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Re-Examination of the Esophageal Squamous Cell Carcinoma Model in Rats Induced by N-Nitrososarcosine Ethyl Ester Precursors

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Studies of the molecular mechanisms of esophageal cancer development have to be carried out on sufficient amount of tumor material, obtained under conditions of controlled exposure to carcinogenic factors. Esophageal cancer models on laboratory animals serve an indispensable source of this material. One of these models is esophageal cancer induction in rats by N-nitroso compound precursors. Despite adequate reproduction of human esophageal cancer, this model in fact has not been used since the 1990ies. Re-examination of esophageal cancer model, induced by N-nitrososarcosine ethyl ester precursors, is carried out and its efficiency in induction of squamous cell carcinoma is confirmed.

Key Words: *esophageal cancer model; N-nitrosocompounds; N-nitrososarcosine precursors*

The prevalence esophageal cancer (EC) ranks eight among tumor diseases all over the world [3]. Esophageal tumors are in the majority of cases highly aggressive and their treatment is difficult because of asymptomatic course of the disease at the early stages and hence, untimely diagnosis. The main types of EC are adeno- and squamous cell carcinoma, which is the most prevalent in the world, including Russia [7].

Studies of the molecular mechanisms of EC development have to be carried out on sufficient volumes of tumor tissues obtained under conditions of controlled exposure to carcinogenic factors. Various EC models on laboratory animals serve as sources of this material. Esophageal tumors are most often induced in rodents by N-nitrosocompounds (NNC), primarily by N-nitrosomethylbenzylamine. The carcinogenic effects of NNC are well known, but the number of squamous cell carcinomas they induce in the rodent EC model is very low. Mainly benign papillomas form, which have no histological analogs in humans and often reach the size causing occlusion of the airways and animal death before the formation of malignant tumors [1,2,4-6]. For this reason NNC precursors seem to be more promising: injection of a combination of sarcosine ethyl ester (SEE) hydrochloride and $NaNO_2$ — N-nitrososarcosine ethyl ester precursors — induces more rapid formation of comparable numbers of papillomas and squamous cell carcinomas [8].

Despite the obvious advantages, the EC model with the use of NNC precursors is not used since the 1990s. In order to evaluate the prospects of this model for studies of EC pathogenesis, we carried out its re-examination.

MATERIALS AND METHODS

Experiments on laboratory animals were carried out in accordance with the requirements of the Ethic Committee of N. N. Blokhin Russian Cancer Research Center.

Male Wistar rats (n=29) aged 8 weeks were randomized into experimental (n=22) and control (n=7)

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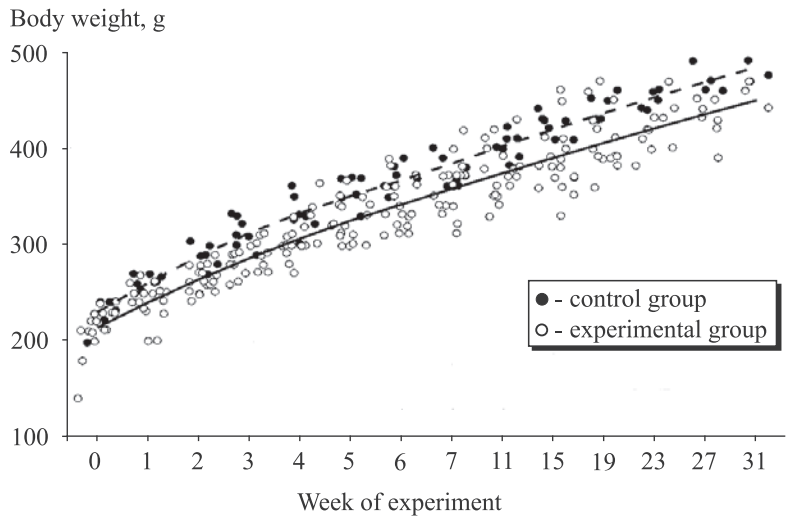

Fig. 1. Dynamics of animal body weight during the experiment. The data are approximated by logarithmic curves: interrupted line, experimental group; continuous line, control group.

groups. Experimental animals received by gavage fresh SEE solution and 1.5% $NaNO_2$ (Sigma-Aldrich) in 0.01 M HCl in a dose of 2 g SEE and 0.3 g $NaNO_2$ per kg every 3 days over 7 weeks. Controls received 0.01 M HCl solution by gavage.

Two and three experimental rats died after weeks 5 and 6 of SEE and $NaNO_2$ gavage, respectively. The remaining animals were sacrificed after SEE and $NaNO_2$ gavage was over: after 8 weeks (3 experimental and 1 control), 12 weeks (3 experimental and 1 control), 16 weeks (4 and 1 rat), 20 weeks (4 and 1 rat), and after 24 weeks (3 and 3 rats).

After autopsy, the esophagus was resected and visually examined and specimens of tissues were collected from all parts of the esophagus with pathological changes and without apparent changes (one sample from the lower, middle, and upper compartments of the esophagus). The samples were embedded in paraffin blocks, sliced (5-μ sections), and stained with hematoxylin and eosin. The samples were analyzed by three qualified pathologists.

RESULTS

Our re-examination of esophageal tumor induction model by oral N-nitrososarcosine ethyl ester precursors was based on the study presenting the most ample description of the model [8]. We used the same dose and protocol, but administered the active substances through a tube (which guaranteed accurate dosage), instead of with drinking water, available for several animals simultaneously.

The time course of body weight increment in experimental animals was about the same as in controls over the entire period of experiment (Fig. 1). Hence, esophageal tumors seemed to cause no appreciable difficulties in eating for animals.

Macroscopic examination of control animals showed esophageal structure characteristic of rodents, without pathological changes, in all animals (Fig. 2, *a*, *b*).

Macroscopic examination of the esophagus in experimental animals showed numerous exophytic tu-

TABLE 1. Induction of Esophageal Tumors by EES and $NaNO_2$

Week after beginning of experiment	Week after EES and $NaNO_2$ treatment	Number of rats	Number of rats with tumors		Total number of tumors		Tumor location*		
			C	P	C	P	U	M	L
15	8	3	2	3	5	11	0	2	14
19	12	3	1	2	1	5	0	1	5
23	16	4	4	3	8	14	1	3	18
27	20	4	4	4	13	19	0	7	25
31	24	3	1	3	1	8	0	1	8
Total:		17	12	15	28	57	1	14	70

Note. C: carcinoma; P: papilloma. U, M, L: upper, middle, and lower thirds of esophagus, respectively. *Summary for carcinomas and papillomas.

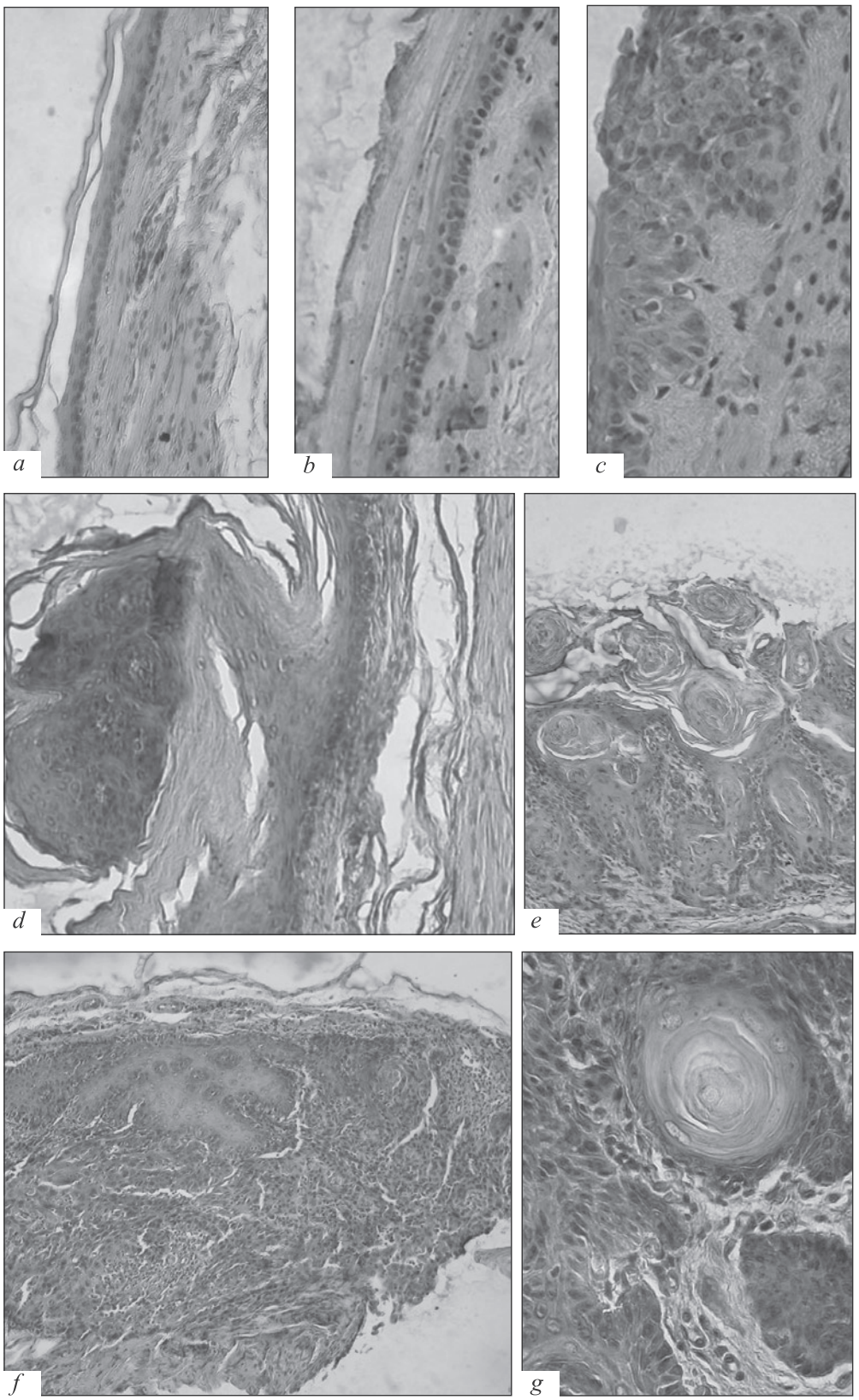

Fig. 2. Morphological changes in the esophagus, ×100 (*a*, *b*, *d*, *e*, *f*), ×200 (*c*, *g*). *a*, *b*) Normal squamous epithelium of the esophagus in control rats; *c*) focus of dysplasia with disordered cell differentiation and intensive proliferation; *d*) papilloma on a wide base protruding into esophageal lumen; *e*) papilloma with manifest signs of hyperkeratosis — formation of horny "pearls"; *f*) well-differentiated squamous cell carcinoma without manifest hornification; *g*) well-differentiated squamous cell carcinoma with hornification.

mors, located mainly in the lower and middle thirds of the esophagus, irrespective of the time passed since the end of SEE and $NaNO_2$ treatment (Table 1). Microscopic studies showed squamous-cell papillomas and carcinomas with and without hornification. In addition, microscopy showed numerous foci of dysplasia in specimens of macroscopically normal tissues (Fig. 2, *c-g*; Table 1).

Tissue with signs of dysplasia differed from normal tissue by intense proliferation and disordered cell differentiation. Count of basal cells increased, the cells became larger and polymorphic, the number of mitoses increased (Fig. 2, *c*). Dysplasia was detected in specimens of macroscopically normal tissues from the lower third of the esophagus in all experimental animals starting from the first autopsy. Papillomas consisted of connective tissue of the stroma, characterized by squamous epithelium growth and containing several fine-walled blood vessels (Fig. 2, *d*, *e*). Two papillomas, collected 12 and 20 weeks after the end of SEE and $NaNO_2$ treatment, had signs of hornification — formation of keratin "pearls", disorders in cell polarity, and emergence of large pyknotic nuclei (Fig. 2, *e*). Malignant tumors were mainly presented by well-differentiated squamous cell carcinomas with hornification (n=17) and without it (n=10) (Fig. 2, *f*, *g*). Carcinomas with hornification were characterized by moderate cellular and nuclear polymorphism and by gradual progress of hornification from periphery to the center of tumor complexes. In addition to well-differentiated carcinomas, there was one solitary poorly-differentiated squamous cell carcinoma with sharply manifest cellular polymorphism and atypia with the minimum signs of hornification.

Our protocol of experiment led to induction of 37% carcinomas and 63% papillomas (an average of 5 tumors per animal: 1.65 carcinomas and 3.35 papillomas), which was close to the results of a previous study [8]: 33 and 67%, respectively (4.4 tumors per animal: 1.45 carcinomas and 2.85 papillomas). On the other hand, our data differed from the results of the above [8] study. We observed no stable increase in the number of papillomas and carcinomas with time, all induced tumors were exophytic, all carcinomas (27 of 28) and 75% papillomas (43 of 57) were located in the lower part of the esophagus (Table 1). The causes of these differences are not clear, presumably, they could be attributed to different protocols of the active substances administration.

The fact that the numbers of papillomas and carcinomas did not increase with time seemed to indicate that the tumors formed in animals earlier than was presumed previously [8], probably before the first autopsy. Hence, it seems that the number of tumors would have been the same at earlier autopsies, but the number of animals in our study was insufficient to choose the optimal term of experiment.

Comparison of our data with the results attained on other models [1,2,4-6,8] indicated that the use of precursors of N-nitrososarcosine ethyl ester induced the greatest number of malignant tumors with the least possible number of undesirable papillomas.

Hence, our re-examination persuasively demonstrated that EC model in rats, induced by N-nitrososarcosine ethyl ester precursors, is easily reproducible and recommended for induction of a sufficient number of squamous-cell esophageal carcinomas.

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