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SITE SPECIFIC CANCER INDUCTION BY INTRA -ESOPHAGEAL COTTON NODE RETENTION AND CARCINOGEN LADEN DRINKING WATER

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Objective: To reveal the relationship between the restriction of esophagus and the esophageal carcinogenesis. Methods: Male Wistar rats weighing 200±20g (N=50) observed as experimental animals. Using carcinogen N-ethyl-N-butyl-nitrosamine (EBN), or N-nitrosomethyl-N-propylamine (MPN), esophageal carcinogenesis was induced. In some rats, a cotton node was detained in the thoracic segment of the esophageal lumen to make artificial restriction. The rats were divided into 6 groups. Group EC or MC were those treated with the artificial restriction and EBN, or MPN. Group E, M or C included those treated only with EBN, MPN, or cotton node. Group U was untreated control. The rats were sacrificed, and the esophagus from the 6 groups of rats were compared. Results: On naked eye examination, the esophageal lesion was the most in EC group, followed by MC group. About 70% of the lesions were within 3mm from the thread node. The E or M group only had a few lesions. There was no observable lesion in the C and U groups. Histological examination found that the hyperplasia, hyperkeratosis, papilloma, and dysplasia were significantly more in EC and MC groups than E and M groups. Severe dysplasia and carcinoma *in situ* were only noticed in the EC and MC groups. Conclusion: It is suggested that the artificial restriction promotes the esophageal carcinogenesis. The effect is related with increased contact with carcinogen and injury at the area of the restriction.

Key words: Artificial restriction, Esophageal carcinoma, Chemically induced, Rat

Esophageal carcinoma is one of the most common neoplasms in China.[1] The significance of environmental carcinogens especially those contained in foods and beverages were stressed; though its relationship with race or inheritance were reported.[2,3] Most of the esophageal carcinomas were located in the three anatomic restrictive segments.[4] Carcinoma is also more common in the restriction area of the esophageal scar.[5] It is suggested that the carcinogen in the food may stay longer in the segments, have more chance to contact with esophageal mucosa, promoting its carcinogenesis.[6] We used the rat esophageal restriction model by intra-esophageal retention of cotton node combined with carcinogen administration to confirm the hypothesis.

MATERIALS AND METHODS

Reagents

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Carcinogen: N-ethyl-N-butyl-nitrosamine(EBN), N-nitrosomethyl-N-propylamine (MPN) were products of Sigma Chemicals (Catalog No. 1758, 7260). The carcinogens were mixed into the drinking water at the concentration of 0.25mg/L for EBN, and 14mg/L for MPN. The water was renewed twice a week.

Animals and Grouping

Male Wistar rats weighing 200±20g (N=50) were used. The rats were of special pathogen free grade, purchased from Charles River Japan Inc. (Kanagawa, Japan).

The rats were randomly divided into 6 groups (Table 1). Group E, M or C included those treated only with EBN, MPN, or cotton node esophageal restriction. Group EC or MC rats were those treated with the artificial restriction and EBN, or MPN. The untreated were group U rats.

Table 1. Grouping and treatment of the rats

Grouping	Intra-esophageal cotton node	Carcinogen
E (N=10)	No	EBN
M (N=10)	No	MPN
C (N= 5)	Yes	No
EC (N=10)	Yes	EBN
MC (N=10)	Yes	MPN
U (N= 5)	No	No

Preparation of Esophageal Restriction Model

We operated on the rats to put a cotton node in the middle part of the esophagus of groups C, EC and MC or only a shame operation on other groups. The operation procedures were as follows: cotton thread with the diameter of lmm was used to tie up a node (diameter=3mm). Under anesthesia, the node was put into the thoracic segment of the esophagus of the rat (6—8cm from front teeth) with the induction of a plastic tube and operation silk thread. The node was connected with one end to No. 0 operation silk thread. Other end of the thread was put though the sidewall of the pharynx to the subcutis of the neck and fixed there, finally. The shame operation rat was put through the same procedure without retention of the thread. The rats were left for a week before carcinogen treatment.

Preparation of Histological Section

At the end of 20-week-treatment, the rats were sacrificed under ethanol ethyl anesthesia. The esophagus from the 6 groups of rats was taken out and cut open longitudinally. The number, location and size of the esophageal lesion were recorded and sampled for histological examination. The samples were fixed in 15% buffered formalin solution, embedded in paraffin, sectioned and stained with H.E. routinely.

RESULTS

Macroscopic observation found that the esophagus with lesion and the number of the lesion were significantly more in the group EC and MC than that of group E and M rats. No obvious lesion was noticed in the group C and U rats (Table 2).

Table 2. Macroscopic lesion number of the rat groups

Group	No. of Rats with lesion	No. of the lesion
E	1	1
M	2	3
C	0	0
EC	8	23
MC	6	14
U	0	0

Analyzing the relationship between the cotton node and the macroscopic lesion, we found that about 70% of lesions were within 3mm to the node (Table 3).

Table 3. The distance between node and lesion

Group	Less or Equal to 3mm(%)	Over 3mm (%)	Total
EC	16 (69.6)	7 (30.4)	23*
MC	11 (78.6)	3 (21.4)	14*

* U>2.58, $P<0.001$

Histological examination found that over 85% of the lesions were hyperplasia, hyperkeratosis, papilloma, or mild to moderate dysplasia. These lesions were significantly more in EC and MC groups

than E and M groups. Severe dysplasia and carcinoma *in situ* were noticed only in the EC and MC groups (Table 4).

Table 4. Histology of the esophageal lesion

Group	Hyperplasia to hyperkeratosis	Papilloma, mild or moderate dysplasia	Severe dysplasia to carcinoma *in situ*
EC	14	6	3
MC	9	3	2
E	0	1	0
M	2	1	0

DISCUSSION

In human beings, the esophagus has three restricted segments: the conjunction with the pharynx, the segments of the esophagus pass through the aorta arch or the diaphragm. These segments were most commonly affected by the esophageal carcinoma.[4]

There are many theories on the carcinogenesis, direct continuous irritation or injury of the irritant or carcinogen on special place inducing the procedure; the carcinogen may have special affinity on selected organ.[5-7] The mechanism for the distribution of esophageal carcinoma was not clarified on the experimental animals.

The carcinogenic effects of nitrosamines were reported in 1960's.[7] The effects were confirmed in experimental animals. Different derivatives of the nitrosamines can induce carcinoma of various organs on the animals. So that some authors suggested that different functional groups of the nitrosamines might lead the chemicals to reach different organs or to selectively adhere on and enter into particular cell types resulted in organ or cell specific carcinogenesis.[7]

On the other hand, the epidemiologic study of both eastern and western countries found that the esophageal carcinoma was concentrated in the three constrictive segments.[2,4,8] It is suggested that the distribution may also be related with the increased contact and action on the esophageal wall due to reduced speed of the carcinogen or irritant through the restricted segment. The Iran people are fond of hot tea and the esophageal cancer is common in the population.[9] Increased incidence of carcinoma is also observed in the stricture area of chemically erotic esophageal scar.[5] These evidences are all in favor of the suggestion on the carcinogenic effects of local irritant.

In order to confirm the importance of local irritant of carcinogen on the carcinogenesis of the esophagus, we made an artificial esophageal restriction model through intra-lumen cotton node retention. Our experiment showed that the rats with the esophageal restriction had significantly more visible esophageal lesions than that of the non-constricted rats. More than 69.6% (EC group) or 78.6% (MC group) of the lesions were no more than 3mm from the node. Severe dysplasias, carcinoma *in situ* were only identified in the restriction with carcinogen groups under microscopic observation. In this experiment, two nitrosamines with different function groups showed the same tendency, though the effect of EBN was stronger than that of MPN. The results showed that the artificial restriction promotes the esophageal carcinogenesis, related with its direct action on its contacting area.

There was a report on the animal model of esophageal restriction.[6] The author put a metal ring around the esophagus through an open-thoracic surgery. But the operation is too complex and invasive, can not be used commonly. The rat esophageal restriction model in the present paper needs not complex operation, and is easy to master. We think that the model is suitable for research on esophageal cancer.

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