J. Kusaka, H. Yoshida and M. Sato

Second Department of Oral and Maxillofacial Surgery, Tokushima University School of Dentistry, 3-18-15 Kuramoto-cho, Tokushima 770, Japan

The chemopreventive effect of oral and intraperitoneal (i.p.) administration of hexamethylene bisacetamide (HMBA) on 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced tumour formation in hamster cheek pouches was investigated. Male Syrian hamsters were treated by painting both cheek pouches with a 0.5% solution of DMBA twice weekly for 11 weeks. In addition to DMBA application, Group 1 hamsters were given 1% HMBA continuously in the drinking water and Group 2 hamsters received i.p. injection of HMBA at a dose of 500 mg/kg three times per week during the experiment. Group 3 animals received DMBA application alone. Thirteen weeks after the start of the experiment, the numbers of cheek pouch tumours and tumour volume were significantly decreased by oral but not i.p. administration of HMBA. Low levels of HMBA were detected in the plasma of the hamsters which were given 1% HMBA in drinking water. These results indicate that oral administration of HMBA can act as a chemopreventive agent against hamster cheek pouch tumorigenesis. Copyright © 1996 Elsevier Science Ltd

Keywords: chemoprevention, hexamethylene bisacetamide, hamster cheek pouch, 9,10-dimethyl-1,2-benzanthracene, oral cancer

Oral Oncol, Eur J Cancer, Vol. 32B, No. 4, pp. 246–250, 1996.

INTRODUCTION

Malignant conversion of oral premalignant lesions, i.e. leucoplakia and erythroplakia, and development of secondary primary tumours in cured cancer patients are the major problems in oral cancer [1]. Chemopreventive trials for oral leucoplakia have been conducted using β-carotene, vitamin E, selenium and 13-*cis*-retinoic acid; significant activity of retinoids in reverse oral premalignancy has been demonstrated [2–8]. However, treatment with effective high doses of retinoic acid is associated with marked toxicity [5, 8].

Hexamethylene bisacetamide (HMBA), a polar planar compound, has been reported to induce terminal differentiation of malignant cells [9–11]. Extensive *in vitro* studies have provided a basis for the application of HMBA to clinical therapy of human cancer, but studies which have examined HMBA in the treatment of advanced malignancies have shown the toxicity of HMBA to be dose-limiting, such that no tumour regression was observed at clinically achievable doses [12–14]. However, it was also reported that the induction of rat mammary tumours was inhibited by intraperitoneal (i.p.) administration of HMBA at doses of 1000 and 1500 mg/kg body weight throughout the experimental period [15]. This suggests that HMBA can be used as a chemopreventive agent, although its single use is not effective for the treatment of cancer, and that long-term administration of the agent would be essential for greater clinical benefit. For chemopreventive therapy, oral administration of HMBA would have practical advantages over intravenous injection. Recent studies have shown that the bioavailability and toxicity of HMBA, administered through a nasogastric tube, are acceptable [16, 17]. The chemopreventive activity of orally administered HMBA has not been examined intensively. In the present study, we examined whether oral or i.p. administration of HMBA could prevent the DMBA-induced tumour formation in the hamster cheek pouch.

Correspondence to Y. Yura.
Received 6 Oct. 1995; provisionally accepted 30 Oct. 1995; revised manuscript received 13 Dec. 1995.

MATERIALS AND METHODS

Animals and chemicals

Male Syrian hamsters aged 5 weeks were purchased from Japan SLC, Inc. (Shizuoka, Japan). The hamsters were housed four to five per cage in an air-conditioned room at 24 ± 2°C with a 12-h light–dark cycle and they were given animal chow (CL-2; Clea Japan Inc., Tokyo) and tap water *ad libitum*. DMBA, purchased from Wako Pure Chemical Industries (Osaka, Japan), was dissolved in a 1:20 mixture of acetone and heavy mineral oil (Sigma Chemical Co., St. Louis, Missouri). HMBA (Sigma Chemical Co.) was dis-

solved in drinking water for oral administration. For i.p. administration, HMBA was dissolved in 0.9% NaCl and the concentration was adjusted to 10%. Acetic anhydride, 1,5-diaminopentane and 1,7-diaminoheptane were purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). Pyridine was obtained from Wako.

Experimental design

A preliminary experiment was undertaken to determine the maximum tolerated dose of HMBA. HMBA at a concentration of 1, 2 or 5% was given in the drinking water to normal hamsters for 13 weeks. HMBA was also adminstered by i.p. injection at a dose of 500 or 1500 mg/kg body weight three times per week for 3 weeks. Five animals were given each dose and the body weights of the hamsters were measured weekly.

To determine the effects of HMBA on DMBA-induced tumour formation, 39 hamsters were divided equally into three groups. All animals were treated by painting both cheek pouches twice weekly for 11 weeks with a 0.5% solution of DMBA dissolved in acetone–heavy mineral oil mixture. In addition to DMBA application into the cheek pouches, hamsters in Group 1 were given 1% HMBA in drinking water continuously and those in Group 2 received i.p. administration of HMBA at a dose of 500 mg/kg body weight three times per week. DMBA application and HMBA administration were initiated simultaneously and HMBA was given until termination of the experiment, although DMBA application was terminated at 11 weeks. Group 3 hamsters received DMBA alone. Thirteen weeks after commencement of the experiment, the hamsters were killed by cardiac puncture performed under general anaesthesia with pentobarbital.

Pathological examination

At the end of the experiment, cheek pouches of the animals were removed, opened longitudinally, washed in phosphate buffered saline, and stretched on pieces of cardboard. The mucosa was inspected and gross visible tumours (>1 mm in diameter) were counted and measured. Cheek pouch tumours >3 mm in diameter were excised together with the surrounding mucosa and were subjected to histological examination. After paraffin embedding, sections 4-μm thick were stained with haematoxylin and eosin for light microscopic examination. The cheek pouch lesions were histologically classified as hyperplasia, papilloma or squamous cell carcinoma as described previously [18, 19].

Measurement of plasma HMBA level

For determination of the plasma HMBA level, normal hamsters, aged 18 weeks, were given either 1 or 5% HMBA in drinking water for 7 days and then heparinised blood samples were collected by cardiac puncture under general anaesthesia. The plasma HMBA levels were also measured after i.p. administration of HMBA at a dose of 500 or 1500 mg/kg body weight. Blood samples were collected at 1 or 6 h after the injection of HMBA. Blood samples were placed on ice and spun down, and the plasma was stored at −70°C. Plasma HMBA concentrations were assessed by our modification of the gas chromatographic method of Kelly *et al.* [20]. Pentamethylene bisacetamide was synthesised by acetylating 1,5-diaminopentane with a 1:1 mixture of acetic anhydride and pyridine and used as an internal standard. The samples were ultracentrifuged by centrifugation in an Amicon MPS-1 (Amicon Corp., Danvers, Massachusetts) and the ultrafiltrate was further concentrated 2-fold by vacuum centrifugation. The gas chromatographic system used a Shimadzu GC-7AG gas chromatograph (Shimadzu Seisakushyo Ltd, Kyoto, Japan) and a 2.1 m × 3 mm (inner diameter) glass column packed with 5% Advance-DS on 80/100 mesh Shinchrom A (Shimadzu). The assay had a detection limit of 10 μg/ml. To measure the plasma HMBA at the level of the detection limit, samples were concentrated 2-fold prior to analysis. Data were means of three determinations.

Statistical analysis

Statistical comparisons were performed using Student's *t*-test for the number and volume of tumours and χ^2 test for the incidence of papillomas and carcinomas. A *P*-value of 0.05 or less was considered significant.

RESULTS

Determination of the maximum tolerated doses of HMBA in normal hamsters

To determine the toxic oral doses, normal hamsters were given various concentrations of HMBA in drinking water for 13 weeks. No hamsters died during HMBA administration. At 13 weeks after the start of HMBA treatment, the mean body weights of the hamsters which were given 1, 2 or 5% HMBA were 139.6, 119.8, and 100.8 g, respectively, and that of the hamsters which did not receive HMBA was 145.3 g. There was a dose-dependent decrease in body weight; significant differences were observed between the untreated hamsters and those which received 2% HMBA ($P<0.05$) or 5% HMBA ($P<0.001$). The weights of liver, kidneys and submandibular glands were also decreased by 5% HMBA (Table 1). HMBA given i.p. at a dose of 1500 mg/kg body weight was toxic, and 60% (3/5) of the treated hamsters died within 3 weeks; such toxicity was not observed at the lower dose. Mean body

Table 1. Effect of HMBA on the weights of liver, kidneys and submandibular glands of normal hamsters

Treatment*	No. of hamsters	Liver (g) (mean ± S.D.)	Kidneys (mg) (mean ± S.D.)	Submandibular glands (mg) (mean ± S.D.)
None	5	9.3 ± 1.2†	1072 ± 60†	576 ± 62‡
1% HMBA	5	9.0 ± 0.9†	1028 ± 83	579 ± 59‡
2% HMBA	5	8.1 ± 0.6	1020 ± 105	512 ± 27†
5% HMBA	5	7.3 ± 0.8	968 ± 39	437 ± 50

*Normal hamsters were given HMBA in drinking water for 13 weeks.
†Significantly different from 5% HMBA group ($P<0.05$).
‡Significantly different from 5% HMBA group ($P<0.01$).

weights of the hamsters which received i.p. injections of HMBA at the doses of 500 and 1500 mg/kg body weight and survived for 3 weeks were 113.5 and 109.0 g, respectively; that of untreated hamsters was 110.8 g.

Effect of HMBA on the body weight of the DMBA-treated hamsters and their liver, kidney and submandibular gland weights

Based on the findings described above, the concentration of HMBA for oral administration was adjusted to 1% (Group 1) and that for i.p. injection was 500 mg/kg body weight (Group 2), and the effect of HMBA on DMBA-induced tumour formation in the hamster cheek pouches was examined. Group 3 hamsters were treated with DMBA alone. The total HMBA dose in each Group 1 hamster was 9.4 g and that in Group 2 animals was 2.7 g. Mean final body weights of the hamsters in Groups 1, 2 and 3 were 139.5, 147.9 and 138.9 g, respectively. There were no significant differences in the final body weights, or in the weights of liver and kidneys among the experimental groups (Table 2). However, the mean weight of submandibular glands in Group 1 was significantly greater ($P<0.001$) than those of Groups 2 and 3 (Table 2). The mean weight of the submandibular glands of normal hamsters, aged 18 weeks, was 576 mg. Thus, the decrease in weight of the submandibular glands in the DMBA-treated hamsters was found to be prevented by oral HMBA administration.

Effects of HMBA on DMBA-induced tumour formation in the hamster cheek pouches

Cheek pouch tumours were demonstrated in all hamsters. However, the mean numbers of cheek pouch tumours in Groups 1–3 were 13.0, 19.2 and 22.1, respectively (Table 3). More cheek pouch tumours were induced in Group 3 as compared with Groups 1 and 2, and the difference between Groups 1 and 3 was significant ($P<0.01$). The mean tumour volume in Group 3 was also significantly greater ($P<0.05$) than that in Group 1. Intraperitoneal administration of HMBA resulted in slight decreases in tumour number and tumour volume, but there were no significant differences between Groups 2 and 3.

Histological examination of cheek pouch tumours

All of the cheek pouch tumours >3 mm in diameter, a total of 138, were examined histologically. Most of the tumours were found to be squamous cell carcinomas (Table 4). When these carcinomas were further classified as well-, moderately-, and poorly-differentiated carcinomas, 42–75% of the carcinomas were well-differentiated. Although the incidence of well-differentiated carcinomas in Group 1 hamsters was the highest, the differences among the experimental groups were not significant.

Plasma HMBA concentrations in HMBA-treated hamsters

After continuous oral administration of 1 or 5% HMBA for 7 days, the plasma HMBA concentrations were 6.7 and 38.0 μg/ml, respectively (Table 5). When hamsters received a single i.p. injection of HMBA at a dose of 1500 mg/kg body weight, the plasma concentrations at 1 and 6 h after injection were 140.5 and 32.0 μg/ml, respectively. At a dose of 500 mg/kg, the plasma concentration of HMBA 1 h after injection was 34.5 μg/ml, but was below the level of detection at 6 h after injection.

DISCUSSION

The effects of oral administration of HMBA on experimental animals had not been extensively investigated. When

Table 2. Effect of HMBA on the weights of liver, kidneys and submandibular glands of DMBA-treated hamsters

Group	Treatment*	No. of hamsters	Liver (g) (mean ± S.D.)	Kidneys (mg) (mean ± S.D.)	Submandibular glands (mg) (mean ± S.D.)
1	DMBA + 1% HMBA (oral)	13	8.9 ± 2.0†	993 ± 110	522 ± 61‡
2	DMBA + 500 mg/kg HMBA (i.p.)	13	8.4 ± 1.1†	1014 ± 85	200 ± 36‡
3	DMBA	13	7.4 ± 1.2	978 ± 180	191 ± 48

*All hamsters were treated by painting both cheek pouches twice weekly with a 0.5% solution of DMBA for 11 weeks. In addition to DMBA, hamsters in Groups 1 and 2 were given oral and i.p. administration of HMBA, respectively, for 13 weeks. Group 3 hamsters were given DMBA alone.
†Significantly different from Group 3 ($P<0.05$).
‡Significantly different from Groups 2 and 3 ($P<0.001$).

Table 3. Incidence, number and volume of cheek pouch tumours in DMBA-treated hamsters

Group	Treatment*	No. of hamsters	No. of hamsters with tumour	No. of tumours/ hamster (mean ± S.D.)	Total tumour volume/ hamster (mm^3) (mean ± S.D.)
1	DMBA + 1% HMBA (oral)	13	13	13.0 ± 6.6†	111.6 ± 176.8‡
2	DMBA + 500 mg/kg HMBA (i.p.)	13	13	19.2 ± 8.4	197.4 ± 218.0
3	DMBA	13	13	22.1 ± 9.2	322.7 ± 250.7

*Hamsters were treated as described in Table 2.
†Significantly different from Group 3 ($P<0.01$).
‡Significantly different from Group 3 ($P<0.05$).

Table 4. Histological features of the cheek pouch tumours in DMBA-treated hamsters

Group	Treatment*	No. of tumours examined†	No. of carcinomas/ No. of tumours (%)	No. of well-differentiated carcinomas/No. of carcinomas (%)	No. of moderately-differentiated carcinomas/No. of carcinomas (%)	No. of poorly-differentiated carcinomas/No. of carcinomas (%)
1	DMBA + 1% HMBA (oral)	24	23/24 (96)	18/23 (75)	4/23 (17)	1/29 (3)
2	DMBA + 500 mg/kg HMBA (i.p.)	35	29/35 (83)	15/29 (42)	12/29 (34)	2/29 (6)
3	DMBA	79	67/79 (85)	51/67 (65)	14/67 (18)	2/67 (3)

*Hamsters were treated as described in Table 2.
†Tumours >3 mm in diameter were examined histologically.

Table 5. Plasma level of HMBA

HMBA treatment*		Route	Concentration (μg/ml)†
1% solution for 7 days		oral	6.7
5% solution for 7 days		oral	38.0
500 mg/kg	1 h after injection	i.p.	34.5
	6 h after injection	i.p.	UD
1500 mg/kg	1 h after injection	i.p.	140.5
	6 h after injection	i.p.	32.0

*Normal hamsters were given either continuous oral administration of HMBA in drinking water or a single i.p. injection of HMBA.
†Mean of 3 determinations. Samples were concentrated by 2-fold and analysed as described in Materials and Methods.
UD, undetectable.

normal hamsters were given HMBA in drinking water at different concentrations, significant decreases in body weight were observed at concentrations of 2 and 5%. We also found that HMBA at a dose of 1500 mg/kg body weight was toxic in hamsters. Thus, we examined the effects of HMBA on DMBA-induced tumour formation at a HMBA concentration of 1% in drinking water and at an i.p. dose of 500 mg/kg body weight.

We and other researchers have demonstrated that application of a 0.5% solution of DMBA into the hamster cheek pouch two or three times per week results in tumour formation within 12 weeks [18, 19, 21]. When examined at 13 weeks after the start of the experiment, as expected, all hamsters had developed cheek pouch tumours. However, the mean tumour number in the hamsters which were given oral HMBA (Group 1) was significantly reduced ($P<0.01$) as compared with the hamsters which received DMBA application alone (Group 3); the decrease by i.p. administration of HMBA was not significant (Group 2). The mean tumour volume was also reduced in Group 1, indicating that oral HMBA can inhibit the growth of tumours. These effects may not be ascribed to the general effect of HMBA, because the body weight was not significantly reduced by this concentration of HMBA. Thus, we concluded that HMBA, if administered continuously, could exert an inhibitory effect on chemical induction of hamster cheek pouch tumours. To our knowledge, this is the first report that indicates the inhibitory activity of HMBA in oral carcinogenesis [22].

Group 1 hamsters were given HMBA in drinking water continuously, whereas Group 2 hamsters received i.p. injection of HMBA three times per week. The total dose of HMBA for each animal in Group 1 was 3.5 times greater than that in Group 2. The results of the present study may be simply explained by the amounts of HMBA administered to the hamsters. Ward *et al.* [17] reported that HMBA was rapidly absorbed from the gastrointestinal tract, with a mean measured bioavailability of $99 \pm 15\%$. Consistent with their results, we demonstrated the presence of HMBA in the plasma of hamsters which were given 1% HMBA in drinking water for 7 days, although the level was relatively low. On the other hand, in the hamsters which received 500 mg/kg HMBA i.p., HMBA level in the plasma dropped below the level of detection at 6 h after injection. This i.p. dose of HMBA did not significantly reduce the number of cheek pouch tumours (Table 3). HMBA may have inhibited tumour induction if its plasma level had been maintained at a constant level during the experimental period.

Concentrations of HMBA less than 0.5 mM, 100 μg/ml have not proven effective in inducing differentiation in any of the cell lines studied [9, 13]. It is unlikely that HMBA delivered through the blood-stream would induce terminal differentiation and growth arrest of tumour cells in hamster cheek pouches. When we examined the features of the induced tumours (>3 mm in diameter), there appeared to be no substantial histological differences in the carcinomas which developed in the animals (Table 4). This indicates that HMBA does not affect the grade of differentiation of the induced carcinomas.

The mechanism by which orally administered HMBA inhibited tumour induction is not known. One possible explanation is that HMBA may act as an inhibitor for poly(ADP-R) polymerase and inhibit the growth of tumour cells [23–25]. Indeed, other inhibitors for poly(ADP-R) polymerase including benzamide, 3-aminobenzamide and 1,2-benzopyrone have been shown to protect cultured mammalian cells from oncogenic transformation induced by various chemical and physical stimuli [25–27]. Furthermore, Tseng *et al.* [24] reported that 200 μM 1,2-benzopyrone in the drinking water reduced both tumour incidence and tumour size in rats which were transplanted subcutaneously with rat cells carrying the inducible ras oncogene. They showed that the inhibitory activity of benzopyrone was 14.4-fold greater than that of HMBA, as assessed by *in vitro* soft agar assay. In the present study, we tested HMBA at a concentration 250 times higher than that of benzopyrone [24] and demonstrated that this concentration of HMBA in drinking water inhibited tumour induction. HMBA and benzopyrone thus may prevent tumour development *in vivo* by inhibiting the enzyme poly(ADP-R) polymerase. Alternatively, HMBA may act only at the applied site, i.e. the upper aerodigestive tract, because 1% HMBA (50 mM) in the drinking water was sufficient to

cause differentiation of transformed cells. HMBA absorbed from the luminal side of the oral mucosa may modify the tumorigenic processes of the epithelial cells, although no apparent effect on the grade of the carcinomas was demonstrated; HMBA may inhibit earlier stages in the development of cheek pouch tumours.

It has been shown that application of DMBA into the hamster cheek pouches results in atrophic changes in the submandibular glands [28]. Probably, DMBA applied into the cheek pouches would be retrogradely transported to the submandibular glands through their excretory ducts where it would exert a toxic effect on the glands. In this study, however, the weights of the submandibular glands of the hamsters which received DMBA and oral HMBA simultaneously were not decreased. A number of HMBA-associated side effects have been reported, but its advantageous effects have not been clarified [13–15, 17]. Orally administered HMBA could protect the submandibular glands from the toxicity of DMBA.

Studies by Hong *et al.* [1] established the short-term activity of high-dose 13-*cis*-retinoic acid in inducing remission of oral leucoplakia and in suppression of secondary primary tumours in the head and neck. The development of other nontoxic chemopreventive agents, possibly acting through different mechanisms, would be helpful in developing new regimens. Breitman and He [29] reported that all-*trans*-retinoic acid and HMBA synergistically induced terminal differentiation of HL60. Combinations of HMBA with 13-*cis*-retinoic acid may maintain the chemopreventive activities and decrease the problems associated with the achievement and maintenance of effective plasma concentrations as single agents.

1. Hong WK, Lippman SM, Wolf GT. Recent advances in head and neck cancer—larynx preservation and cancer chemoprevention: the seventeenth annual Richard and Hinda Rosenthal foundation award lecture. *Cancer Res* 1993, **53**, 5113–5120.
2. Shklar G, Marefat P, Kornhauser A, Trickler DP, Wallace KD. Retinoid inhibition of lingual carcinogenesis. *Oral Surg Oral Med Oral Pathol* 1980, **49**, 325–332.
3. Hong WK, Endicott J, Itri LM, *et al.* 13-*Cis*-retinoic acid in the treatment of oral leukoplakia. *N Engl J Med* 1986, **315**, 1501–1505.
4. Stich HF, Rosin MP, Hornby AP. Remission of oral leukoplakias and micronuclei in tobacco/betel quid chewers treated with β-carotene and with β-carotene plus vitamin A. *Int J Cancer* 1988, **42**, 195–199.
5. Schwartz JL, Flynn E, Shklar G. The effect of carotenoids on the antitumour immune response *in vivo* and *in vitro* with hamster and mouse immune effectors. *Ann NY Acad Sci* 1990, **587**, 92–109.
6. Lippman SM, Hong WK. Retinoid chemoprevention of upper aerodigestive tract carcinogenesis. In DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Important Advances in Oncology*. Philadelphia, JB Lippincott, 1992, 93–109.
7. Benner SE, Winn RJ, Lippman SM, *et al.* Regression of oral leukoplakia with alpha-tocopherol: a community clinical oncology program (CCOP) chemoprevention study. *J Natl Cancer Inst* 1993, **85**, 44–47.
8. Hong WK, Lippman SM, Itri LM, *et al.* Prevention of secondary primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. *N Engl J Med* 1990, **323**, 795–801.
9. Reuben RC, Wife RL, Breslow R, Rifkind RA, Marks PA. A new group of potent inducers of differentiation in murine erythroleukemia cells. *Proc Natl Acad Sci USA* 1976, **73**, 862–866.
10. Rabson AS, Stern R, Tralka TS, Costa J, Wilczek J. Hexamethylene bisacetamide induces morphologic changes and increased synthesis of procollagen in cell line from glioblastoma multiforme. *Proc Natl Acad Sci USA* 1977, **74**, 5060–5064.
11. Palfrey C, Kimhi Y, Littauer UZ, Reuben RC, Markes PA. Induction of differentiation in mouse neuroblastoma cells by hexamethylene bisacetamide. *Biochem Biophys Res Commun* 1977, **76**, 937–942.
12. Egorin MJ, Sigman LM, Van Echo DA, Forrest A, Whitacre MY, Aisner J. Phase I clinical and pharmacokinetic study of hexamethylene bisacetamide (NSC 95580) administered as a five-day continuous infusion. *Cancer Res* 1987, **47**, 617–623.
13. Young CW, Fanucchi MP, Walsh TD, *et al.* Phase I trial and clinical pharmacological evaluation of hexamethylene bisacetamide administration by ten-day continuous intravenous infusion at twenty-eight-day intervals. *Cancer Res* 1988, **48**, 7304–7309.
14. Conley BA, Forrest A, Egorin MJ, Zuhowski EG, Sinibaldi V, Van Echo DA. Phase I trial using adaptive control dosing of hexamethylene bisacetamide (NSC 95580). *Cancer Res* 1989, **49**, 3436–3440.
15. Chun HG, Leyland-Jones B, Hoth D, *et al.* Hexamethylene bisacetamide: a polar–planar compound entering clinical trials as a differentiating agent. *Cancer Treat Rep* 1986, **70**, 991–996.
16. Page JG, Grieshaber CK, Kostello MD. Bioavailability and oral toxicity studies with hexamethylene bisacetamide (HMBA: NSC-95580) in beagle dogs. *Proc Am Assoc Cancer Res* 1986, **27**, 274.
17. Ward FT, Kelley JA, Roth JS, *et al.* A phase I bioavailability and pharmacokinetic study of hexamethylene bisacetamide (NSC 95580) administered via nasogastric tube. *Cancer Res* 1991, **51**, 1803–1810.
18. Yura Y, Harada K, Tsujimoto H, Yoshida H, Sato. Effect of sialoadenectomy and epidermal growth factor administration on 9,10-dimethyl-1,2-benzanthracene-induced tumor formation in hamster cheek pouch. *Oral Surg Oral Med Oral Pathol* 1993, **76**, 723–728.
19. Harada K, Yura Y, Tsujimoto H, Kusaka J, Yoshida H, Sato M. Effect of local administration of epidermal growth factor on 9,10-dimethyl-1,2-benzanthracene-induced tumour formation in hamster cheek pouch. *Oral Oncol, Eur J Cancer* 1995, **31B**, 27–31.
20. Kelley JA, Roth JS, Litterst CL. Gas chromatographic determination of hexamethylene bisacetamide in plasma and urine. *Anal Lett* 1985, **18**, 1043–1062.
21. Shklar G. Experimental oral pathology in the Syrian hamster. *Prog Exp Tumor Res* 1972, **16**, 518–538.
22. Tanaka T. Chemoprevention of oral carcinogenesis. *Oral Oncol, Eur J Cancer* 1995, **31B**, 3–15.
23. Milo GE, Kurian P, Kirsten E, Kun E. Inhibition of carcinogen-induced cellular transformation of human fibroblasts by drugs that interact with the poly(ADP-ribose) polymerase system. *FEBS Lett* 1985, **179**, 332–336.
24. Tseng A Jr, Lee WMF, Kirsten E, *et al.* Prevention of tumorigenesis of oncogene-transformed rat fibroblasts with DNA site inhibitors of poly (ADP ribose) polymerase. *Proc Natl Acad Sci USA* 1987, **84**, 1107–1111.
25. Alderson T. Poly(ADP-ribosylation) processing as a target for the anti-tumor effects of the cell differentiating agent, hexamethylenebisacetamide, and the N^6-substituted adenosines. *Anti-Cancer Drugs* 1991, **2**, 543–548.
26. Nakayasu M, Shima H, Aonuma S, Nakagama H, Nagao M, Sugimura T. Deletion of transfected oncogenes from NIH 3T3 transformants by inhibitors of poly (ADP-ribose) polymerase. *Proc Natl Acad Sci USA* 1988, **85**, 9066–9070.
27. Shima H, Nakayasu M, Aonuma S, Sugimura T, Nagao M. Loss of the MYC gene amplified in human HL-60 cells after treatment with inhibitors of poly(ADP-ribose)polymerase or with dimethyl sulfoxide. *Proc Natl Acad Sci USA* 1989, **86**, 7442–7445.
28. Cohen CI, Stahl SS. The response of hamster submaxillary glands to experimentally induced carcinoma in the cheek pouch. *J Dent Res* 1964, **43**, 461.
29. Breitman TR, He R. Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60. *Cancer Res* 1990, **50**, 6268–6273.

Acknowledgements—This work was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture of Japan (04807146). We thank Dr Y. Toyooka (Maruishi Pharmaceutical Co.) for helpful suggestions in preparing pentamethylene bisacetamide.