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ONCOLOGY

Inhibitory Effect of Peptide Vilon on the Development of Induced Rat Urinary Bladder Tumors in Rats

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The effect of peptide vilon (Lys-Glu) on urinary bladder carcinogenesis in rats was studied. Urinary bladder tumors were induced with a selective carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine. The tumors developed in 56% vilon-treated animals and in 75.5% controls. Vilon 2-fold decreased the incidence of preneoplastic and early neoplastic changes in urinary bladder mucosa and significantly inhibited carcinogenesis.

Key Words: *urinary bladder; carcinogenesis; N-butyl-N(4-hydroxybutyl) nitrosamine; peptides; vilon*

Pronounced stimulating effects of vilon on the immune system, tissue regeneration, and inhibition of inflammatory processes were demonstrated in many experimental studies [2,7]. Vilon prolonged the life-span and decreased the incidence of spontaneous tumors in mice [6]. The purpose of the present study was to examine the effect of this peptide on the formation of induced urinary bladder (UB) tumors.

MATERIALS AND METHODS

The experiments were conducted with 100 random-bred albino male rats (Rappolovo Breeding Center, Russian Academy of Medical Science) weighing 120-140 g. The animals were kept in plastic cages (5 rats per cage) with automatic water bowls and received standard granulated forage and water *ad libitum*. All animals were weighed monthly, starting from month 1 of the experiment.

All rats received selective UB carcinogen N-butyl-N(4-hydroxybutyl)nitrosamine (BBNA; 0.04%) with water via automatic water bowls for 12 weeks. In total, each rat received 1 g BBNA.

Experimental rats (n=50) simultaneously with BBNA received vilon (1 μg/kg body weight, intramuscularly into the right thigh) for 6 months (5-day courses with 4 weeks intervals, total dose 30 μg/rat).

Control rats (n=50) were injected with physiological saline. Dead animals were subjected to autopsy. After the end of the experiment (49 weeks) all animals were killed and macroscopic and microscopic studies were performed for detailed examination of UB pathology. For microscopy, 5-7 μ sections were prepared. The preparations were stained with hematoxylin and eosin. The analysis of UB changes was based on Classification of Tumors in Laboratory Animal [8]. The results were processed statistically [1].

RESULTS

No differences in physiological state and behavior of both rat groups were noted. One control animal died 2 months after the start of the experiment. No changes in internal organs, including UB were revealed except enteritis.

Since neoplastic processes in UB mucosa were multicentric and tumors developed asynchronously in

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one rat, which affected the degree of malignization in different mucosa regions, pathological state was evaluated not by tumor size and number, but by the degree of their malignancy.

Most control animals (n=34) were sacrificed on week 49 of the experiment. Preneoplastic and neoplastic changes in UB were found in 15 of 16 animals died before this term.

Apart from changes in UB, colitis associated with sclerosis, paralytic dilatation, and papillomatosis of the large intestine was observed.

The first rat with UB changes died on week 21 of the experiment. UB mucosa looked rough with numerous small 0.1-0.2-cm nodules. Microscopic examination revealed diffuse hyperplasia of the transitional epithelium with multiple papilloma. Three rats died by week 30: diffuse and focal hyperplasia were found in 2 of them, one rat had focal squamous cell papilloma. Six more rats died by week 40: two of them had UB carcinoma, two transitional cell papilloma, and two rats had diffuse and focal hyperplasia. By week 45 of the experiment, carcinoma was found in 2 rats, while 3 others had transitional cell papillomas with invasive growth or *in situ* carcinoma structures.

Thus, the degree of UB tumor malignancy increased with increasing the duration of treatment. On week 49, autopsy of 34 rats revealed the following changes in UB: 26 animals had benign and malignant tumors with metastases into internal organs, 8 rats had early preneoplastic changes like diffuse or focal hyperplasia of UB mucosa. In total, during 49 weeks of the experiment, proliferative changes in UB mucosa manifested as diffuse and nodal hyperplasia accompanied by the formation of Brunn nests, *Cystitis cystica* and *Cystitis glandularis* phenomena were observed in all 49 animals survived until the appearance of the first tumor 21 weeks.

UB dissection showed multifocal lesion most pronounced at late stages of the tumor process. Each rat had on average 2.5 tumor nodes. Frequently, tumor nodes occupied the entire bladder lumen. Sometimes the tumors grew through all layers of UB wall, spread on the serous membrane, and metastasized into the mesentery and paraaortic lymph nodes.

Papillary transitional cell tumors were often associated with inverted papilloma structures. In these cases, endophytic growth with deep invaginations and the formation of lateral secondary and tertiary trabeculae in loose and swollen connective tissue was observed. The transitional epithelium was transformed into squamous epithelium or formed pseudoglandular structures. Inverted papillomas were also malignant and formed large fields of atypical polymorphic urothelium with high mitotic index and infiltrative growth.

Preneoplastic and early neoplastic changes in UB mucosa (diffuse and focal hyperplasia and micropapillary hyperplasia) were noted in 12 rats (Table 1). In all cases mucosa was changed. Of 37 rats with tumors, 13 animals had definitely malignant tumors, while 24 rats had benign or relatively benign tumors with initial invasive growth of *in situ* carcinoma structures.

In vilon-treated group, 2 rats died on week 22 of the experiment: one rat had general peritonitis and another had toxic enteritis. Hyperplasia of the UB mucosa (0.1 mm thickenings) was found on the lateral surface of UB in both animals.

Thereafter, focal and diffuse hyperplastic changes were found in 3 rats at different terms of the experi-

TABLE 1. Effect of Vilon on BBNA-Induced Carcinogenesis

Parameter	Control		Vilon	
	abs.	%	abs.	%
Initial number of animals	50	—	50	—
Survived to the appearance of the first tumor	49	—	50	—
Without UB tumor	0	—	4*	—
Preneoplastic and early neoplastic changes	12	24.5	18	39
Number of animals with UB tumors				
total	37	75.5	28	56*
benign	13	35.5	14	50
in situ carcinoma	11	29.7	4	14.3*
malignant	13*	35.2	10	35.7
Number of UB tumors per animal	2.6 (102/40)	—	1.5 (51/34)	—
Total UB mucosa lesion	6	—	3	—

Note. *p<0.05 compared to the control. In parentheses: number of tumor nodes/number of rats with tumors and early neoplastic changes. *Metastases to paraaortic lymph nodes and mesentery.

ment up to week 45: benign transitional cell papillomas in 2 and UB carcinomas in 2 animals.

Forty-one rats survived by week 49. In contrast to the control group, macro- and microscopic examination revealed no hyperplastic changes in 4 rats; 18 rats (39%, including rats died from the start of the experiment) showed only initial hyperplastic or early preneoplastic changes (Table 1). Similarly to the control group, neoplastic process started with diffuse or focal hyperplasia of the transitional epithelium associated with the formation of Brunn nests containing pseudoglandular structures and vegetations of transitional epithelium. No atypical cells and enhanced mitotic activity were found.

Comparison of UB lesions in the experimental and control groups revealed later epithelium malignization in vilon-treated rats, which was especially pronounced during evaluation of morphologic changes in the subgroup with relatively benign tumors (*in situ* carcinoma foci, invasive tumor growth). These changes were observed in 11 (29.7%) and 4 (14.3%, $p<0.05$) animals in the control and vilon-treated groups, respectively. Mucosa lesions were fewer (1.5 tumor nodes per rat).

The number of animals with papillomas did not differ from the control (14 and 13 animals, respectively), while the number of rats with malignant tumors slightly decreased (10 *vs.* 13 animals in the control). Morphology of malignant tumors was similar to that in the control: 6 rats had papillary transitional cell carcinoma with inverted polyp-like vegetations, while 4 rats had squamous cell carcinoma.

Our previous studies on the induction of UB tumors in rats with various carcinogens revealed definite stages in the formation and development of tumors, their malignization, and latency depending on specific activity, dose, and duration of treatment with the inductor. These facts are very important for correct evaluation of additional factor stimulating or inhibiting the specific effect. Studies of dose dependency showed that 2-fold increase in the total dose of BBNA (from 840 to 1680 mg/rat) resulted in 100% UB lesion, while the degree of malignancy increased from 68.4 to 93.7%. Early hyperplastic changes disappeared, the number of transitional cell papillomas and relatively benign tumors (*in situ* carcinoma) decreased to 6.2%, while the incidence of carcinomas increased to 75% [3].

These and previous data [4] suggest that UB-specific carcinogens determine the dynamics of morphological changes: weakening of the carcinogenic effect was accompanied by deceleration of urothelium malignization and accumulation of preneoplastic and early neoplastic changes. The carcinogenic effect can be attenuated by decreasing the dose of carcinogen or by treatment with antineoplastic agents. For instance, experiments with sodium selenite, an inhibitor of carcinogenic effect of BBNA, showed that this substance decelerated, but not its completely inhibited urothelium malignization [5].

By our opinion, the intensity and nature of early preneoplastic and neoplastic changes in UB reflect many processes of oncogenesis, its initiation, promotion and progress. Typically, on week 49 of the experiment minimum lesions or their absence were observed in 18 (44%) of 41 vilon-treated rats including 4 rats without UB pathology, whereas in the control group minimum changes were observed only in 8 (23%) of 34 rats. Thus, vilon injections against the background of carcinogen administration 2-fold decreased the incidence of pathological changes in UB.

These findings suggest that immunomodulator vilon inhibited both the initial stages of carcinogenesis and tumor progression, *i.e.* realization of the tumor process.

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