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EFFECT OF KRESTIN ON BLADDER TUMOR INDUCTION IN RATS BY *N*-BUTYL-*N*-(4-HYDROXYBUTYL) NITROSAMINE

Kimio FUJITA, Hiroko FUJITA, Kazuo SUZUKI, Atsushi TAJIMA and Yoshio ASO

From the Department of Urology, Hamamatsu University School of Medicine, Handacho 3600, Hamamatsu, Shizuoka, 431-31, Japan

INTRODUCTION

A variety of polysaccharides have been reported to have antitumor activity mediated by their ability to stimulate immunologic host defence mechanisms. Krestin, or PS-K, is one of such polysaccharides isolated from basidiomycetes. Experimental and clinical studies have reported the antitumor effect of the substance[4,7,8]. All these studies deal with established cancer cells. The possible role of the polysaccharide on the host defence mechanism against neogenesis of malignancy is interesting.

MATERIALS AND METHODS

Animals. The animals used were ACI/N rats, weighing 200 to 250 g, purchased from Hoshino Animal Co., Tokyo.

Chemicals. N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) was obtained from Izumi Chemical Co., Yokohama. Krestin (PS-K) was supplied from Kureha Chemical Ind., Tokyo.

Experimental procedure. Fourty-eight rats were devided to 3 groups. The first group was control group which did not receive neither BBN nor Krestin. The second group received BBN only and the third group received both BBN and Krestin.

BBN was dissolved in deionized water as a 0.025% solution and administered ad libitum for 12 weeks. The total dose taken by an animal was 540 mg on the average. Krestin was diluted in saline and was injected intraperitoneally at a dose of 50 mg/kg of body weight once two days during the exposure period.

Four rats from each group were sacrificed at 6, 12, 24 and 28 weeks each after the beginning of the experiment. Bladder was distended by 0.5 ml of 10% formalin and fixed, then embedded in paraffin and sections were stained with hematoxylin and eosin.

Histological grading. The generated tumors were classified according to their histological findings as follows;

Grade 0: Papillary tumor covered by regular transitional epithelium indistinguishable from that of the normal bladder and not more than six layers thick.

Grade 1A: Papillary tumor including inverted type and squamous metaplasia. Cells do not show any tendency to anaplasia and the basement membrane is intact. Mitotic figures are either extremely rare or entirely absent.

Grade 1B: Same as Grade 1A but undifferentiated cells and mitotic figures are occasionally found.

Grade 2: Prominently overgrowing tumor. Cells show pleomorphism. Mitotic figures are frequently observed.

Grade 3: Highly aggressive tumor, showing evidence of anaplasia and invasion.

RESULTS

Any animals fed with water without BBN did not develop bladder tumor throughout the experimental period. Animals BBN and sacrificed at 6 and 12

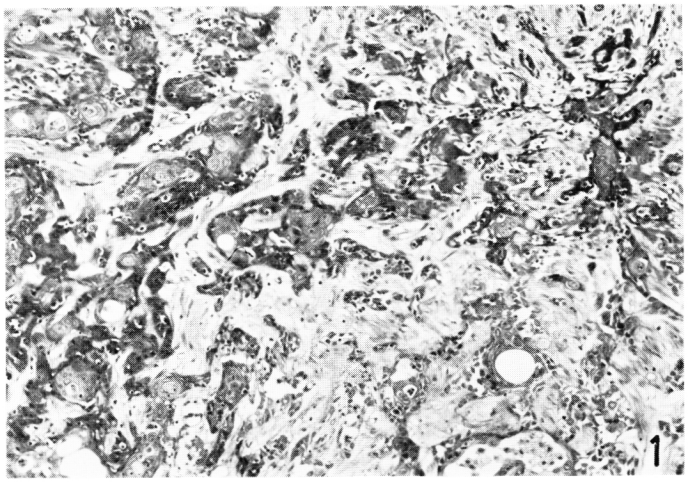

Fig. 1. An anaplastic, highly aggressive tumor arised from the bladder of a rat without Krestin. Squamous transformation is characteristic.

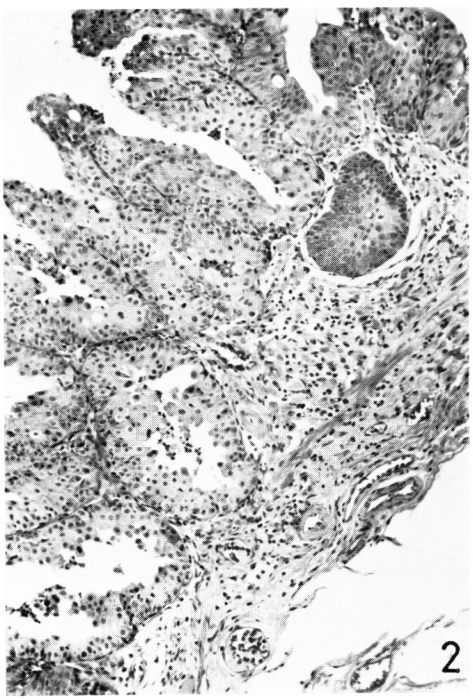

Fig. 2. Papillary tumor of a rat received Krestin. Profound mononuclear cell infiltration and degenerating tumor cells are observed.

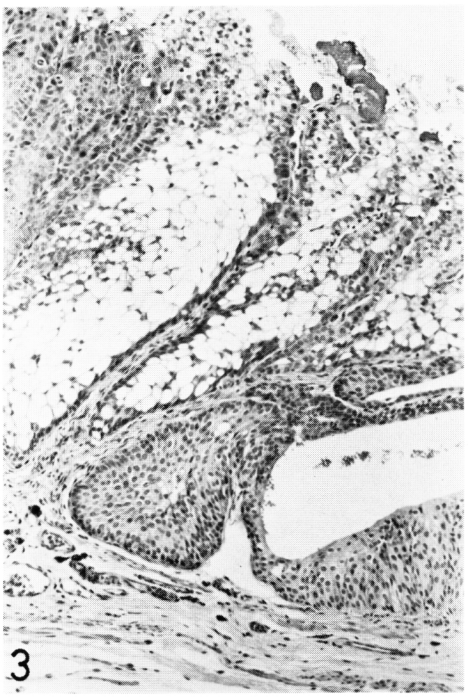

Fig. 3. A conspicous vacuolated area. Although the mechanism of this phenomenon is not known, it may be a process of malignant cells being eliminated.

Table 1. Size of the Bladder Tumors.

In cases of multiple tumors, the volumes of tumors were collected into one assumptive mass.

Group	Size				Total
	Under 5mm	5-10mm	10-20mm	Over 20mm	
BBN	4	2		2	8
BBN+Krestin	5	2	1		8

Table 2. Grade of the Bladder Tumors.

Group	Grade				Total
	0	1	2	3	
		A B			
BBN		2	4	2	8
BBN+Krestin	1	4 2	1		8

weeks developed many papillary hyperplastic foci of Grade 0. The number and size of the hyperplastic foci showed no difference between the Krestin group and the group without Krestin. The difference between the two groups was observed in animals explored at 24 weeks or later. Highly aggressive tumor developed in two animals not received Krestin (Fig. 1). One of them died at 26 weeks and the other was included in the animals sacrificed at 28 weeks.

Animals explored later than 24 weeks were classified according to the grading and size of bladder tumors they developed. The tumors, although essentially being of transitional cell type, showed marked tendency to squamous transformation. The results were shown in Table 1 and Table 2. Anaplastic tumor was less frequent among animals received Krestin ($p<0.05$). Highly aggressive tumor was not found in the group. On histological examination characteristic findings for the Krestin group were marked lymphocyte infiltrations (Fig. 2) and vacuolization of tumor cells (Fig. 3).

DISCUSSION

Many substances were suggested to have antitumor effect as the results of immune stimulation. Krestin is a protein-bound polysaccharide derived from coriolus vesicolor, Quel. of basidiomycetes[4]. The antitumor effect of the agent is considered to be exerted through a host-mediated immune mechanism, because the effect is observed even when the agent is administered to animals before the experimental tumor inoculation. It restores the depressed antibody-forming capacity of tumor-bearing animals[7]. It stimulates lymphocytes and induces them into blastogenesis in vitro and, in vivo, causes hyperplasia of T-cell area in spleen and lymph nodes[8]. The polysaccharide is now commercially available and clinically used as an adjuvant therapy for malignancy.

BBN is known to have a selective carcinogenic action on the urinary bladder. Many investigators have used the substance for rats and mice to generate bladder tumor[2,5,6,9]. Our present observation suggests the possible effect of Krestin on prevention of the tumor-genesis. To our regret, we could not clearly demonstrate the mechanism through which the animals with Krestin inhibit the development of the tumor. Immunological indicies we studied, such as cell surface markers of lymphocytes, blastogenesis by lectins, and hemolytic plaque forming cells, were extremely complicated and could not be explained reasonably. Nevertheless, the histological observations suggest the inhibitory effect of the substance on the development of the tumor, probably through the defence surveillance mechanism of the animals. Marked mononuclear cell infiltration and vacuolated tumor cells may be the expression of the surveillance mechanisms which eliminate malignant cells[1,3,10].

SUMMARY

ACI/N rats were exposed to N-butyl-N-(4-hydroxybutyl) nitrosamine and developed hyperplasia, metaplasia and subse-

quent malignancies in the bladder. However, animals simultaneously administered Krestin, a protein-bound polysaccharide isolated from basidiomycetes, showed an inhibitory effect on the development of malignancy. It is an evidence that an immunostimulating antitumor agent have a preventive effect on oncogenesis.

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和文抄録

N-Butyl-N-(4-hydroxybutyl) Nitrosamine による膀胱発癌に対する Krestin の効果

浜松医科大学泌尿器科学教室（主任：阿曽佳郎教授）

藤田公生・藤田弘子

鈴木和雄・田島惇

阿曽佳郎

ACI/N ラットを1群16匹とし，膀胱発癌剤である N-butyl-N-(4-hydroxybutyl) nitrosamine を 0.025% 溶液として12週間自由飲水させた．免疫賦活作用をもつ多糖体とされている Krestin を 50 mg/kg 隔日腹腔内注射した群を発癌予防群とした．発癌予防群においては悪性度の高い腫瘍の発生が抑制される傾向がみられ，発癌剤投与開始から24ないし28週に検索したラット8匹中 Grade 2 以上の腫瘍の発生していたのは1匹であった．