Metastases Following Biopsy of Oral Carcinoma in Hamsters and the Role of Local Prebiopsy Bleomycin

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Abstract. *Background: This animal study researches the effect of biopsy on metastasis of oral carcinoma. Materials and Methods: Sixty hamsters developed oral tumors after treatment with 9,10-dimethyl-1,2-benzanthracene and were then divided into six groups. Animals of groups 1 and 2 did not receive any treatment, while groups 3-6 were biopsied at the end of the 14th week and groups 5 and 6 also received a prebiopsy intratumoral injection of bleomycin. Animals of groups 1, 3, 5 and 2, 4, 6 were sacrificed at the 17th and 19th week respectively. Specimens of tumors, ipsilateral cervical lymph nodes, lungs and livers were obtained from all animals and histologically examined. Results: All animals developed oral squamous cell carcinomas. In group 4, four out of ten animals showed metastases to the cervical lymph nodes, and one out of four showed a distant metastasis to the lung. Conclusion: Delay of treatment following biopsy can increase the risk of cervical lymph node metastases which can be reduced by an intratumoral administration of bleomycin.*

It is well known that the most frequent human oral malignancy is the squamous cell carcinoma (SCC). Oral squamous cell carcinoma (OSCC) has a tendency to metastasize through the local lymphatic drainage before distant spread. OSCC spreads first to the lymph nodes of the neck. The three treatment modalities of OSCC include surgery, radiotherapy and chemotherapy. It is standard practice that a biopsy of the suspicious lesion is the first essential step for any clinician in order to confirm the diagnosis (1).

Chemical carcinogenesis with 9,10-dimethyl-1,2-benzanthracene (DMBA) in the hamster buccal pouch can induce squamous cell carcinomas, which can subsequently produce cervical lymph node and lung metastases (2-8). An experimental model of oral carcinogenesis with DMBA was established in Syrian golden hamsters in order to study the effect of biopsy on neck lymph node metastasis and distant metastasis to lungs and liver.

Interestingly, it has been shown that an intratumoral injection of bleomycin (BLM) combined with electrochemotherapy on chemically induced tongue cancer in hamsters induced rapid necrosis of the tumor and subsequent rapid tumor volume reduction (9). Based on these data, an intratumoral injection of BLM was administered before the biopsy of carcinomas induced in hamster cheek pouch with DMBA in order to investigate any changes on the regional lymph node and distant organ metastases pattern.

Materials and Methods

Sixty male Syrian golden hamsters (*Mesocricetus auratus*), ten weeks of age and weighing approximately 100 g each, were used in this study. The hamsters were handled in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Tumor induction. The left cheek pouch of animals (anesthetized with ether) were painted three times per week for 14 weeks with a 0.5% solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) (Sigma, St. Louis, MO, USA) dissolved in paraffin oil, using a #4 camel hair brush. The pouches of all animals were examined weekly in order to observe the growth of tumors on the mucosa. At the end of the 14 weeks, all animals developed tumor (size 2-3 cm) in the left cheek pouch. The animals were then randomly divided into six groups:

Group 1: Control-3 weeks (n=10). Following a three-week period without application of the carcinogen, the animals were sacrificed by an overdose of ketamine hydrochloride (>30 mg/kg). The tumour of the cheek pouch and the ipsilateral cervical lymph nodes were excised. The lungs and the liver of the animals were also removed.

Group 2: Control-5 weeks (n=10). Following a five-week period without application of the carcinogen, the animals were sacrificed and specimens as described for animals in Group 1 were taken.

Group 3: Biopsy-3 weeks (n=10). At the end of 14 weeks, the tumors in the pouch were biopsied under anesthesia (20 mg/kg ketamine hydrochroride and 1 mg/kg xylazine hydrochroride given

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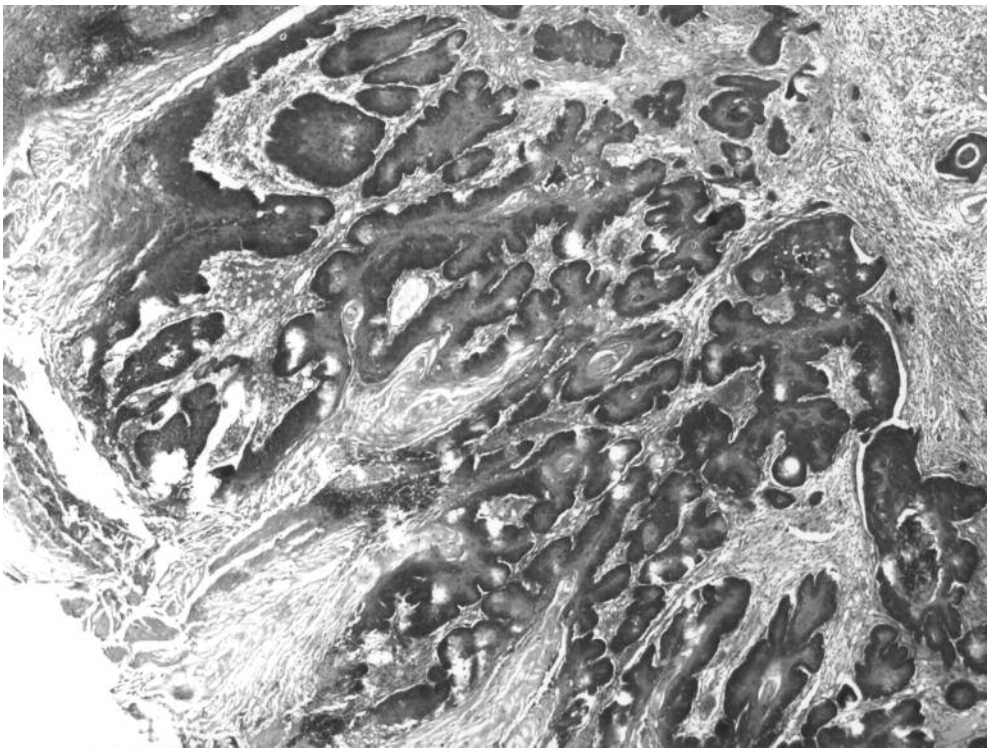

Figure 1. *Well-differentiated SCC of hamster buccal pouch (H&E x4).*

i.p.). Sterile #15 scalpel blades were used to biopsy the tumour. A tissue specimen, approximately 0.5 cm, was removed. Following a three-week period, the animals were sacrificed and the tumor, the ipsilateral cervical lymph nodes, the lungs and the liver were removed.

Group 4: Biopsy-5 weeks (n=10). The tumors in the pouch were biopsied under anesthesia. Following a five-week period, the animals were sacrificed and the tumor, the ipsilateral cervical lymph nodes, the lungs and the liver were removed.

Group 5: Bleomycin/biopsy-3 weeks (n=10). At the end of 14 weeks, 0.04 ml of 100% bleomycin hydrochloride was injected at the base of the tumor. Five minutes later, the tumors were biopsied under anesthesia. Following a three-week period, the animals were sacrificed and the tumor, the ipsilateral cervical lymph nodes, the lungs and the liver were removed.

Group 6: Bleomycin/biopsy-5 weeks (n=10). The administration of bleomycin hydrochloride was followed by biopsy of the tumor. Following a five-week period, the animals were sacrificed and the tumor, the ipsilateral cervical lymph nodes, the lungs and the liver were removed.

After their excision, the tumors, cervical lymph nodes, lungs and livers of all animals in groups 1-6 were given a number and examined blindly. All specimens were fixed in 10% formalin, embedded in paraffin and serial sections were cut and stained with hematoxylin and eosin for histological examination.

Statistical analysis. Statistical differences between groups were evaluated by χ^2 test with Yates correction. Statistical significance was set at 5% ($p<0.05$).

Results

The animals in the control groups 1 and 2 had developed squamous cell carcinomas in their cheek pouch at 17 and 19 weeks respectively. Additionally, all animals in groups 3-6 developed SCC in their cheek pouch at the time of biopsy which was present at the time of death. The tumors were exophytic and had rough surfaces. The majority of the tumors were well differentiated; however some were moderately differentiated (Figure 1).

In groups 1, 2 and 3, the lymph nodes of all animals showed no evidence of metastasis (0%). Furthermore, all animals in these three groups were free of distant metastases to the lungs and liver. In group 4, at the 5th week following biopsy of the tumors, four out of ten animals showed histological signs of metastatic deposits of SCC to the ipsilateral cervical lymph nodes (Figure 2). There was significant difference compared with groups 2 and 3 ($p<0.05$). One of the animals with lymph node metastasis also exhibited a distant metastasis to the lung (Figure 3), but this difference was not significant.

In groups 5 and 6, neither cervical lymph node nor distant metastases were seen microscopically in any of the animals. In group 6, at the 5th week following BLM administration and biopsy of the tumors, none of the animals showed

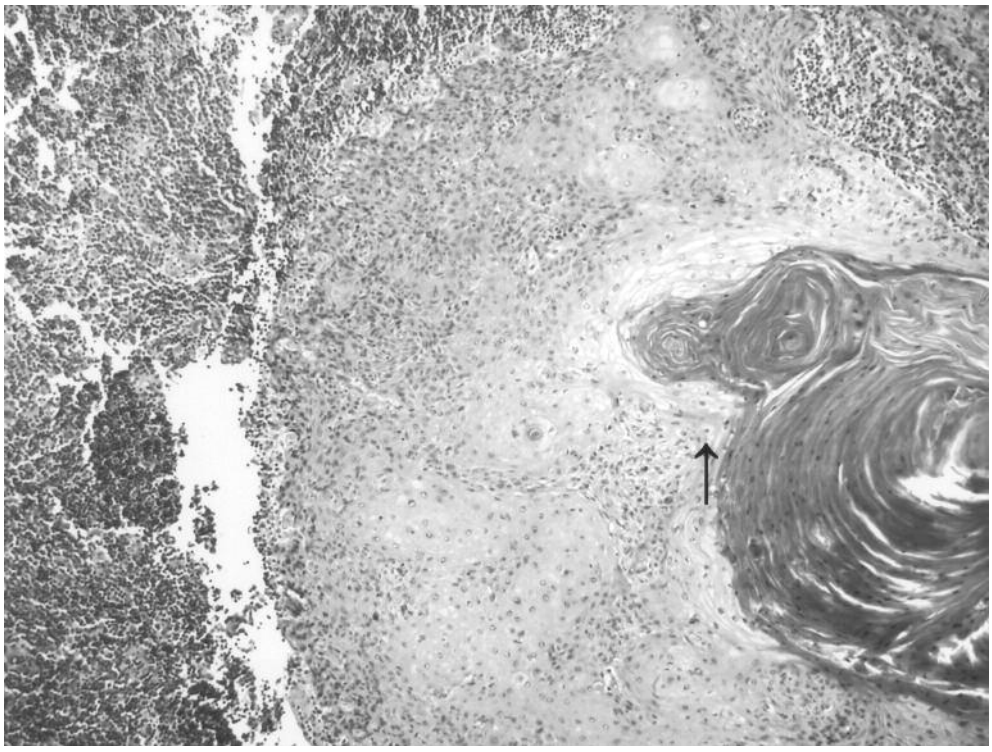

Figure 2. *Metastasis of SCC in a cervical lymph node (black arrow) (H&E x10).*

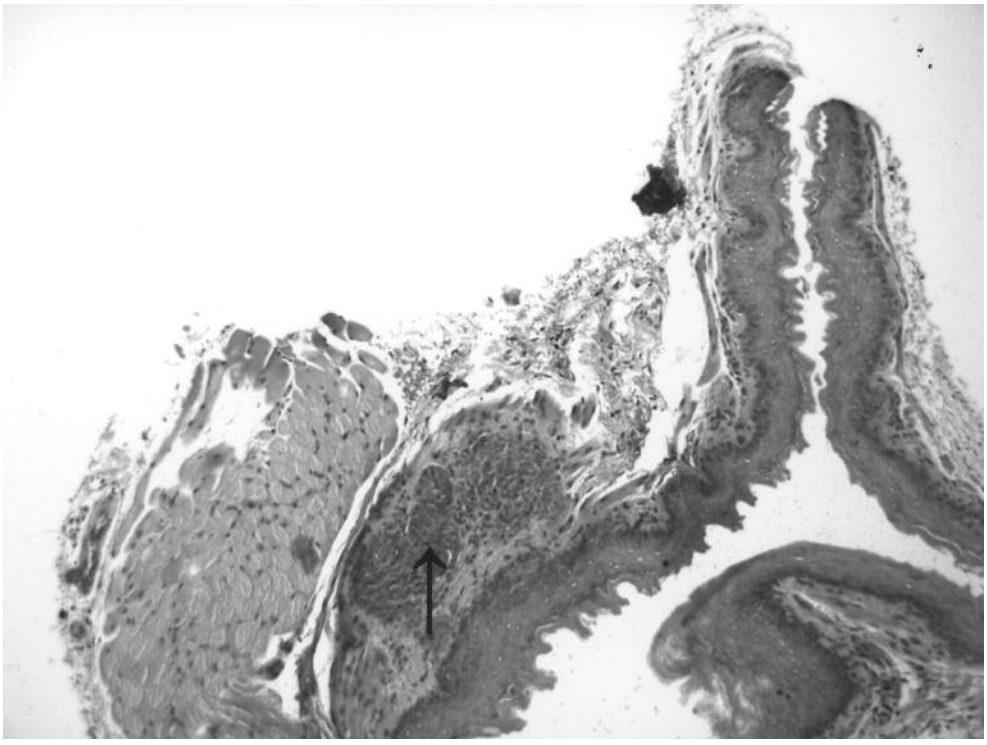

Figure 3. *Metastasis of SCC in the lung, poorly differentiated (black arrow) (H&E x10).*

Table I. *Neck lymph nodes, lung and liver metastases in the experimental groups.*

Groups	No. of hamsters			
	Total	With lymph node metastasis	With lung metastasis	With liver metastasis
Group 1 (Control-3 weeks)	10	0	0	0
Group 2 (Control-5 weeks)	10	0	0	0
Group 3 (Biopsy-3 weeks)	10	0	0	0
Group 4 (Biopsy-5 weeks)	10	4*	1	0
Group 5 (Bleomycin/biopsy-3 weeks)	10	0	0	0
Group 6 (Bleomycin/biopsy-5 weeks)	10	0#	0	0

*$p<0.05$ *vs.* group 2 & 3; #$p<0.05$ *vs.* group 4.

histological signs of metastatic deposits of SCC to cervical lymph nodes (significant as compared to group 4, $p<0.05$).

The results of the experiments are summarized in Table I.

Discussion

The presence of metastasis to the cervical lymph nodes from hamster buccal pouch carcinoma induced with DMBA is controversial in the literature. There are some reports which have supported lymph node metastasis in this model of carcinogenesis (2, 5, 10, 11) in spite of a number of others which have failed to demonstrate lymph node metastasis (12-14).

Kage *et al.* (2) showed that the surgical excision of the hamster cheek pouch carcinoma is efficient in producing unequivocal lymph node metastasis. Seven out of sixteen animals (43%) developed lymph node metastasis 7 weeks following the surgical excision of the carcinoma. Craig (10) has also reported a similar percentage (48%) of lymph node metastasis following a longer period of observation. It is quite interesting in the present study that the incisional biopsy in the same model of experimental oral carcinogenesis showed no neck lymph node metastasis at 3 weeks following the biopsy of the carcinomas, but four out of the ten animals exhibited lymph node metastasis at 5 weeks postbiopsy. The incidence of metastasis was 40%, despite our shorter experimental period (19 weeks) compared to the studies of Kage *et al.* (2) and Craig (10) (21 and 30 weeks, respectively).

However, Tsiklakis *et al.* (5) failed to demonstrate that incisional biopsy influenced cervical lymph node metastasis, even though metastasis to regional lymph nodes occurred frequently. There was also no significant difference in metastasis between the control group and the experimental group of incisional biopsy at 20 weeks of the experimental period of their study.

Regarding distant metastasis in the lung and liver, both studies by Kage *et al.* (2, 3) failed to produce metastasis to the distant organs in the control groups. Only one animal in the group which had received intraperitoneal injection of hydrocortisone for 3 weeks had cervical lymph node and lung metastasis simultaneously. Although spontaneous lung carcinoma could have occurred, this is extremely unlikely based on the work of Pour and Althoff (15), which studied the incidence of spontaneous lung carcinoma in hamsters and found none among 1,200 animals investigated. Furthermore, Tsiklakis *et al.* (5) reported no distant metastasis to the lungs or the liver, neither in control groups nor in groups that received cortisone. It is interesting in the present study that one animal had simultaneous metastasis to the cervical lymph node and to the lung found at 5 weeks post-biopsy without the administration of steroids.

Literature research revealed that the local use of BLM for dysplastic and malignant lesions has been previously reported. Topical application of BLM in dimethylsulfoxide has been evaluated in open clinical trials and resulted in regression of leukoplakia (16-19). Resolution of dysplastic leukoplakia was reported after local injection of BLM weekly for 8 weeks (20). Topical BLM may prevent the potential progression of leukoplakia through dysplasia to carcinoma (21). Topical bleomycin has also been used for resolving Kaposi's sarcoma of the skin (22). The intratumoral injection of BLM combined with electrochemotherapy on the chemically induced tongue cancer in hamster induced rapid necrosis and volume reduction of the tumor (9). More recently, the intratumoral injection of BLM following by electroporation found to be effective in patients with head and neck and skin tumors (23, 24). Interestingly, in the present study, the intratumoral injection of BLM before the incisional biopsy of the carcinomas prevented neck lymph node metastasis in the group of animals which had tumor resection and lymph node removal at 5 weeks postbiopsy.

In conclusion there are a couple of interesting findings in the present study. The "delay" of treatment up to 5 weeks following the biopsy of a carcinoma induced in hamster cheek pouch with DMBA can increase the risk of neck lymph node metastasis. Furthermore, the prebiopsy intratumoral administration of bleomycin can reduce the risk of cervical lymph node metastasis, as shown in this experimental model of oral carcinogenesis.

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